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Design and synthesis of privileged scaffolds for targeting RNA

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Chemistry

by

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2014
The Dissertation of Kevin D. Rynearson is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2014
DEDICATION

I would like to dedicate my thesis to my amazing wife, Tiffany J. Rynearson. She has been a wonderful source of support throughout my graduate career. She has inspired me to strive for greatness in all of my endeavors and to never give up in spite of adversity. She is beautiful, kind and intelligent. She has been understanding and compassionate through the stress, and she has made sacrifices for my success. I love you.

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3. “Screening for Inhibitors of the Hepatitis C Virus Internal Ribosome Entry

4. “1,3-Diazepanes of Natural Product-Like Complexity from Cyanamide-
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PRESENTATIONS

1. Poster presentation in the American Chemical Society 245th ACS National
Design and synthesis of privileged scaffolds for targeting RNA

by

Kevin D. Rynearson

Doctor of Philosophy in Chemistry

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Professor Thomas Hermann, Chair

A series of novel benzoazoles was designed and synthesized with the goal of binding to subdomain IIa of the Hepatitis C virus (HCV) internal ribosome entry site (IRES) RNA and inhibiting viral translation. The benzoazoles were prepared with fundamental traits which bias these ligands for interaction with RNA. The benzoazoles were constructed around a recognition element contained in a known subdomain IIa binding ligand that forms discrete hydrogen bonds to the RNA target. This motif within a novel benzoazole core was anticipated to act as a molecular anchor which orients various substituents, allowing for the exploration of the chemical environment in the subdomain IIa binding cavity. The novel ligands were evaluated by a FRET assay and an in vitro translation assays to determine binding affinity and target inhibition.
Structure activity relationships of the substitution patterns were explored. Functional elements which conferred activity were combined around a common benzoxazole scaffold with the goal of creating improved inhibitors.
Chapter 1

Synthesis of Oxazoline Analogs of Streptolidine Lactam
Introduction

Initially relegated to role of a transient messenger between DNA and protein, ribonucleic acid (RNA) has emerged as a crucial macromolecule to processes such as transcriptional regulation, translational regulation, protein interaction and catalysis. This fundamental shift makes RNA an attractive drug target due to the expansive role RNA plays in essential biological functions. Small molecule ligands which selectively bind to RNA and abrogate function offer a potential avenue for the development of novel therapeutics.

RNA, like protein, offers a rich landscape for small molecule interaction. As RNA folds, regions often are not fully base paired resulting in perturbations from a simple duplex. These unpaired regions form complex secondary structures such as hairpins, bulges and loops. The elegant architectures formed by mismatched bases afford potential binding pockets for small molecules to disrupt function. However, unlike proteins which display regions of both positive and negative potentials, RNA offers a surface with varying degrees of anionic character. Consequentially, ligands designed to bind to RNA must complement the electrostatic nature of RNA.[1-3]

Despite a secondary structure which creates a landscape rich with potential binding sites, RNA is an underexploited target for small molecules. Antibiotics such as aminoglycosides, macrolides, tetracyclins and oxazolidinones indeed exert their antibacterial effects by binding to ribosomal RNA (rRNA); however, targeting other types of non-ribosomal RNA has proven to be difficult.[4]
The challenges within this field are to expand beyond rRNA targets while improving selectivity of small molecules for their cognate RNA with the goal of creating a paradigm for target specific RNA binding ligands.

**Streptolidine Lactam**

Antibiotics which block bacterial protein synthesis by binding to rRNA share common structural motifs despite chemical diversity of these natural products. Ligand selectivity and affinity are provided by the dense arrangement of hydrogen-bond donors and acceptors within a rigid molecular scaffold. General affinity of the ligand for RNA is further enhanced by the presence of basic moieties which result in non-specific electrostatic interactions with the phosphate backbone. Streptolidine lactam 1, a non-proteogenic amino acid constituent of streptothricin antibiotics, captures this paradigm with an array of hydrogen-bonding sites which line the periphery of this rigid heterocycle (Figure 1.1). The absence of multiple basic sites within the streptolidine lactam should lead to increased selectivity by limiting non-specific electrostatic interactions. However, the presence of the highly basic aminoimidazole within the streptolidine lactam negatively impacts its drug-like properties.
The aminoimidazole moiety has proved to be adept at the recognition of nucleobases within RNA folds. Hepatitis C virus (HCV) binding ligands illustrate the critical role that the aminoimidazole group plays in binding to an RNA target.\(^{[8-10]}\) Streptothricin antibiotics which contain the aforementioned aminoimidazole motif (Figure 1.2) have been shown to bind the bacterial ribosome and block the translocation of the peptidyl-tRNA complex and thereby inhibit protein synthesis.\(^{[11-12]}\) However, the clinical use of broad spectrum streptothricin antibiotics is prevented due to mammalian toxicity. The inherent toxic nature of the streptothricin antibiotics is attributed to indiscriminate protein binding and nephrotoxicity resulting from the strong basicity of the aminoimidazole moiety contained within the streptolidine lactam.\(^{[13-14]}\) The electronic similarity of the aminoimidazole to guanidine renders it quite basic with a $pK_a$ of approximately 10.
Figure 1.2: Structure of the streptothricin family of antibiotics (4) which contain the streptolidine lactam. The number of β-lysyl groups may vary from $n = 1$ to $n = 7$. The aminoimidazole moiety is highlighted in red.

In order to retain the hydrogen-bonding interaction sites of the streptolidine lactam while tempering the basicity of the aminoimidazole group, oxazoline analogs 2 and 3 were designed (Figure 1.1). Oxazoline 2 was previously synthesized in our lab to evaluate the potential of this core as a selective RNA binding ligand.\textsuperscript{[15]} In an effort to further explore RNA-ligand interactions, ring-expanded azepane oxazoline 3 was designed to accentuate the non-planarity of the RNA ligand by increasing the ring size of the lactam. The interruption of planarity disfavors nonspecific intercalation and is generally considered beneficial for target selectivity in nucleic acid binding ligands. Additionally, seven-membered rings remain conspicuously absent from recently reported fragment libraries directed at RNA targets despite offering a rigid non-planar scaffold for the assembly of directed functional groups capable of interacting with RNA nucleobases.\textsuperscript{[16]}
Scheme 1.1: One-pot synthesis resulting in oxazoline lactam 5 and rearranged 1,3-diazapane 6 from protected epoxy-δ-lactam 7 in a 2.5:1 ratio in a 63% yield.

Reagents and conditions: a) NaNH, DMSO, 10 °C to R.T.; b) MeOH, H2O, 2 steps 63%.

During the course of the synthesis of oxazoline 5, a novel rearrangement was observed providing structurally complex diazepane 6.[15] This unprecedented rearrangement permitted access to two functionally diverse scaffolds which maintain the requisite hydrogen-bonding faces for RNA recognition from a single precursor. Synthetic efforts toward azepane oxazoline 3 allow the examination of the broader applicability of this rearrangement toward a ring expanded substrate.

Retrosynthetic Analysis

Inspection of the streptolidine lactam reveals the potential for both cis and trans-fused ring isomers. A synthetic route toward the trans-fused analogs was developed since this motif is more common in natural product isolates.[17] The synthesis of the desired trans-fused azepane oxazoline 3 was envisaged through the nucleophilic opening of epoxy-ε-lactam 8 with cyanamide followed by deprotection (Scheme 1.2).[18-19] The regioselective control of the ring opening would be the result of the steric bulk provided by the silyl-protected hydroxyl,
favoring an attack at the less hindered α-position. The epoxy-ε-lactam 8 would be generated from electron poor alkene 9 using peroxides. Preparation of protected hydroxylactam 9 would be accomplished via allylic oxidation of unsaturated lactam 11 to ketolactam species 10 which would then be reduced and protected. Beckmann rearrangement of ketoxyime 12 would provide direct access to lactam 11. Finally, ketoxyime 12 would be made by the straightforward reaction of commercially available cyclohexenone 13 with hydroxylamine.

![Reaction Mechanism](image)

**Scheme 1.2:** Initial retrosynthetic analysis of azepane oxazoline 3.

Synthesis of epoxy-ε-lactam 8 commenced with the facile condensation of cyclohexenone and hydroxylamine hydrochloride providing ketoxyime 12 (Scheme 1.3). Beckmann rearrangement in molten polyphosphoric acid followed by allylic oxidation catalyzed by manganese acetate dihydrate of ketoxyime 12 allowed straightforward access to precursor 10. Protected hydroxylactam 9 was readily obtained by 1,2-reduction utilizing Luche conditions followed by silyl-protection of hydroxylactam 14. Efforts to form epoxy-ε-lactam 8 from protected hydroxylactam 9 using various peracids, peroxides, and oxone were
unsuccessful. Hydroxylactam 14 was also subjected to various epoxidation conditions to explore the role of sterical factors upon this reaction. Similarly, epoxide formation upon the deprotected substrate was not observed suggesting steric effects do not play a major role in impeding this reaction. Additionally, epoxidation of ketolactam 10 was undertaken anticipating that the strongly electron deficient alkene would be more reactive. Again, the alkene failed to be oxidized when exposed to various epoxide forming conditions implying that the flanking functional groups have a profound impact on the chemical reactivity of these species. Despite the preparation of precursors 9, 10 and 14, the deactivating nature of the amide adjacent to the alkene rendered these compounds all but inert toward epoxidation necessitating the development of an alternative synthetic route.

Scheme 1.3: Initial route toward the preparation of epoxy-ε-lactam precursor 8.

Reagents and conditions: a) NH$_2$OH•HCl, NaOAc, H$_2$O, EtOH; b) Polyphosphoric acid, 120 °C, 2 steps 44%; c) Mn(III)Ac•2H$_2$O, TBHP, EtOAc, 69%; d) NaBH$_4$, CeCl$_3$, MeOH, 0 °C, 85%; e) tert-Butyldimethylsilyl chloride, imidazole, DMF, 0 °C to R.T., 68%.
The inability to prepare epoxy-ε-lactam 8 through unsaturated hydroxylactam 9 required modification of the synthetic route. The final transformation providing azepane oxazoline 3 would be accomplished as previously mentioned through the opening of epoxide 8 with cyanamide (Scheme 1.4). However, the alternative synthetic pathway to epoxy-ε-lactam precursor 8 would depend upon the introduction of the epoxide prior to ring expansion. Epoxy-ε-lactam 8 would be prepared by the Schmidt rearrangement of epoxy ketone 15. Access to epoxy ketone 15 would be accomplished by the epoxidation followed by protection of α,β unsaturated ketone 16. The formation of the epoxide was predicted to yield a pair of syn enantiomers as a result of coordination of the peroxide with hydroxyl of the substrate. Hydroxy ketone 16 would be synthesized by the ring opening of epoxide 17. Acidic hydrolysis of commercially available 1-methoxy-1,4-cyclohexadiene (19) followed by the epoxidation of β,γ unsaturated ketone 18 would permit the construction of epoxide 17.

Scheme 1.4: Revised retrosynthetic analysis of azepane oxazoline 3.
Synthesis of Azepane Oxazoline Analog of Streptolidine Lactam

Synthesis of azepane oxazoline 3 was undertaken according to the revised retrosynthetic analysis in which the epoxidation would be carried out prior to ring expansion. Preparation of azepane oxazoline 3 was initiated by the acid catalyzed hydrolysis of 1-methoxy-1,4-cyclohexadiene\(^{[20-22]}\) followed by epoxidation of the resulting unsaturated ketone with mCPBA which gave straightforward access to epoxy ketone 17\(^*\) as a racemic mixture (indicated by \(\ast\)) \((\text{Scheme 1.5})\)^{[23-26]} Base induced ring opening of precursor 17\(^*\) supplied allylic alcohol 16\(^*\) as a racemic mixture\(^{[23-26]}\) which was then oxidized to hydroxy epoxide 20\(^*\) using tert-butylhydroperoxide in the presence of Triton B. As anticipated, hydroxy epoxide 20\(^*\) was isolated as a mixture of syn enantiomers\(^{[27-32]}\) The observed diastereoselectivity of the epoxidation has been previously demonstrated and is likely the result of the peroxide forming a hydrogen bond to the hydroxyl face of the substrate prior to reaction favoring the formation of the syn product\(^{[33]}\) Precursor 20\(^*\) was then efficiently converted to silyl-protected epoxy ketone 15\(^*\) using tert-butyldimethylsilyl chloride. However, classical Schmidt rearrangement conditions to access epoxy-\(\varepsilon\)-lactam 8 were unsuccessful owing to the harsh reaction conditions necessary to affect this transformation. The evaluation of milder conditions capable of instigating the Schmidt rearrangement were investigated but proved fruitless.
Scheme 1.5: Revised route toward the preparation of epoxy-ε-lactam precursor 8. * indicates product was isolated as a racemic mixture.

Reagents and conditions: a) HClO₄, H₂O, CCl₄; b) mCPBA, DCM, 0 °C to R.T.; c) basic alumina, DCM, diethyl ether, 3 steps 56%; d) tert-Butylhydroperoxide, Triton B, THF, 0 °C, 83%; e) tert-Butyldimethylsilyl chloride, DIPEA, DMAP, DMF, 0 °C to R.T., 84%.

Direct access to epoxy-ε-lactam 8 from the protected epoxy ketone 15* was not possible by way of the Schmidt rearrangement which is likely the result of the substrates instability under the reaction conditions. Guided by the literature precedent for the conversion of epoxy naphthoquinone into an epoxy benzazepine, hydroxy epoxide 20* was oxidized to meso-diketoepoxide 21 with the aim that this molecular fortification would allow the substrate to survive the Schmidt rearrangement (Scheme 1.6).[34] Additionally, the oxidation of hydroxy epoxide 20* results in an achiral intermediate rendering the stereochemical outcome of previous steps of no consequence with respect to the synthesis of epoxy-ε-lactam 8. Subsequent exposure of meso-diketoepoxide 21 to classical
Schmidt rearrangement conditions was able to induce ring expansion to ketone 22*, however in very low yield.

Scheme 1.6: Final route developed to prepare epoxy-ε-lactam precursor 8. Additional oxidation step to meso-diketone 21 yielded a substrate capable of undergoing the Schmidt rearrangement. * indicates product was isolated as a racemic mixture.

Reagents and conditions: a) PDC, NaOAc, DCM, 86%; b) NaN₃, AcOH, H₂O, H₂SO₄ (cat), 5 °C 62% based on recovered starting material; c) NaBH₄, EtOH, H₂O, 89%; d) tert-butyldimethylsilyl chloride, DIPEA, DMAP, DMF, 0 °C to R.T., 81%.

Dissatisfied with the yield from the Schmidt rearrangement, various reaction conditions were explored with the goal of optimizing this transformation. Experimental conditions which limit the amount of sulfuric acid used in the Schmidt rearrangement were hypothesized to result in improved yields. Novel conditions in which acetic acid was employed as the solvent with catalytic amounts of sulfuric acid gave moderate yields. The optimized reaction conditions toward epoxy-ε-lactam 8 maintain the acidic nature of the solvent required for the reaction while restricting the use of corrosive sulfuric acid which causes substrate
and product degradation. Furthermore, acetic acid allowed better control of the heat generated by the reaction resulting in decreased charring during the addition of the azide.[35]

With a satisfactory route to functionally complex precursor 8, synthesis toward azepane oxazoline 3 was continued. Reduction of ketolactam 22* using sodium borohydride provided facile access to hydroxy lactam 23* (Scheme 1.6). Surprisingly, the reaction proceeded stereoselectively furnishing exclusively the anti-product as a racemic mixture confirmed by crystal structure analysis. The presence of the epoxide in the substrate was expected to influence the reducing agent to the less hindered face, however not to the extent observed. Stereoselective reductions such as this have been previously reported in which linear α,β-epoxy ketones react with sodium borohydride in the presence of calcium or lanthanum ions give rise exclusively to anti products. The ions in the reaction are suspected to form a rigid bidentate complex with the epoxy ketone that exposes the less hindered face to hydride attack.[36] The epoxy ketone motif within a rigid lactam 22* may serve a similar purpose by adopting a convex/concave structure that favors the reduction at the more accessible convex surface resulting exclusively in the formation of the anti product. Subsequent protection of hydroxylactam 23* using tert-butyldimethylsilyl chloride yielded the precursor 8* for oxazoline formation.
Scheme 1.7: Preparation of azepane oxazoline analog 3* of streptolidine lactam. Precursor 24* and analog 3* were isolated in a 1:5 ratio in 64% yield from a single pot. * denotes product was isolated as a racemic mixture.

Reagents and conditions: a) NaNH, DMSO, 10 °C to R.T.; b) H₂O, 2 steps 64%.

Epoxy-ε-lactam 8* was reacted with cyanamide in dimethyl sulfoxide in an analogous fashion to the previously synthesized oxazoline analog 2 of the streptolidine lactam (Scheme 1.7). The nucleophilic epoxide ring opening proceeded regioselectively as predicted with the attack occurring at the less hindered carbon α to the carbonyl of the amide. However, distinct from the six-membered analog, the main product was desilylated azepane oxazoline 3*, and rearranged side products were not observed. The reason for the desilylation of the ring-expanded analog is unclear since the same reaction conditions furnished silylprotected oxazoline 5 from the corresponding six-membered epoxy lactam 7.

The crystal structure of the hydrochloride salt of azepane oxazoline 3* revealed the protonation of the oxazoline nitrogen atom (Figure 1.3). However, the crystal structure of six-membered oxazoline 5 obtained under neutral crystallization conditions does not display the same protonation suggesting that the proton attached to the nitrogen of the oxazoline of 3* is likely an artifact of the crystallization conditions. Therefore, the pKₐ value of the oxazoline nitrogen of
analog 3* is similar to that of the six-membered oxazoline 5. Based on the crystal structure, azepane oxazoline 3* maintains the dense arrangement of hydrogen bond donors and acceptors of the streptolidine lactam, but within a less basic and non-planar heterocyclic system.

Figure 1.3: Crystal structure of azepane oxazoline 3* as a hydrochloride salt. The chloride ion is indicated as a green sphere.

Rearrangement of Seven-membered Analog

The ring expanded analog of streptolidine lactam was anticipated to be more conducive to rearrangement since the larger ring should exhibit greater flexibility, however no rearranged product was observed. Comparing the epoxy lactam precursors reveals opposing stereochemistry which likely impacts the substrates ability to rearrange (Figure 1.4). The syn relationship between the epoxide and the silyl protected hydroxyl in the smaller analog appears be necessary to facilitate the rearrangement. Conversely, the anti conformation of the larger analog may act to preclude the rearrangement. To determine the effect
of stereochemistry upon the rearrangement, a syn analog of epoxy-$\varepsilon$-lactam 8* had to be prepared.

![Structural comparison of epoxy lactam precursors of six and seven-membered analogs. * denotes product was isolated as a racemic mixture.](image)

**Figure 1.4:** Structural comparison of epoxy lactam precursors of six and seven-membered analogs. * denotes product was isolated as a racemic mixture.

Preparation of the syn epoxy lactam was undertaken from hydroxy epoxide precursor 20* (**Scheme 1.8**). Facile protection of the hydroxyl with benzyl bromide resulted in precursor 26*. The protected epoxy ketone 26* was subjected to the optimized conditions for inducing the Schmidt rearrangement resulting in the desired syn protected epoxy lactam 27*. The successful conversion of the precursor 26* to lactam 27* using the Schmidt rearrangement demonstrates the superior stability of the benzyl protected substrate when compared to its silyl protected analog.

![Synthetic route to prepare syn epoxy-$\varepsilon$-lactam precursor 27*. * denotes product was isolated as a racemic mixture.](image)

**Scheme 1.8:** Synthetic route to prepare syn epoxy-$\varepsilon$-lactam precursor 27*. * denotes product was isolated as a racemic mixture.

Reagents and conditions: a) Benzyl bromide, Ag$_2$O, THF, 80%; b) NaN$_3$, AcOH, H$_2$O, H$_2$SO$_4$ (cat), 5 °C, 58% based on recovered starting material.
The syn protected epoxy lactam 27* was exposed to cyanamide in dimethyl sulfoxide followed by aqueous work up anticipating the formation of the rearranged product. However, the only product observed was azepane oxazoline 28*. This result indicates that the rearrangement is not favorable for larger ring systems suggesting that this reaction is unique to δ-lactam 7. Even tough, epoxy lactam 28* was not able to rearrange, the stereochemistry may still play an important role in the rearrangement of δ-lactam 7.

Scheme 1.9: Nucleophilic ring opening of epoxy lactam 27* furnishing azepane oxazoline 28*. * denotes product was isolated as a racemic mixture.

Reagents and conditions: a) NaNHCN, DMSO, 10 °C to R.T.; b) H₂O, 2 steps 62%.

Conclusions

Despite numerous synthetic challenges, ring expanded azepane oxazoline analog 3* of the natural streptolidine lactam scaffold contained within the streptothricin antibiotics was successfully prepared. The synthesis of azepane oxazoline analog 3* relied upon novel Schmidt rearrangement conditions that utilize acetic acid as a solvent. The crystal structure analysis of the oxazoline analog shows that the scaffold retains the beneficial networks hydrogen-bond donors and acceptors that mediate RNA recognition around a rigid non-planar
heterocyclic core that disfavors non-specific intercalation between nucleic acids. Additionally, the incorporation of the oxazole within the streptolidine lactam results in an analog with attenuated basicity. Unfortunately, explorations into a novel rearrangement discovered for the 6-membered azepane oxazoline analogs were unfruitful indicating that the observed reaction is specific to the substrate and cannot be applied broadly as a synthetic method. The above synthetic procedure towards azepane oxazole analog 3* provides a starting point for the preparation of RNA-targeting ligands which contain a highly functionalized seven-membered heterocyclic core that is scarce among previously known RNA-binding molecules.
Materials and Methods

(Z)-Cyclohex-2-en-1-one oxime (12)

![Figure 1.5](Z)-cyclohex-2-en-1-one oxime (12).

To a solution of 2-cyclohexen-1-one (10.33 mmol, 1.00 mL) in methanol (20 mL) and water (3.33 mL), sodium acetate (20.66 mmol, 1.69 g) was added with constant magnetic stirring. Then hydroxylamine hydrochloride (20.66 mmol, 1.44 g) was added to the solution. Once the solution becomes homogenous, the reaction vessel is placed under argon atmosphere and sonicated for seven hours at room temperature. Upon completion of the reaction, the resulting solution is transferred to a separatory funnel, water (50 mL) is added and the contents are extracted with dichloromethane (8 x 75 mL). The organic layers are combined, dried over sodium sulfate and concentrated at reduced pressure. Silica gel column chromatography of the pink oil (10:1 ethyl acetate/hexanes) provided a mixture of E and Z isomers of cyclohex-2-en-1-one oxime as white crystals.

12: $^1$H NMR (400 MHz, CDCl$_3$) δ 9.83 (br s, 1H, OH), 6.21 (m, 1H), 6.14 (d, 1H, J = 8.0 Hz), 2.62 (t, 2H, J = 4.8 Hz), 2.17 (m, 2H), 1.75 (m, 2H); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 156.7, 136.7, 124.4, 25.3, 22.5, 21.0; Crystal structure available.
1,5,6,7-Tetrahydro-2H-azepin-2-one (11)

Figure 1.6: 1,5,6,7-tetrahydro-2H-azepin-2-one (11).

Polyphosphoric acid (7.5 mL) was added to a mixture of E and Z isomers of cyclohex-2-en-1-one oxime (19.97 mmol, 2.22 g). The resulting viscous solution was placed under argon atmosphere and heated to 120 °C with constant magnetic stirring. The reaction was kept at 120 °C for four hours. Then ice cold water (65 mL) was added to the molten mixture with constant magnetic stirring providing a dark brown liquid. The reaction solution was then transferred to a separatory funnel and extracted with dichloromethane (8 x 60 mL). The combined organic layers were then dried over sodium sulfate and concentrated at reduced pressure. Silica gel column chromatography of the resulting brown oil (20:1 ethyl acetate/methanol) provided pure 11 as a faint yellow oil in 44% yield over 2 steps from 2-cyclohexen-1-one.

11: $^1$H NMR (400 MHz, CDCl$_3$) δ 8.01 (br s, 1H, NH), 6.12 (dt, 1H, J = 12.0 Hz, J' = 5.2 Hz), 5.71 (d, 1H, J = 12.0 Hz), 3.08 (t, 2H, J = 6.0 Hz), 2.27 (m, 2H), 1.80 (m, 2H); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 171.6, 141.4, 125.6, 41.2, 30.8, 28.4.
6,7-Dihydro-1H-azepine-2,5-dione (10)

![Chemical structure of 6,7-dihydro-1H-azepine-2,5-dione (10)](image)

**Figure 1.7**: 6,7-dihydro-1H-azepine-2,5-dione (10).

To freshly distilled ethyl acetate (20.0 mL) over 3Å molecular sieves, 1,5,6,7-tetrahydro-2H-azepin-2-one (11) (3.78 mmol, 0.42 g) was added. A solid addition funnel containing manganese (III) acetate dihydrate (0.38 mmol, 0.09 g) was connected to the reaction flask and the system was placed under argon atmosphere. tert-Butyl hydrogen peroxide (18.90 mmol, 2.05 mL) was then added with constant magnetic stirring at room temperature. The reaction was kept for two hours, and then manganese (III) acetate dihydrate was added to the solution. The heterogeneous reaction mixture was stirred at room temperature for 48 hours and then filtered over a thin pad of celite which was washed with dichloromethane (100 mL). The filtrate was dried over sodium sulfate and concentrated under reduced. Silica gel column chromatography of the crude brown oil (15:1 ethyl acetate/methanol) provided pure 10 as an off white crystalline solid in 69% yield.

**10**: $^1$H NMR (400 MHz, CDCl$_3$) δ 8.14 (br s, 1H, NH), 6.46 (d, 1H, J = 11.2), 6.33 (d, 1H, J = 11.2 Hz), 3.50 (q, 2H, J = 5.6 Hz), 2.79 (t, 2H, J = 6.0 Hz); $^{13}$C NMR
(400 MHz, CDCl₃) δ 200.1, 168.4, 135.3, 134.4, 45.3, 37.3; Crystal structure available.

5-Hydroxy-1,5,6,7-tetrahydro-2H-azepin-2-one (14)

Figure 1.8: 5-hydroxy-1,5,6,7-tetrahydro-2H-azepin-2-one (18).

To a solution of 6,7-dihydro-1H-azepine-2,5-dione (10) (3.60 mmol, 0.45 g) in methanol (10 mL), cerium (III) chloride (4.00 mmol, 0.99 g) was added with constant magnetic stirring. Then sodium borohydride (4.00 mmol, 0.15 g) was added in small portions over a period of 10 minutes. The reaction was kept at room temperature for 10 minutes, and then water (25 mL) was added to the reaction mixture. The reaction was then concentrated under reduced pressure. Silica gel column chromatography of the resulting yellow oil provided pure 14 as a colorless oil in an 85% yield.

14: ¹H NMR (400 MHz, CDCl₃) δ 6.72 (br s, 1H, NH), 6.31 (dd, 1H, J = 11.6 Hz, J' = 5.2 Hz), 5.82 (d, 1H, J = 11.6 Hz), 4.45 (q, 2H, J = 4.8 Hz), 3.28 (m, 1H), 3.16 (m, 1H), 2.76 (br s, 1H, OH), 2.17 (m, 1H), 1.99 (m, 1H); ¹³C NMR (400 MHz, CDCl₃) δ 170.1, 142.7, 124.4, 69.0, 37.5, 29.9.
5-((tert-butyldimethylsilyl)oxy)-1,5,6,7-Tetrahydro-2H-azepin-2-one (9)

Figure 1.9: 5-((tert-butyldimethylsilyl)oxy)-1,5,6,7-tetrahydro-2H-azepin-2-one (9).

To a solution of 5-hydroxy-1,5,6,7-tetrahydro-2H-azepin-2-one (14) (4.33 mmol, 0.55 g) in dimethylformamide (12 mL), tert-butyldimethylsilyl chloride (10.81 mmol, 1.63 g) and a catalytic amount of 4-dimethylaminopyridine were added with constant magnetic stirring. The reaction solution was then placed under argon atmosphere and cooled to 0 °C. N,N-Diisopropylethylamine (10.81 mmol, 1.88 mL) was added, and the ice bath was subsequently removed allowing the mixture to warm to room temperature where it was kept for 12 hours. Then dichloromethane (10 mL) was added and the reaction solution was transferred to a separatory funnel. The reaction mixture was washed with 1N HCl (10 mL) followed by saturated sodium bicarbonate solution (10 mL). The organic layer was dried over sodium sulfate and concentrated in vacuo. Silica gel column chromatography of the brown residue (10:1 ethyl acetate/hexanes) provided pure 9 as an off-white solid in a 68% yield.[43]

9: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.03 (br s, 1H, NH), 6.19 (dd, 1H, $J = 11.2$ Hz, $J'$ = 5.6 Hz), 5.82 (d, 1H, $J = 11.2$ Hz), 4.47 (q, 2H, $J = 5.2$ Hz), 3.37 (m, 1H), 3.13
Commercially available 1-methoxy-1,4-cyclohexadiene (19) (0.109 mol, 12.0 g) was added to a solution of carbon tetrachloride (30 mL) and water (75 mL). To this biphasic mixture, six drops of 70% perchloric acid were added with constant magnetic stirring. The reaction was kept for 24 hours at room temperature. The mixture was then transferred to a separatory funnel, and the carbon tetrachloride layer was collected and dried over sodium sulfate. The solvent was removed under reduced pressure to give 18 as a colorless oil which was reacted without further purification.\textsuperscript{[20-22]}

18: \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 5.78 (m, 1H), 5.64 (m, 1H), 2.76 (m, 2H\textsubscript{4}), 2.38 (m, 4H); \textsuperscript{13}C NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\) 210.1, 127.1, 124.5, 40.0, 38.9, 25.8; HRMS mass calculated for C\textsubscript{6}H\textsubscript{8}ONa (M+Na\textsuperscript{+}) 119.0467, found 119.0468; Delta 0.8 ppm.
rac 7-Oxabicyclo[4.1.0]heptan-3-one (17*)

![Structure of rac 7-Oxabicyclo[4.1.0]heptan-3-one (17*)](image)

**Figure 1.11:** rac 7-oxabicyclo[4.1.0]heptan-3-one (17*).

A solution of cyclohex-3-enone (18) (0.109 mol, 10.5 g) in dichloromethane (250 mL) was placed under argon atmosphere and cooled to 0°C. Separately, a solution of 3-chloroperoxybenzoic acid (0.12 mol, 27.6 g) in dichloromethane (250 mL) was prepared. The 3-chloroperoxybenzoic acid solution was transferred to the cyclohex-3-enone solution drop wise via cannula over a period of 45 minutes with constant magnetic stirring. After standing for 1 hour at 0°C the ice bath was removed and the reaction kept for 16 hours at room temperature. The mixture was then transferred to a separatory funnel and washed over 5% Na₂SO₃ (250 mL), saturated NaHCO₃ (250 mL), water (250 mL) and saturated NaCl (200 mL) respectively. The organic layer was collected and dried over sodium sulfate. The solvent was removed under reduced pressure to give racemic 17* as a pale yellow oil which was reacted without further purification.[23-26]

17*: HRMS mass calculated for C₆H₉O₂ (M+H)^+ 113.0597, found 113.0598; Delta 0.9 ppm.
Racemic 7-oxabicyclo[4.1.0]heptan-3-one (17*) (0.109 mol, 12.2 g) was dissolved in a 1:1 mixture of diethyl ether (65 mL) and dichloromethane (65 mL). Basic alumina (activity I, 0.382 mol, 38.9 g) was added with constant magnetic stirring. The reaction was kept under argon for 2.5 hours at room temperature. Then the mixture was filtered and the filtrate washed thoroughly with dichloromethane (50 mL). Removal of the solvent under reduced pressure furnished crude racemic 16* as a yellow oil. Silica gel column chromatography of the residue (1:1 hexanes/ethyl acetate) yielded pure racemic 16* as a colorless oil in 56% yield (6.8 g) over 3 steps from 1-methoxy-1,4-cyclohexadiene (18).[23-26]

16*: ¹H NMR (400 MHz, CDCl₃) δ 6.77 (d, 1H J = 9.2 Hz), 5.70 (dd, 1H, J = 9.2, 2.4 Hz), 4.53 (dt, 1H, J= 2.4 Hz), 4.35 (br s, 1H, OH), 2.31 (m, 1H), 2.17 (m, 1H), 2.09 (m, 1H), 1.72 (m, 1H); ¹³C NMR (400 MHz, CDCl₃) δ 200.1, 154.7, 128.7, 66.0, 35.5, 32.3; HRMS mass calculated for C₆H₉O₂ (M+H)⁺ 113.0597, found 113.0595; Delta -1.8 ppm.
(1R/S,5R/S,6R/S)-5-Hydroxy-7-oxabicyclo[4.1.0]heptan-2-one (20*)

![Molecule](image)

20*

**Figure 1.13:** (1R,5R,6R)-5-hydroxy-7-oxabicyclo[4.1.0]heptan-2-one and (1S,5S,6S)-5-hydroxy-7-oxabicyclo[4.1.0]heptan-2-one (20*).

To a solution of racemic 4-hydroxycyclohex-2-enone (16*) (43.7 mmol, 4.9 g) in tetrahydrofuran (35 mL) and benzyltrimethylammonium hydroxide (0.87 mmol, 0.40 mL of 40% solution in MeOH) under argon, tert-butylhydroperoxide 5.5M in decane (0.131 mol, 23.8 mL) was added drop wise while stirring at 0°C. The reaction was kept for 2 hours at 0°C. A saturated solution of ammonium chloride (30 mL) was added and the mixture was extracted with diethyl ether (6 x 25 mL). The combined ether layers were dried over sodium sulfate and concentrated in vacuo. Silica gel column chromatography of the residue (1:1 hexanes/ethyl acetate) yielded racemic 20* as a white crystalline solid in 83% yield (4.6 g).[27-31]

20*: ¹H NMR (400 MHz, CDCl₃) δ 4.22 (q, 1H, J = 4.4 Hz), 3.62 (d, 1H, J = 2.4 Hz), 3.48 (br s, 1H, OH), 3.26 (dd, 1H, J = 4.4 Hz, J' = 2.4 Hz), 2.47 (m, 1H), 2.11 (m, 1H), 1.94 (m, 1H), 1.84 (m, 1H); ¹³C NMR (400 MHz, CDCl₃) δ 204.6, 66.7, 58.8, 56.4, 34.5, 24.8; Crystal structure available.
7-Oxabicyclo[4.1.0]heptane-2,5-dione (21)

![Structure of 7-Oxabicyclo[4.1.0]heptane-2,5-dione (21)](image)

**Figure 1.14:** 7-oxabicyclo[4.1.0]heptane-2,5-dione (21).

To a solution of pyridinium dichromate (17.9 mmol, 6.7 g) in dichloromethane (35 mL), sodium acetate (8.96 mmol, 0.73 g) was added while stirring under argon at 0°C. Racemic 5-hydroxy-7-oxabicyclo[4.1.0]heptan-2-one (20*) (43.7 mmol, 4.9 g) was dissolved in dichloromethane (10 mL) and added drop wise to the reaction via syringe. After standing for 1 hour at 0°C the mixture was kept for 12 hours at room temperature. The solution was then filtered over a short plug of silica gel in order to remove excess pyridinium dichromate. The plug was thoroughly washed with dichloromethane (200 mL), and the resulting solution was concentrated *in vacuo*. Silica gel column chromatography of the residue (2:1 hexanes/ethyl acetate) yielded 21 as a faint yellow oil in 86% yield (4.7 g).[37,38]

**21:** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.60 (s, 2H), 2.99 (dt, 2H, $J = 11.2$ Hz, $J' = 6.0$ Hz, $J'' = 5.6$ Hz), 2.42 (dt, 2H, $J = 11.2$ Hz, $J' = 6.0$ Hz, $J'' = 5.6$ Hz); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 202.4, 58.9, 33.2.
**rac 8-Oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*)**

![Structural formula](image)

**Figure 1.15:** *rac 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*).

To a solution of 7-oxabicyclo[4.1.0]heptane-2,5-dione (21) (3.08 mmol, 0.39 g) in water (2.0 mL) and glacial acetic acid (4.05 mL) sodium azide (7.71 mmol, 0.50 g) was added while vigorously stirring and then cooled to 5 °C. Ice cold concentrated sulfuric acid (7 mL) was added at a rate of 4 drops per minute. Evolution of nitrogen was observed. Upon completion of sulfuric acid addition, the reaction was kept for 20 minutes at 0 °C. Ice water (4 mL) was added with constant magnetic stirring after which the mixture was transferred to a separatory funnel and extracted with dichloromethane (10 x 10 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. Silica gel column chromatography of the orange oil (1:2 hexanes/ethyl acetate) yielded racemic 22* as a crystalline white solid in 62% yield (0.27 g) based on recovered starting material. **CAUTION:** This reaction, which generates toxic and explosive hydrazoic acid *in situ*, and should be performed in a well-ventilated hood and behind a safety shield. Extreme caution during the course of sulfuric acid addition is necessary to obtain optimum yields and avoid charring of the product.[34,39]
**22**: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.23 (br s, 1H, NH), 4.45 (m, 1H), 3.81 (d, 1H, J = 3.6 Hz), 3.75 (d, 1H, J = 3.6 Hz), 3.04 (m, 1H), 2.77 (m, 2H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 203.0, 168.9, 59.4, 55.9, 43.6, 35.2; HRMS mass calculated for C$_6$H$_8$NO$_3$ (M+H)$^+$ 142.0499, found 142.0501; Delta 1.4 ppm; Crystal structure available.

(1$R$/S,6$S$/R,7$R$/S)-6-Hydroxy-8-oxa-3-azabicyclo[5.1.0]octan-2-one (23$^*$)

![Chemical Structure](image)

Figure 1.16: (1$R$,6$S$,7$R$)-6-hydroxy-8-oxa-3-azabicyclo[5.1.0]octan-2-one and (1$S$,6$R$,7$S$)-6-hydroxy-8-oxa-3-azabicyclo[5.1.0]octan-2-one (23$^*$).

A solution of sodium borohydride (0.57 mmol, 0.16 g) in 80% aqueous ethanol (2.7 mL) was added drop wise over a period of 15 minutes to racemic 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22$^*$) (1.55 mmol, 0.22 g) in ethanol (20 mL) at 0 °C with continuous magnetic stirring. After 10 minutes 0.1M HCl (10 mL) was added with constant stirring at 0 °C. The mixture was transferred to a separatory funnel and extracted with dichloromethane (10 x 10 mL). The combined organic layers were concentrated to approximately 25 mL, washed over saturated sodium bicarbonate (20 mL), and dried over sodium sulfate. The solution was further concentrated in vacuo. Silica gel column chromatography of
the residual yellow oil (20:1 ethyl acetate/methanol) furnished racemic 23* as a crystalline white solid in 89% yield (0.20 g).\[42]\n
23*: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 2.16 (dt, 1H, $J = 9.6$ Hz, $J' = 5.2$ Hz), 1.98 (d, 1H, $J = 4.4$ Hz), 1.91 (dt, 1H, $J = 6.8$ Hz, $J' = 4.8$ Hz), 1.74 (s, 1H, OH), 1.68 (t, 1H, $J = 5.2$ Hz, $J' = 4.4$ Hz), 1.60 (dt, 1H, $J = 6.8$ Hz, $J' = 4.4$ Hz), 0.54 (dt, 1H, $J = 9.6$ Hz, $J = 4.4$ Hz), 0.08 (dt, 1H $J = 9.6$ Hz, $J = 4.8$ Hz); $^{13}$C NMR (400 MHz, CD$_3$OD) $\delta$ 169.4, 68.6, 55.8, 50.1, 34.8, 30.4; HRMS mass calculated for C$_6$H$_{10}$NO$_3$ (M+H)$^+$ 144.0658, found 144.0655; Delta 2.0 ppm; Crystal structure available.

(1$R$/S,6$S$/R,7$S$/R)-6-((tert-butyldimethylsilyl)oxy)-8-Oxa-3-azabicyclo[5.1.0]octan-2-one (8*)

![Diagram of the compound](image)

Figure 1.17: (1$R$/S,6$S$/7$S$)-6-((tert-butyldimethylsilyl)oxy)-8-oxa-3-azabicyclo[5.1.0]octan-2-one and (1$S$/6$R$/7$R$)-6-((tert-butyldimethylsilyl)oxy)-8-oxa-3-azabicyclo[5.1.0]octan-2-one (8*).

To a solution of racemic 23* (2.61 mmol, 0.37 g) in dimethylformamide (15 mL), tert-butyldimethylsilyl chloride (6.53 mmol, 0.98 g) and a catalytic amount of 4-dimethylaminopyridine were added with constant magnetic stirring. The
reaction solution was then placed under argon atmosphere and cooled to 0 °C. *N*-*N*-Diisopropylethylamine (6.53 mmol, 1.14 mL) was added, and the ice bath was subsequently removed allowing the mixture to warm to room temperature where it was kept for 12 hours. Then ice cold water (20 mL) was added with constant magnetic stirring after which the reaction solution was transferred to a separatory funnel and extracted with dichloromethane (6 x 20 mL). The combined organic layers were dried over sodium sulfate and concentrated *in vacuo*. Silica gel column chromatography of the resulting red oil (2:1 hexanes/ethyl acetate) yielded racemic mixture of 8* as a crystalline white solid in 81% yield (0.54 g).[^43]

**8**: ^1^H NMR (400 MHz, CD$_3$OD) δ 7.36 (br s, 1H), 4.01 (dt, 1H, J = 8.8 Hz, J’ = 4.0 Hz), 3.48 (d, 1H, J = 4.0 Hz), 3.37 (dd, 1H, J = 8.8 Hz, J’ = 4.0 Hz), 3.17 (m, 2H), 2.05 (m, 1H), 1.67 (m, 1H), 0.87 (s, 9H), 0.09 (d, 6H); ^13^C NMR (400 MHz, CD$_3$OD) δ 170.9, 70.7, 58.8, 53.5, 36.6, 33.6, 25.9, 18.2, -4.6; HRMS mass calculated for C$_{12}$H$_{23}$NO$_3$SiNa (M+Na)$^+$ 280.1340, found 280.1339; Delta -0.4 ppm.
(3aR/S,8R/S,8aR/S)-2-Amino-8-((tert-butyldimethylsilyl)oxy)-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (24*)

(3aR/S,8R/S,8aR/S)-2-Amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*)

![Chemical structures](image)

Figure 1.18: (3aS,8S,8aS)-2-amino-8-((tert-butyldimethylsilyl)oxy)-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one and (3aR,8R,8aR)-2-amino-8-((tert-butyldimethylsilyl)oxy)-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (24*). (3aS,8S,8aS)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one and (3aR,8R,8aR)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*).

A solution of sodium hydrogencyanamide (0.56 mmol, 0.36 g) in dimethyl sulfoxide (1.5 mL) was prepared and cooled to 10 °C. A solution of racemic 8* (0.51 mmol, 0.13 g) in dimethyl sulfoxide (1.95 mL) was then added via syringe with constant magnetic stirring. The reaction was kept for 1 hour at 10 °C and then allowed to warm to room temperature and kept for an additional 23 hours. After concentration in vacuo water (4 mL) was added followed by titration with 0.1M HCl at 0 °C to acidify. The mixture was transferred to a separatory funnel,
extracted with chloroform (8 x 3 mL) and both layers were collected. The water layer was concentrated and the residue recrystallized in a 3:1 methanol/water mixture to give racemic 3* as a white crystalline solid in 53% yield (0.080 g). The chloroform layers were concentrated in vacuo. Silica gel column chromatography of the orange residue (10:1 ethyl acetate/methanol) yielded racemic 24* as a white solid in 11% yield.[15]

24* : 1H NMR (400 MHz, CD3OD) δ 4.59 (d, 1H, J = 11.6 Hz), 4.14 (dt, 2H, J = 11.2 Hz, J' = 5.2 Hz), 3.92 (t, 1H, J = 11.2 Hz), 3.36 (dt, 1H, J = 9.2 Hz, J' = 2.8 Hz), 3.15 (dt, 1H, J = 8.8 Hz, J =2.8), 2.04 (m, 1H), 1.56 (m, 1H), 0.94 (s, 9H), 0.15 (d, 6H); 13C NMR (400 MHz, DMSO-D6) δ 172.8, 161.7, 85.9, 74.3, 66.7, 37.3, 37.1, 26.5, 18.6, -3.7, -4.1; HRMS mass calculated for C13H25N3O3Si (M+H)+ 300.1738, found 300.1739; Delta 0.3 ppm.

3*: 1H NMR (500 MHz, D2O) δ 5.06 (d, 1H, J = 12.0 Hz), 4.62 (t, 1H, J = 12.0 Hz), 4.14 (dt, 1H, J = 8.0 Hz, J' = 4.0 Hz), 2.95 (m, 2H), 1.56 (dt, 1H, J = 16.0 Hz, J' = 4.0 Hz), 0.86 (m, 1H); 13C NMR 500 MHz, D2O) δ 168.3, 163.3, 86.9, 70.2, 58.7, 36.8, 33.7; HRMS mass calculated for C7H11N3O3Na (M+Na)+ 208.0693, found 208.0695; Delta 1.0 ppm; Crystal structure available.
General Techniques

Commercial reagents were obtained (Sigma Aldrich, Acros, Alfa Aesar, Fisher) and used without any further purification. Non-aqueous reactions were carried out under anhydrous conditions using oven-dried glassware under an inert atmosphere in dry, freshly distilled solvents. All air-sensitive reaction solutions were transferred via syringe or stainless steel cannula. Reactions were monitored by TLC, which was performed on pre-coated silica gel 60 F254 plates supplied by EMD, visualized utilizing UV light, and developed using ceric ammonium molybdate stain (CAM) with heat or ninhydrin stain with heat. Organic solvents were removed by rotary evaporation below 30 °C at approximately 15 mmHg. Flash column chromatography was executed with silica gel 60 (230-400 mesh) supplied by Silicycle and eluting solvents are indicated in the text. Yields refer to chromatographically and spectroscopically (\(^1\)H NMR, \(^{13}\)C NMR) homogeneous materials, unless otherwise stated. NMR spectra were recorded on Varian Mercury 400 MHz, Jeol Unity 500 MHz, and Agilent VNMR S 500 MHz outfitted with an XSens cold probe using deuterochloroform, deuteromethanol, deuterowater or deuterodimethyl sulfoxide (Cambridge Isotope) as solvents. The chemical shifts are given in ppm relative to the standard reference TMS or residual undeuterated solvent. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublet, dt = doublet of triplet. Low resolution mass spectra were obtained on a ThermoFinnigan LCQDECA-MS spectrometer. High resolution mass spectra (HRMS) were
recorded on a VG 7070 HS or a VG ZAB-ZSE mass spectrometer. Crystals were mounted in nylon loops using Paratone oil and then placed on the diffractometer under a nitrogen stream at 100K. All crystallographic data has been deposited at the CCDC, 12 Union Road, Cambridge CB21EZ, UK.
Spectral Data

Nuclear Magnetic Resonance

Spectrum 1.1: \((1R/S,5R/S,6R/S)-5\text{-hydroxy}-7\text{-oxabicyclo[4.1.0]heptan-2-one (20*)} \) \(^1\)H NMR (400 MHz, CDCl\(_3\)).
Spectrum 1.2: (1R/S,5R/S,6R/S)-5-hydroxy-7-oxabicyclo[4.1.0]heptan-2-one (20*) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 1.3: 7-oxabicyclo[4.1.0]heptane-2,5-dione (21) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 1.4: 7-oxabicyclo[4.1.0]heptane-2,5-dione (21) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 1.5: rac 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22\textsuperscript{*}) \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}).
Spectrum 1.6: *rac* 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*) $^{13}$C NMR (400 MHz, CDCl$_3$).
**Spectrum 1.7:** (1R/S,6S/R,7R/S)-6-hydroxy-8-oxa-3-azabicyclo[5.1.0]octan-2-one (23*) $^1$H NMR (400 MHz, CD$_3$OD).
**Spectrum 1.8:** \((1R/S, 6S/R, 7R/S)-6\text{-hydroxy-8-oxa-3-azabicyclo[5.1.0]octan-2-one (23\text{*})}^{13}\text{C NMR (400 MHz, CD}_3\text{OD).}

![Diagram of the molecule](image-url)
Spectrum 1.9: (1R/S, 6S/R, 7S/R)-6-((tert-butyldimethylsilyl)oxy)-8-oxa-3-azabicyclo [5.1.0] octan-2-one ($8^*$) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 1.10: (1R/S,6S/R,7S/R)-6-((tert-butyldimethylsilyl)oxy)-8-oxa-3-aza-bicyclo[5.1.0] octan-2-one (8*) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 1.11: (3aR/S,8R/S,8aR/S)-2-amino-8-((tert-butyldimethylsilyl)oxy)-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (24*) ¹H NMR (400 MHz, CD₃OD).
Spectrum 1.12: (3aR/S,8R/S,8aR/S)-2-amino-8-((tert-butyldimethylsilyl)oxy)-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (24*) $^{13}$C NMR (400 MHz, CD$_3$OD).
Spectrum 1.13: (3aR/S,8R/S,8aR/S)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*) \(^1\)H NMR (400 MHz, D\(_2\)O).
**Spectrum 1.14:** (3aR/S,8R/S,8aR/S)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*) $^{13}$C NMR (400 MHz, D$_2$O).
Crystal Structures

**Spectrum 1.15**: Crystal structure of (Z)-cyclohex-2-en-1-one oxime (12).

**Spectrum 1.16**: Hydrogen bonding pattern observed in crystal structure of (Z)-cyclohex-2-en-1-one oxime (12).
**Spectrum 1.17:** Crystal packing observed in crystal structure of (Z)-cyclohex-2-en-1-one oxime (12).

**Spectrum 1.18:** Alternative view of crystal packing observed in crystal structure of (Z)-cyclohex-2-en-1-one oxime (12).
**Spectrum 1.19:** Crystal structure of 6,7-dihydro-1H-azepine-2,5-dione (10).

**Spectrum 1.20:** Hydrogen bonding pattern observed in crystal structure of 6,7-dihydro-1H-azepine-2,5-dione (10).
**Spectrum 1.21:** Crystal packing observed in crystal structure of 6,7-dihydro-1H-azepine-2,5-dione (10).

**Spectrum 1.22:** Alternative view of crystal packing observed in crystal structure of 6,7-dihydro-1H-azepine-2,5-dione (10).
**Spectrum 1.23:** Crystal structure of $(1R,5R,6R)$-5-hydroxy-7-oxabicyclo[4.1.0]heptan-2-one and $(1S,5S,6S)$-5-hydroxy-7-oxabicyclo[4.1.0]heptan-2-one ($20^*$).

**Spectrum 1.24:** Hydrogen bonding pattern observed in crystal structure of $(1R,5R,6R)$-5-hydroxy-7-oxabicyclo[4.1.0]-heptan-2-one and $(1S,5S,6S)$-5-hydroxy-7-oxabicyclo[4.1.0]-heptan-2-one ($20^*$).
**Spectrum 1.25:** Crystal packing observed in crystal structure of (1\(R\),5\(R\),6\(R\))-5-hydroxy-7-oxabicyclo[4.1.0]-heptan-2-one and (1\(S\),5\(S\),6\(S\))-5-hydroxy-7-oxabicyclo[4.1.0]-heptan-2-one (20\(^*\)).

**Spectrum 1.26:** Alternative view of crystal packing observed in crystal structure of (1\(R\),5\(R\),6\(R\))-5-hydroxy-7-oxabicyclo[4.1.0]-heptan-2-one and (1\(S\),5\(S\),6\(S\))-5-hydroxy-7-oxabicyclo[4.1.0]-heptan-2-one (20\(^*\)).
**Spectrum 1.27:** Crystal structure of *rac* 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*).

![Crystal structure of rac 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*)](image1)

**Spectrum 1.28:** Hydrogen bonding pattern observed in crystal structure of *rac* 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*).

![Hydrogen bonding pattern observed in crystal structure of rac 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*)](image2)
**Spectrum 1.29:** Crystal packing observed in crystal structure of rac 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*).

**Spectrum 1.30:** Alternative view of crystal packing observed in crystal structure of rac 8-oxa-3-azabicyclo[5.1.0]octane-2,6-dione (22*).
**Spectrum 1.31:** Crystal structure of \((1R,6S,7R)-6\text{-hydroxy-8-oxa-3-azabicyclo}[5.1.0]\text{octan-2-one}\) and \((1S,6R,7S)-6\text{-hydroxy-8-oxa-3-azabicyclo}[5.1.0]\text{octan-2-one}\) (23*).

**Spectrum 1.32:** Hydrogen bonding pattern observed in crystal structure of \((1R,6S,7R)-6\text{-hydroxy-8-oxa-3-azabicyclo}[5.1.0]\text{octan-2-one}\) and \((1S,6R,7S)-6\text{-hydroxy-8-oxa-3-azabicyclo}[5.1.0]\text{octan-2-one}\) (23*).
**Spectrum 1.33:** Crystal packing observed in crystal structure of $(1R,6S,7R)$-6-hydroxy-8-oxa-3-azabicyclo-$[5.1.0]$octan-2-one and $(1S,6R,7S)$-6-hydroxy-8-oxa-3-aza-bicyclo$[5.1.0]$octan-2-one ($23^*$).

**Spectrum 1.34:** Alternative view of crystal packing observed in crystal structure of $(1R,6S,7R)$-6-hydroxy-8-oxa-3-azabicyclo-$[5.1.0]$octan-2-one and $(1S,6R,7S)$-6-hydroxy-8-oxa-3-aza-bicyclo$[5.1.0]$octan-2-one ($23^*$).
**Spectrum 1.35:** Crystal structure of (3aS,8S,8aS)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one and (3aR,8R,8aR)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*).

![Diagram of the crystal structure](image)

**Spectrum 1.36:** Hydrogen bonding pattern observed in crystal structure of (3aS,8S,8aS)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one and (3aR,8R,8aR)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*).

![Diagram showing hydrogen bonding pattern](image)
**Spectrum 1.37:** Crystal packing observed in crystal structure of (3aS,8S,8aS)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one and (3aR,8R,8aR)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*).

**Spectrum 1.38:** Alternative view of crystal packing observed in crystal structure of (3aS,8S,8aS)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one and (3aR,8R,8aR)-2-amino-8-hydroxy-6,7,8,8a-tetrahydro-3aH-oxazolo[4,5-c]azepin-4(5H)-one (3*).
References


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Chapter 2

2-Aminobenzoxazoles as Translation Inhibitors of HCV Internal Ribosomal Entry Site
Introduction

Hepatitis C (HCV) is an infectious disease which insidiously affects the liver often resulting in cirrhosis or hepatocellular carcinoma many years after infection. HCV is estimated to infect 150-200 million people worldwide, and more than 350,000 people die every year from HCV related liver diseases.[1] Since there is no vaccine available for HCV, this infection remains the leading reason for liver transplantation.[2] HCV is transmitted through contact with virus contaminated blood usually stemming from intravenous drug use. Persistent HCV infection occurs in approximately 80% of those exposed to the virus.[3] The standard of care for HCV infection was initially a cocktail of pegylated interferons and ribavirin which suffered from limited efficacy and serious side effects.[4] The recent development of viral protease inhibitors, boceprevir and telaprevir, are better tolerated than previous therapies but are still only effective on a segment of the HCV infected population (genotype 1).[5] As a result, novel and complementary therapies for the broader treatment of HCV are needed.

HCV is a small positive-stranded RNA virus belonging to the Flaviviridae family.[6] The viral genome consists of a single open reading frame of approximately 9600 nucleobases flanked by a 5'-UTR (untranslated region) and a 3'-UTR which are critical to viral replication (Figure 2.1). The highly conserved 5'-UTR contains an internal ribosomal entry site (IRES) which is responsible for the recruitment of the host cell’s 40S ribosomal subunit and initiation of translation in a cap-independent manner.[7-9] The IRES represents an interesting
target for the development of selective small molecule antiviral therapies due to its high sequence conservation, as well as, its unique biological function in viral replication.\cite{10-13}

**Figure 2.1:** Organization HCV RNA genome illustrating structured untranslated regions located at both the 5’ and 3’ ends of the RNA. The structural and non-structural gene regions of the open reading frame are indicated by blue and green, respectively.

**Internal Ribosomal Entry Site of HCV**

The IRES is a highly structured non-translated domain comprised of approximately 340 nucleobases located within the 5’-UTR of HCV. The IRES is responsible for the initiation of protein synthesis through binding to the human 40S ribosomal subunit and circumventing the need for most initiation factors. Unlike canonical protein synthesis in eukaryotes which requires a multi-step process involving 5’ cap recognition, assembly of a full complement of initiation
cofactors and scanning\cite{14}, the IRES directly recruits the 40S ribosomal subunit and eukaryotic initiation factor 3 (eIF3), ultimately resulting in the assembly of functional ribosomes which initiate viral translation\cite{7-9}.

The HCV IRES contains three independently folding domains (II-IV) essential to IRES function which are connected by flexible regions of single stranded RNA (Figure 2.2)\cite{15}. The process of viral translation initiation is dependent upon the unique interactions of domains II, III and IV with the host’s ribosome. Viral translation initiation starts with the IRES binding to the 40S ribosomal subunit with high affinity through interactions facilitated by the basal portion of domain II\cite{16}. During binding, the hairpin loop of domain IV melts revealing the start codon, in addition to ~13 nucleotides of the coding region and exposes the viral translation initiation site\cite{17-19}. The completely conserved hairpin of IRES subdomain IIb then engages in interactions with the ribosome which instigate conformational changes that guide the viral translation initiation site into the ribosomal decoding groove\cite{20-22}. Then, the apical portion domain III provides a platform for the recognition and binding of eukaryotic initiation factor 3 (eIF3). This association promotes the binding of the ternary complex (eIF2-Met-tRNA\textsubscript{Met}.GTP), resulting in the formation of the IRES·48S complex\cite{9,16}. Hydrolysis of eIF2-bound GTP and subsequent eIF2 release is then carried out by domain II of the IRES. The subsequent joining of the 60S ribosomal subunit results in the formation of a functional 80S ribosomal assembly thereby completing translation initiation\cite{9,16,21,23}. Despite a fundamental understanding of
IRES mediated translation, the minutiae of the manipulation of the ribosomal machinery through the various stages of initiation remains incomplete.

Figure 2.2: Secondary structure of the HCV IRES. The position of subdomain IIa is indicated by a gold box.

The complex process of IRES translation initiation requires domains II-IV work in cooperative fashion to coerce the ribosome into producing viral polyprotein precursor. Since each modular domain is critical to function, the IRES should be rich with potential binding sites for therapeutic intervention. However, the IRES has no enzymatic activity or explicit active site, making it a difficult drug target.\textsuperscript{[24]} Furthermore, limited RNA-focused medicinal chemistry efforts have led to an incomplete understanding of the fundamental principles that govern the selective recognition of RNA by small molecules.
Comprehensive biochemical studies of the IRES have shown regions which play critical roles in viral translation initiation. Chemical probing has demonstrated domain III to be essential to binding with high affinity to both the 40S ribosomal subunit and eIF3. Furthermore, loops within domain III have been discovered to be necessary for the formation of a functional binary complex with robust translational activity.\textsuperscript{[9,16]} Nonetheless, interruption of IRES binding to the 40S subunit or eIF3 is unlikely using a small molecule given the extend surfaces utilized in this macromolecular recognition event. IRES domain IV, which harbors the start codon, is also involved in 40S binding. Sequestration of the start codon through small molecule stabilization of domain IV could compromise viral translation, yet has not been demonstrated. In contrast, IRES domain II is not required for 40S binding, but is essential to viral translation initiation. Mutant IRES, lacking domain II, bind with wild type affinity, but suffer from impaired translational activity.\textsuperscript{[17]} Moreover, small molecules which bind to domain II and inhibit IRES function by compromising translation have been recently reported.\textsuperscript{[25]} Domain II of the IRES offers an exciting possibility for the development of novel antiviral therapies using small molecules to interfere with biological function.

**HCV IRES Domain II**

HCV IRES domain II is divided into two portions, a basal domain IIa containing an internal loop and an apical domain IIb containing a hairpin (Figure 2.2).\textsuperscript{[16]} Both portions within domain II display regions of high conservation which
stem from specific structure giving rise to function (Figure 2.3). NMR studies of domain II folding in the presence of metal ions established the overall structure of domain II as an L-shape.\textsuperscript{[26]} A high resolution image of the bent L-shape architecture was provided by crystal structure analysis of subdomain IIa, which revealed the discreet placement of three magnesium ions as a part of the RNA fold.\textsuperscript{[27]} The distinctive 90° bend of domain II is stabilized by a combination of stacking, hydrogen bonding and metal ion participation.

The bent architecture of domain IIa is of significant consequence to proper IRES function. Cryo-EM reconstructions of the HCV IRES-40S complex implicate subdomain IIa to be essential for the precise positioning of the distal subdomain IIb hairpin in the ribosomal E-site. Once situated within the E-site, subdomain IIb
makes specific contacts with ribosomal protein rpS5 which induces conformational changes in the 40S subunit that close the mRNA binding cleft around the coding RNA within.[17] Additionally, subdomain IIb promotes release eIF2 committing the 48S complex into 80S assembly, which underscores the fundamental importance of the proper folding of subdomain IIa.[23] After translation initiation subdomain IIb must be removed from the E-site allowing the P-site RNA to translocate for translation to take place. Conformational changes within the flexible subdomain IIa may also facilitate the release of hairpin IIb from the E-site allowing translation to occur.[28, 29] Ligands or mutations that result in conformational changes within subdomain IIa by interrupting the L-shaped bend compromise IRES function and inhibit viral protein synthesis through the disorientation of distal hairpin IIb.[28-30]

Inhibitors of HCV IRES Subdomain IIa

The structure adopted by subdomain IIa of the HCV IRES is analogous to a macromolecular hinge responsible for proper placement of hairpin IIb culminating in viral polyprotein synthesis. Small molecules which bind to subdomain IIa and perturb the structure should inhibit IRES driven translation and offer a therapeutic avenue toward the development of novel antiviral drugs. Indeed, small molecules have been shown to bind to subdomain IIa of the IRES and compromise translation (Figure 2.4).[28-30] Mass spectrometry-based high throughput screening against a 29-mer oligonucleotide of IRES subdomain IIa
led to the identification of aminobenzimidazole 1 as a ligand with modest affinity (~100 μM) for the target structure. Chemical optimization of the initial ligand 1 led to the synthesis of structurally complex aminobenzimidazole derivative 2 with greatly improved affinity for subdomain IIa with a $K_D$ of 0.86 μM.[25] Further insight into the interaction between inhibitor 2 and subdomain IIa was gained from a co-crystal structure which revealed that compound 2 captures subdomain IIa in an elongated linear complex (Figure 2.5).[31] This radical departure from the native 90° bend abrogates the function associated with subdomain IIa. Studies using FRET (Förster resonance energy transfer) measurements of a fluorescently labeled oligonucleotide corresponding to subdomain IIa confirm the picture portrayed by the co-crystal structure. In solution, subdomain IIa is observed in an extended form in the presence of benzimidazole inhibitor 2. Additionally, FRET measurements in presence of competitor RNA molecules demonstrated that benzimidazole 2 is selective for subdomain IIa.[28] Despite the wealth of structural and chemical data, synthetic access to aminobenzimidazole 2 remains a challenge, complicated by a long synthetic route with multiple low yielding steps.[25, 32]
Figure 2.4: Structure of the HCV translation inhibitors that bind to subdomain IIa. Aminobenzimidazole inhibitors 1 and 2 were initially identified by Isis Pharmaceuticals.\cite{25} Diaminopiperidine 3 was discovered previously in our lab.\cite{29}

Figure 2.5: Model for the mode of action of translation inhibitors targeting subdomain IIa of the IRES. The native form of the subdomain IIa RNA is a bent motif shown in the center that is stabilized by three magnesium ions represented as green spheres. Dynamic changes in the L-shaped structure may be necessary to release the ribosome after translation initiation. Benzimidazole inhibitor 2 captures an elongated form of the RNA which disrupts IRES function resulting in inhibition of viral translation as shown on the left. Diaminopiperidine inhibitor 3 binds in competition with the magnesium ions of the natively folded RNA arresting flexibility resulting in inhibition of IRES function perhaps by preventing ribosome release as illustrated on the right.
Inhibition of the IRES function through subdomain IIa is not confined to small molecules which capture its impotent elongated form. Inspired by the aminoglycoside antibiotics, a series of simplified mimetics consisting of a 3,5-diaminopiperidine (DAP) dipeptides were investigated as RNA inhibitors. Fluorescence and FRET measurements show ligand 3 binding to subdomain IIa with \( \sim 7 \mu \text{M} \) affinity and capturing the native L-shape of the RNA (Figure 2.5). Competition experiments with high concentration of magnesium indicate that the inhibitor-subdomain IIa interaction is strongly governed by electrostatics. This binding mode suggests that inhibitor 3 directly interacts with sites occupied by one or both magnesium ions that stabilize the bend formed by subdomain IIa. Distinct from benzimidazole 2, which captures an elongated confirmation of subdomain IIa, the DAP ligands arrests the bent form suggesting that small molecules that interfere with the native articulation of subdomain IIa will act as inhibitors of IRES driven translation.

**Structure Based Design of Novel Ligands for Targeting IRES Subdomain IIa**

Ligands which bind to RNA and abrogate function share common structural motifs despite chemical diversity of these compounds. Ligand selectivity and affinity for RNA are provided by the dense arrangement of hydrogen-bond donors and acceptors around a rigid planar molecular scaffold. Faces of hydrogen bond donors and acceptors allow the recognition of unpaired nucleobases while the planarity permits access to spaces between the bases within RNA folds. General affinity for RNA is further enhanced by the presence of
basic moieties within the ligand which contribute to non-specific electrostatic interactions with the phosphate backbone. Compounds conforming to this paradigm should be biased toward interaction with RNA.

Based on the co-crystal structure of ligand 2 bound to subdomain IIa, binding is governed by several specific interactions (Figure 2.6). The basic benzimidazole moiety of compound 2 is protonated creating a pair of hydrogen bonds deep within the binding pocket formed by subdomain IIa to the Hoogsteen face of G110. Stacking interactions are observed between ligand 2, and the adjacent nucleobases which form the floor and ceiling of the binding cavity. The basic dimethyl amino side chains are also protonated resulting in favorable electrostatic interaction with the negatively charged RNA. In addition, one of the protonated dimethyl amino side chains forms a single hydrogen bond to the phosphate backbone. Further stabilization of the binding pocket is observed by the formation of RNA base triples which engage in interactions across the RNA helix. Three magnesium ions are also present in linear conformation adopted by the RNA in complex with ligand 2 further stabilizing the unusual motif.
Figure 2.6: Crystal structure of subdomain IIa of the IRES in complex with benzimidazole inhibitor 2. (A) View of binding pocket formed by subdomain IIa. The ligand 2 is shown in yellow sticks and the Mg$^{2+}$ ions are indicated by green spheres. (B) Summary of interactions of ligand 2 with the binding site. Hydrogen bonds are shown as dashed lines. Stacked lines (≡) indicate stacking of bases and intercalation of the ligand. Formation of non-Watson-Crick base pairs is shown with solid lines and symbols according to Leontis and Westhof.\[31\]

Based on the wealth of structural data and using benzimidazole 2 as source of inspiration, a family of ligands was designed to explore fundamental interactions which govern binding to HCV IRES subdomain IIa. A benzoxazole motif was selected to replace the benzimidazole as the core scaffold. The benzoxazole core is a less basic motif aimed at improving the drug-like qualities of the designed ligands. Additionally, the benzoxazole scaffold possesses the requisite functionalities to recognize the deep binding pocket of the subdomain IIa RNA through the previously observed hydrogen bonding and stacking of
benzimidazole 2 (Figure 2.7). However, the reduced basicity of the core may influence the protonation state of the benzoazole thereby reducing the strength of the interaction within the binding cavity. This series of ligands will determine the ability of the benzoazole core to act a recognition element which binds to subdomain IIa and orients adjacent functionalities within the binding pocket.

A single chain will be added to the benzoazole core with varying head groups to investigate the nature of the interactions at the edge of the binding pocket. The head groups are planned to facilitate new hydrogen bonding or electrostatic interactions with the goal of augmenting ligand affinity through the incorporation of molecular motifs which vary in size, composition and character. The length of the side chain attached to the head group will likewise be varied to include two and three carbons analogs, ensuring that the larger head groups will interact near the binding cavity. Furthermore, the consequence of shifting the side chain to the exocyclic nitrogen will be determined by assessing ligand binding.

To simplify the synthetic scaffold of benzimidazole 2, the chroman ring (ring A) was omitted in this series of novel benzoazoles. Additionally, the hydrogen bond acceptor of ring A was replaced by a hydrogen bond donor resulting in general scaffold 4. The introduced amine at the 6-postion was aimed at discovering new hydrogen bonding interactions within the binding cavity. This series of benzoazoles was designed to gain a deeper insight into the binding of small molecules to subdomain IIa of the HCV IRES, as well as enrich our understanding of fundamental RNA-ligand interaction.
Figure 2.7: Comparison of known the HCV translation inhibitor 2 with novel benzoxazole 4. The retained core scaffold is highlighted in red. The side chain length of benzoxazole 4 varies by a single methylene unit ($n = 1$ or $2$). The various head groups are listed.

The novel ligands will be assembled around benzoxazole core scaffold 4 using a convergent synthetic approach allowing the efficient assembly of diverse compounds capable of exploring the character of the molecular space in the RNA binding pocket. The modular assembly of simplified ligands facilitates the development of a structure activity relationship (SAR) toward the creation of superior ligands for the recognition of subdomain IIa of the HCV IRES. The elaboration of ligands will focus on enabling favorable RNA-ligand interactions while stressing simplified synthetic access to small molecules with drug-like qualities.
Retrosynthesis of Benzoxazole Ligands

The synthesis of the 6-aminobenzoxazole family of compounds (4) was envisaged by reduction of the corresponding 6-nitrobenzoxazole 5 (Scheme 2.1). Direct coupling of mercaptoxazole 6 with various alkylamino head groups would allow access to intermediate 5. The mercaptoxazole 6 would be prepared from commercially available 2-amino-5-nitrophenol and carbon disulfide (Scheme 2.2). The alkylamino head groups would be synthesized by nucleophilic substitution from the corresponding bromoalkylphthalimide with a secondary amine head group. The free amine head group would then be generated by the hydrazinolysis of the phthalimide reminiscent of the Gabriel synthesis. The convergent approach toward the synthesis of 6-aminobenzoxazoles allows the expedient preparation of molecules which explore complex interactions between the ligand and subdomain IIa of the IRES.

Scheme 2.1: Retrosynthetic analysis of 6-aminobenzoxazole 4. The side chain length of intermediates 4, 5 and 7 vary by a single methylene unit (n = 1 or 2).
Scheme 2.2: Retrosynthetic analysis of mercaptobenzoxazole 6 and primary amine 7. The length of the alkyl chain within precursors 7, 9 and 10 vary by a single methylene unit (n = 1 or 2).

Synthesis of Benzoxazole Ligands for Targeting IRES Subdomain IIa

The mercaptobenzoxazole 6 was readily prepared in a single step from commercially available 2-amino-5-nitrophenol (Scheme 2.3). The base induced cyclization of 2-amino-5-nitrophenol with carbon disulfide proceeded in good yield despite long reaction times, providing facile access to one of the coupling partners in route to the desired 6-aminobenzoxazoles.[33-35]

Scheme 2.3: Synthesis of mercaptobenzoxazole 6.

Reagents and conditions: a) KOH, CS₂, H₂O, EtOH, 80 °C, 77%.

General synthesis of the alkylamino head groups commenced with the nucleophilic substitution of bromoalkylphthalimide with a secondary amine, providing straightforward access to amino phthalimide 9 (Scheme 2.4).[36-38] The
mild hydrazinolysis of intermediate 9 resulted in the desired primary amino head groups for coupling.\[^{39-43}\] The addition of a single methylene unit, going from ethyl to a propyl chain between the primary amine and the head group, resulted in higher yields likely due to the decreased volatility of the product. The isolated amino head groups were then coupled to the mercaptobenzoxazole 6 without purification.

Scheme 2.4: Synthesis of alkylamino sidechains. The length of the alkyl chain within precursors 7, 9 and 10 vary by a single methylene unit (n = 1 or 2). The various head groups are listed.

Reagents and conditions: a) HNR\(_2\), K\(_2\)CO\(_3\), CH\(_3\)CN, R.T., 68-96%; b) H\(_2\)NNH\(_2\)H\(_2\)O, EtOH, 80 °C.

The final preparation of the 6-aminobenzoxazoles began with the coupling of mercaptobenzoxazole 6 with an alkylamino head groups 7 (Scheme 2.5). The coupling reaction was enabled by converting mercaptobenzoxazole 6 to a highly reactive 2-chloroxazole species \textit{in situ} using oxalyl chloride. Neutralization of the acid generated by the oxalyl chloride followed by the addition of the synthesized primary amino head group nucleophile resulted in the desired 6-nitrobenzoxazole from a single pot.\[^{44}\] Optimization of this reaction led to the use of dilute reaction
conditions to disfavor the formation of benzoxazole dimers, as well as, precipitates which arrest reaction progress. The coupling reaction favored amines containing the longer propyl chain manifested by higher yields which is likely the result of moving the sterically cumbersome head group further away from the primary amine nucleophile.

![Scheme 2.5: Synthetic route to 6-aminobenzoxazoles 4. The side chain length of intermediates 4, 5 and 7 vary by a single methylene unit (n = 1 or 2).]

Reagents and conditions: a) Oxalyl chloride, DCM, DMF, TEA, 0°C to R.T., 46-88%; b) SnCl₂, HCl, EtOH, 50 °C, 53-64%; c) PtO₂, H₂, R.T. 68-82%.

Facile reduction of 6-nitrobenzoxazoles was envisaged to permit access to the desired 6-aminobenzoxazoles. However, attempts using palladium hydrogenation conditions failed to reduce the nitro group. Alternatively, classical nitro reduction conditions using stannous chloride and hydrochloric acid were successful, but resulted in modest yields owing to the harsh reaction conditions.[45] Isolation of the various reduced 6-aminobenzoxazoles was challenging due to the strongly polar character of the molecules. Additionally, the
6-aminobenzoxazoles were discovered to be unstable at room temperature when exposed to the ambient atmosphere. The electron rich core scaffold is hypothesized to be susceptible to radical oxidation in the presence of air. In order to boost the yield and ease the isolation of 6-aminobenzoxazoles, the classical reduction was replaced by hydrogenation using Adam’s catalyst resulting in an improved method for the preparation of synthetically demanding 6-aminobenzoxazoles.[46]

**Förster Resonance Energy Transfer Assay**

The subdomain IIa bend has been demonstrated to be critical to the placement of apical IIb hairpin during translation initiation. Therefore, ligands which bind to subdomain IIa and disturb proper folding could lead to the development of HCV translation inhibitors.[47] To evaluate the dynamic process of RNA folding, a fluorescently labeled oligonucleotide of subdomain IIa was prepared containing a pair of cyanine dyes at the 5'-termini (Figure 2.8A). Guided by the crystal structure of subdomain, the length of the folded L-shaped RNA bearing the dyes was selected to be shorter than the Förster radius of the dye pair.[27] The resulting viral construct exhibits a maximized sensitivity to changes in the interhelical angle of subdomain IIa, allowing insight into the overall adopted structure of the RNA as measured by Förster resonance energy transfer (FRET).[28]
Figure 2.8: FRET assay monitoring changes in the conformation of subdomain IIa of the HCV IRES accompanied by ligand binding.\cite{28,47} (A) Subdomain IIa RNA labeled with cyanine dyes used for FRET measurements. Nucleotides that deviate from the HCV genotype 1b sequence are shown in outlined font. (B) Observed FRET signal of cyanine dye-labeled subdomain IIa with benzimidazole 2 in the presence of 2 mM Mg\textsuperscript{2+}.

The observed FRET signal can be correlated to the interhelical angle adopted by subdomain IIa. In the absence of divalent metal ions, the internal loop of subdomain IIa does not stably fold, placing the cyanine dyes outside of the Förster radius resulting in a FRET signal that is not detectable. The addition of Mg\textsuperscript{2+} ions manifests a dose-dependent increase in FRET due to subdomain IIa assuming the functional L-shape which brings the dyes into close proximity.
Small molecule ligands which distort the proper folding of the critical bend in subdomain IIa should cause changes in the FRET signal when compared the natively folded subdomain IIa in the presence of Mg\textsuperscript{2+} ions.\textsuperscript{[28,47]} The addition of known translation inhibitor 2, which interacts with subdomain IIa, results in a dose-dependent quenching of FRET suggesting that the ligand captures an elongated form of subdomain IIa which places the dyes outside of the Förster radius (Figure 2.8B). This observation has been confirmed by a co-crystal structure which shows the ligand bound form of subdomain IIa adopting a nearly linear shape that abrogates IRES function (Figure 2.6).\textsuperscript{[31]} Since the conformation adopted by subdomain IIa is critical to function, using FRET measurements to monitor the perturbations caused by the presence of a small molecule ligand could lead to the development novel viral translation inhibitors. Additionally, the dynamic structural information provided by the FRET assay could reveal further insight into role subdomain IIa plays in the complex mechanism of viral translation initiation.

**In vitro Translation Assay**

Further understanding regarding the ligand-RNA interaction will be accomplished using an *in vitro* translation assay (IVT). The IVT assay utilizes a bicistronic dual reporter system composed of a 5' cap which controls the expression of firefly luciferase and a functional IRES which controls the expression of renilla luciferase (Figure 2.9). The bicistronic reporter system
probes the ability of the ligand to discriminate between IRES-driven translation and cap-driven translation by indirectly measuring the relative luminescence resulting from the expression of the downstream reporters. The relative levels luminescence from each reporter can then be correlated to the general affinity and selectivity of the ligand for the IRES. Inhibitors which decrease the relative levels of firefly signal are likely affecting cap-driven translation. Conversely, ligands that reduce the relative signal of renilla are likely interfering with IRES-driven translation. Compounds which result in commensurate decreases in both signals are generally classified as translation inhibitors affecting the ribosome. However, recent research has revealed that inhibitors which affect subdomain IIa of the IRES are capable of sequestering ribosomes resulting in the overall suppression of both signals similar to what would be observed with ribosomal inhibitors.\(^{[48]}\)

In order to remove the uncertainty associated with the bicistronic IVT assay, a counter screen was developed. Ligands which inhibit both signals will be counter screened in a monocistronic version of the IVT assay, containing only a 5’ cap to examine the inhibitors effect upon only cap-driven translation (Figure 2.10). Additionally the downstream luciferase will changed to renilla to ensure that the ligand is not acting as an inhibitor of the reporter enzyme. Ligands which show activity in monocistronic counter screen are not likely inhibitors of the IRES; rather these molecules are interacting with the ribosome or the reporter enzyme, resulting in the observed translation inhibition. This decoupling of the assay will allow the unambiguous assessment of ligands which suppress both signals.
**Figure 2.9:** Schematic showing bicistronic dual reporter of the IVT assay. Firefly luciferase expression is under the control of cap-driven translation. Renilla luciferase expression is under the control of IRES-driven translation.

**Figure 2.10:** Schematic showing monocistronic reporter of the IVT assay counter screen. Renilla luciferase expression is under the control of cap driven translation.

IVT assays have been previously used as a high throughput screen for small molecules that selectively inhibit HCV IRES-driven translation.\(^{[24]}\) However, few active IRES inhibitors have been discovered using IVT assays as a result of the complex nature and inherent limitations of the assay. Regardless, the IVT assay is an ideal complement to the direct and compositionally simple FRET assay. The IVT assay requires complex biological mixtures including high
concentrations of ribosomes that also act as a source of complex competitor RNA which can assist in assessing the promiscuity of the ligand under study.

Biochemical Evaluation of Benzoxazole Ligands for Targeting the IRES

The synthesized benzoxazole ligands were evaluated in the FRET assay with the aim of discovering new ligands that are adept at capturing the translation incompetent extended form of subdomain IIa of the HCV IRES. Many of the ligands showed decreased FRET indicating modest binding affinity for non-functional form of the RNA despite the divergent nature of the side chains and head groups. This general affinity suggests that the core benzoxazole is competent at recognizing the binding cavity formed by subdomain IIa (Table 2.1). Reduction in FRET signal was also consistently observed for ligands containing the longer linker showing a clear preference for the propyl side chain as opposed the shorter ethyl tether. The preference for the longer side chain indicates the presence of a favorable interaction based on the proper placement of the head group at the edge of the binding pocket which is not accessible to the shorter side chain. However, ethyl tethered ligand 27 exhibited good affinity for subdomain IIa with an EC$_{50}$ of 88 μM. The anomalous affinity suggests that this ligand may be binding in an alternative mode to the subdomain IIa RNA.

The nature of the distal interaction of various head groups with the binding cavity remains unclear based on the FRET measurements. Aminobenzoxazoles 11, 13 and 14 show good affinities for subdomain IIa with
EC$_{50}$ values between 40-120 $\mu$M, yet similar compounds 12 and 14 show no appreciable binding activity (Figure 2.10). The interaction of the head group with the binding pocket prefers the six-membered heterocycles which can also accommodate the smaller dimethyl amino head group but not methyl group of the larger piperazine. The absence of affinity of pyrrolidino ligand 12 implies that the conformation assumed by the head group heterocycles may be critical to ligand binding. However, the peculiar nature of the various head groups interactions cannot be described by a general structure activity relationship.

Figure 2.11: Structure of various aminobenzoxazoles evaluated in the FRET and IVT assays. Ligands 11, 13 and 14 showed good affinity for subdomain IIa in both assays.
**Table 2.1:** Ligand affinity measured by FRET and IVT assays.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID (Notebook ID)</th>
<th>FRET (EC&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>IVT (% inhibition at 100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure 1" /></td>
<td>11 (Kr-203)</td>
<td>121 μM</td>
<td>30±8%</td>
</tr>
<tr>
<td><img src="image2" alt="Structure 2" /></td>
<td>12 (Kr-203353)</td>
<td>n.a.</td>
<td>25±5%</td>
</tr>
<tr>
<td><img src="image3" alt="Structure 3" /></td>
<td>13 (Kr-203349)</td>
<td>47 μM</td>
<td>29±5%</td>
</tr>
<tr>
<td><img src="image4" alt="Structure 4" /></td>
<td>14 (Kr-203347)</td>
<td>43 μM</td>
<td>31±4%</td>
</tr>
<tr>
<td><img src="image5" alt="Structure 5" /></td>
<td>15 (Kr-203351)</td>
<td>n.a.</td>
<td>54±2%</td>
</tr>
<tr>
<td><img src="image6" alt="Structure 6" /></td>
<td>16 (Kr-418)</td>
<td>31 μM</td>
<td>52±3%</td>
</tr>
<tr>
<td><img src="image7" alt="Structure 7" /></td>
<td>17 (Kr-303)</td>
<td>52 μM</td>
<td>46±1%</td>
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</table>
Table 2.1, Continued: Ligand affinity measured by FRET and IVT assays.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID (Notebook ID)</th>
<th>FRET (EC&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>IVT (% inhibition at 100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure" /></td>
<td><strong>19</strong> (Kr-105)</td>
<td>110 μM</td>
<td>21±2%</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure" /></td>
<td><strong>21</strong> (Kr-202F)</td>
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<td>42±9%</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure" /></td>
<td><strong>22</strong> (Kr-202Cl)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure" /></td>
<td><strong>23</strong> (Kr-100)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure" /></td>
<td><strong>24</strong> (Kr-101)</td>
<td>n.a.</td>
<td>30±6%</td>
</tr>
<tr>
<td><img src="image6.png" alt="Structure" /></td>
<td><strong>25</strong> (Kr-203255)</td>
<td>500 μM</td>
<td>24±2%</td>
</tr>
<tr>
<td><img src="image7.png" alt="Structure" /></td>
<td><strong>26</strong> (Kr-203253)</td>
<td>210 μM</td>
<td>38±6%</td>
</tr>
<tr>
<td><img src="image8.png" alt="Structure" /></td>
<td><strong>27</strong> (Kr-203249)</td>
<td>88 μM</td>
<td>39±6%</td>
</tr>
</tbody>
</table>
Table 2.1, Continued: Ligand affinity measured by FRET and IVT assays.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID (Notebook ID)</th>
<th>FRET (EC$_{50}$)</th>
<th>IVT (% inhibition at 100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure image" /></td>
<td>28 (Kr-203247)</td>
<td>n.a.</td>
<td>39±4%</td>
</tr>
<tr>
<td><img src="image" alt="Structure image" /></td>
<td>29 (Kr-203251)</td>
<td>n.a.</td>
<td>27±3%</td>
</tr>
<tr>
<td><img src="image" alt="Structure image" /></td>
<td>30 (Kr-202)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

EC$_{50}$ is the concentration required for a fifty percent reduction in the observed FRET signal. IVT measurements were made in the presence of 100 μM of the ligand under study. Percent inhibition is normalized to the relative signal of the luciferase reporter in the absence of ligand. n.a. = no activity.

The prepared ligands were examined in the IVT assay to evaluate translational inhibition and to ascertain the selectivity for the subdomain IIa target. Nearly all of the aminobenzoxazoles evaluated showed moderate binding activity, supporting the design paradigm which aims at biasing ligands for interaction with RNA (Table 2.1). However, ligands which show activity in the IVT assay, but not in the FRET assay, are not acting upon the desired subdomain IIa target. These ligands are likely general translation inhibitors affecting the ribosome, resulting in decreased translational activity. Additionally, the relative small size and planarity of these ligands may be responsible for the suppression
of translational activity through non-specific intercalation. However, determining
the specific mode of translational inhibition for these ligands is not possible due
to the complex cascade of events measured by the IVT assay.

The ligand-RNA interactions identified by the FRET assay were also borne
out by the IVT assay. Moderately FRET-active ligand 11 demonstrated
commensurate levels of inhibition in the IVT assay suggesting that this
compound is selective for subdomain IIa even in the presence of competitor
RNA. However, ligands 13 and 14 display an intermediate level of promiscuity
which explains the strong reduction in activity observed in the IVT assay in
comparison with the FRET assay. Ligands 13 and 14 are likely engaging in non-
specific interactions with the competitor RNA resulting in diminished translation
inhibition.

**Re-evaluation of the Aminobenzoxazole Core**

Initial ligand design was focused around core scaffold 4 with various head
groups attached by a tether of variable length. This effort led to the discovery of
aminobenzoxazole 11 as a selective inhibitor for subdomain IIa of the IRES with
an EC$_{50}$ of $\sim$100 μM as measured by the FRET assay. The activity of
benzoxazole 11 was confirmed by the IVT assay which showed a 30% inhibition
of IRES-dependent translation at a concentration of 100 μM while not affecting
the cap-driven counterpart. To ascertain the role of the amine at the 6-position of
benzoxazole 11 with respect to binding, analogs 16, 17 and 19 were constructed
(Figure 2.11). Additionally, a pair of benoxazoles containing a halogen at the 6-position were synthesized in hopes that the electron withdrawing group would balance the electron rich benoxazole resulting in compounds with increased stability while maintaining good target affinity observed in parental ligand 11. Finally, parental ligand 20 would allow the affinity of the core alone to be established.

![Chemical structures](image)

**Figure 2.12:** Designed analogs of aminobenzoxazole 11. X = Cl or F. Analogs were synthesized using the previously outlined synthetic route starting from the appropriate phenol.

The benoxazole analogs were prepared using the synthetic route outlined above starting from the appropriate phenol (Scheme 2.3 and Scheme 2.5), and the respective affinities for subdomain IIa were measured in the FRET assay. Aminobenzoxazole 20 displayed no reduction in FRET signal indicating that core scaffold alone is not able to capture the elongated form of the RNA. The addition of a dimethyl amino head group attached by a propyl side chain, represented by compound 19, restored activity comparable to the initial lead 11.
underscoring the importance of this substitution with respect to ligand binding. Both ligands 16 and 17, which illustrate the effect of migrating the amine, showed improved binding to subdomain IIa with $EC_{50}$ values of 31 μM and 52 μM, respectively. However, ligand 16 resulted in optimal binding interactions with the target for this series of compounds by placing the amine substituent at the 7-position. Despite the enhanced activity toward the target RNA, ligands 16 and 17 display the same sensitivity to oxidation found in parent ligand 11. Replacing the amine in ligand 11 by a halogen resulted in stable compounds which show no reduction in FRET signal. The complete abrogation of binding indicates that the halogen is deleterious to ligand affinity, perhaps by changing the electronic character of the aromatic system by perturbing the critical hydrogen bonding face of the benzoxazole.

The potency of the aminobenzoxazole analogs was also measured by the IVT assay. The aminobenzoxazole core 20 and halogenated scaffold 21 are modest inhibitors of translation at 100 μM by acting independently of subdomain IIa of the IRES. However, FRET-active ligands 16, 17 and 19 reduced levels of translation moderately by directly binding to the compromised extended conformation of subdomain IIa. Furthermore, ligands 16, 17 and 19 demonstrate improved selectivity over compound 11 based on the consistent potency of the RNA-ligand interaction as measured by the independent assays.
Conclusions

A series of 2-substituted aminobenzoxazoles was synthesized with characteristics intended to bias these ligands for interaction with RNA. Select aminobenzoxazoles were able to bind to subdomain IIa of the HCV IRES and reduce translation, likely in a similar fashion to that observed for known subdomain IIa inhibitor 2. The interaction of the distinct head groups at the edge of the binding pocket was also explored; however, the head group did not facilitate significant improvement in ligand affinity. The placement of the head group within the binding cavity was shown to be vital. Ligands containing the shorter ethyl tether show diminished binding activity when compared to their propyl analogs. The longer propyl linker facilitates a stabilizing ligand-RNA interaction not accessible to the shorter side chain. Additionally, the aminobenzoxazole core alone was shown not to be able to capture the elongated form of subdomain IIa emphasizing the critical role the side chain plays in ligand binding. Replacement of the amine at the 6-position with a halogen (X= Cl of F) results in ligands without affinity for the target RNA revealing the importance of the amine to the recognition of subdomain IIa. The optimal placement of the amine within the benzoxazole scaffold was then determined to be the 7-position resulting in 3-fold improvement in binding.
Materials and Methods

6-Nitrobenzo[d]oxazol-2-amine (KR-100)

![KR-100](image)

**Figure 2.13:** 6-nitrobenzo[d]oxazol-2-amine (KR-100).

Di(imidazole-1-yl)methanimine (12.98 mmol, 1.51 g) was added to a solution of 2-amino-5-nitrophenol (6.49 mmol, 1.00 g) in acetonitrile (40 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for fifteen hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) of the resulting brown solid provided pure **Kr-100** as a yellow solid in an 81% yield.

**KR-100:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.17 (br s, 2H, NH$_2$), 8.16 (d, 1H, $J = 2.4$ Hz), 8.04 (dd, 1H, $J = 8.8$ Hz, $J' = 2.4$ Hz), 7.26 (d, 1H, $J = 8.8$ Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 167.0, 151.6, 147.8, 140.9, 121.6, 114.7, 105.2; HRMS mass calculated for C$_7$H$_6$N$_3$O$_3$ (M+H)$^+$ 180.0404, found 180.0406; Delta 1.1 ppm.
Benzo[d]oxazole-2,6-diamine (KR-101)

Figure 2.14: benzo[d]oxazole-2,6-diamine (KR-101).

To a of solution of 6-nitrobenzo[d]oxazol-2-amine (KR-100) (6.20 mmol, 1.11 g) in methanol (65 mL), 10% palladium on activated carbon (10 wt% of KR-100, 0.11 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for three hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (20 mL). The filtrate was concentrated in vacuo yielding an off-white crystalline solid in a 96% yield. Benzo[d]oxazole-2,6-diamine (KR-101) is air unstable and appropriate measures should be taken to minimize air exposure. Silica gel column chromatography (1:1 hexanes/ethyl acetate) can be utilized to re-isolate KR-101 from a decomposed mixture.

KR-101: \(^1\)H NMR (400 MHz, MeOD-\(d_4\)) \(\delta\) 6.98 (d, 1H, \(J = 8.0\) Hz), 6.73 (d, 1H, \(J = 2.0\) Hz), 6.59 (dd, 1H, \(J = 8.0\) Hz, \(J' = 2.0\) Hz), 4.91 (s, 4H, NH\(_2\)); \(^{13}\)C NMR (400 MHz, MeOD-\(d_4\)) \(\delta\) 162.3, 149.2, 142.2, 134.5, 115.1, 112.1, 97.3; HRMS mass
calculated for C₇H₈N₃O (M+H)⁺ 150.0662, found 150.0665; Delta 2.0 ppm; Crystal structure available.

6-Nitrobenzo[d]oxazole-2-thiol (KR-200)

Potassium hydroxide (35.68 mol, 2.00 g) was added to a solution of 2-amino-5-nitrophenol (32.44 mmol, 5.00 g) in carbon disulfide (64.88 mmol, 3.92 mL), ethanol (27.0 mL) and water (5.4 mL) with constant magnetic stirring. The reaction was placed under argon atmosphere and refluxed for 72 hours. The reaction mixture was then cooled to room temperature and filtered. The filtrand is then rinsed with ice cold ethanol (10 mL), transferred to a separatory funnel, acidified with 1N hydrochloric acid (150 mL), and extracted with ethyl acetate (6 x 160 mL). The organic layer was then dried over sodium sulfate and concentrated under reduced pressure yielding a yellow solid. Silica gel column chromatography of the solid (5:1 hexanes/ethyl acetate) provided pure KR-200 as a yellow powder in a 77% yield.
KR-200: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.36 (d, 1H, $J = 2.0$ Hz), 8.17 (dd, 1H, $J = 8.8$ Hz, $J = 2.0$ Hz), 7.35 (d, 1H, $J = 8.8$ Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 182.4, 148.3, 144.1, 138.0, 122.4, 110.9, 106.7.

$N^1,N^1$-Dimethyl-$N^2$-(6-nitrobenzo[d]oxazol-2-yl)ethane-1,2-diamine (KR-202255)

![KR-202255](image)

Figure 2.16: $N^1,N^1$-dimethyl-$N^2$-(6-nitrobenzo[d]oxazol-2-yl)ethane-1,2-diamine (KR-202255).

Cesium carbonate (9.14 mmol, 2.98 g) was added to a solution of 6-nitrobenzo[d]oxazol-2-amine (KR-100) (7.31 mmol, 1.31 g) in acetonitrile (500 mL). The reaction was placed under argon atmosphere, and 2-chloro-$N,N$-dimethylethan-1-amine (7.39 mmol, 0.79 g) was added with constant magnetic stirring. Then the reaction mixture was heated to 55 °C for six hours. The reaction was cooled to room temperature, filtered, and the filtrand was washed with ethanol (20 mL). The filtrate was concentrated under reduced pressure yielding a red oil. Silica gel column chromatography of the oil (20:1 dichloromethane/methanol) provided pure KR-202255 as a yellow solid in a 59% yield.
**KR-202255**: $^1$H NMR (400 MHz, DMSO-$_d_6$) $\delta$ 8.11 (d, 1H, $J$ = 1.2 Hz), 8.02 (dd, 1H, $J = 8.4$ Hz, $J'$ = 1.2 Hz), 7.27 (d, 1H, $J = 8.4$ Hz), 4.51 (bs, 1H, NH), 3.42 (t, 2H, $J = 6.4$ Hz), 2.45 (t, 2H, $J = 6.4$ Hz), 2.16 (s, 6H); $^{13}$C NMR (400 MHz, DMSO-$_d_6$) $\delta$ 166.2, 151.2, 147.9, 140.9, 121.7, 114.8, 105.1, 58.3, 45.7, 41.1; HRMS mass calculated for C$_{11}$H$_{15}$N$_4$O$_3$ (M+H)$^+$ 251.1139, found 251.1141; Delta 0.8 ppm.

$N^2$-(2-(dimethylamino)ethyl)Benzo[d]oxazole-2,6-diamine (KR-203255)

![KR-203255](image)

Figure 2.17: $N^2$-(2-(dimethylamino)ethyl)benzo[d]oxazole-2,6-diamine (KR-203255).

To a solution of $N^1,N^1$-dimethyl-$N^2$-(6-nitrobenzo[d]oxazol-2-yl)ethane-1,2-diamine (KR-202255) (3.24 mmol, 0.81 g) in methanol (125 mL), PtO$_2$ (0.16 mmol, 0.04 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for twelve hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (75 mL). The filtrate was concentrated *in vacuo* yielding a red oil. Silica gel column chromatography of the oil (10:1 dichloromethane/methanol) provided pure KR-203255 as a faint brown
oil in a 79% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.1-0.3% acetonitrile in water. **KR-203255** is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

**KR-203255FB:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.31 (bs, 1H, NH), 6.88 (d, 1H, J = 8.4 Hz), 6.55 (s, 1H), 6.34 (dd, 1H, J = 8.4 Hz, J = 1.2 Hz), 4.78 (bs, 2H, NH$_2$), 3.28 (t, 2H, J = 6.8 Hz), 2.40 (t, 2H, J = 6.8 Hz), 2.14 (s, 6H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 161.2, 149.6, 144.0, 134.0, 116.0, 110.5, 96.0, 58.7, 45.9, 41.1; HRMS mass calculated for C$_{11}$H$_{17}$N$_4$O (M+H)$^+$ 221.1397, found 221.1396; Delta - 0.5 ppm.

$N^1,N^1$-Dimethyl-$N^3$-(6-nitrobenzo[\textit{d}]oxazol-2-yl)propane-1,3-diamine (KR-202)

![KR-202](image)

**Figure 2.18:** $N^1,N^1$-dimethyl-$N^3$-(6-nitrobenzo[\textit{d}]oxazol-2-yl)propane-1,3-diamine (KR-202).

A solution of 6-nitrobenzo[\textit{d}]oxazole-2-thiol (KR-200) (11.37 mmol, 2.23 g) in dichloromethane (379 mL) and oxalyl chloride (17.05 mmol, 1.46 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (15 mL)
was added drop wise over a period of 45 minutes. CAUTION: The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After four hours, the reaction was again cooled to 0 °C, and triethylamine (34.10 mmol, 4.76 mL) was added drop wise over a period of 25 minutes. Then N,N-dimethylpropane-1,3-diamine (12.50 mmol, 1.57 mL) was added, and the ice bath was removed. The reaction was stirred for fourteen hours at room temperature then concentrated in vacuo yielding a red solid. Silica gel column chromatography of the solid (15:1 dichloromethane/methanol) provided pure KR-202 as a yellow powder in a 72% yield.

**KR-202:** $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.92 (br s, 1H, NH), 8.15 (d, 1H, J = 2.0 Hz), 8.04 (dd, 1H, J = 8.8 Hz, J' = 2.0 Hz), 7.29 (d, 1H, J = 8.8 Hz), 3.38 (t, 2H, J = 6.8 Hz), 2.68 (t, 2H, J = 7.2 Hz), 2.40 (s, 6H), 1.85 (p, 2H, J = 7.2 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 166.1, 151.2, 147.9, 141.0, 121.7, 114.9, 105.2, 55.8, 44.2, 40.9, 25.9; HRMS mass calculated for C_{12}H_{17}N_{4}O_{3} (M+H)$^+$ 265.1295, found 265.1299; Delta 1.5 ppm.
**N²-(3-(dimethylamino)propyl)Benzo[d]oxazole-2,6-diamine (KR-203)**

![KR-203](image)

**Figure 2.19:** N²-(3-(dimethylamino)propyl)benzo[d]oxazole-2,6-diamine (KR-203).

A solution of N¹,N¹-dimethyl-N³-(6-nitrobenzo[d]oxazol-2-yl)propane-1,3-diamine (KR-202) (6.42 mmol, 1.58 g) in ethanol (46.0 mL) was placed under argon atmosphere. Then a solution of stannous chloride dihydrate (39.89 mmol, 9.00 g) in concentrated hydrochloric acid (15.22 mL) was added drop wise with constant magnetic stirring. The reaction mixture was then heated to 50 °C for four hours. The reaction was cooled to 0 °C, and the pH was adjusted to 10 by addition of 1N sodium hydroxide. The resulting heterogeneous mixture was filtered, and the filtrand was washed with ethanol (75 mL). The filtrate was concentrated, and silica gel column chromatography of the resulting brown solid (10:1 dichloromethane/methanol) provided pure KR-203 as a faint pink oil in a 51% yield. KR-203 is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

**KR-203:** ¹H NMR (400 MHz, CDCl₃) δ 7.05 (d, 1H, J = 8.0 Hz), 6.85 (br s, 1H, NH), 6.57 (d, 1H, J = 1.6 Hz), 6.44 (dd, 1H, J = 8.0 Hz, J’ = 1.6 Hz), 3.93 (br s, 2H, NH₂), 3.43 (t, 2H, J = 6.0 Hz), 2.37 (t, 2H, J = 6.4 Hz), 2.18 (s, 6H), 1.73 (p,
2H, J = 6.4 Hz); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 161.6, 149.5, 141.3, 135.6, 116.2, 111.5, 97.1, 58.5, 45.5, 43.0, 26.2; HRMS mass calculated for C$_{12}$H$_{19}$N$_4$O (M+H)$^+$ 235.1553, found 235.1552; Delta -0.4 ppm.

6-Nitro-$N$-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazol-2-amine (KR-202253)

![Figure 2.20: 6-nitro-$N$-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazol-2-amine (KR-202253).](image)

A solution of 6-nitrobenzo[d]oxazole-2-thiol (KR-200) (14.37 mmol, 2.82 g) in dichloromethane (479 mL) and oxaly chloride (17.97 mmol, 1.54 mL) was placed under argon atmosphere and cooled to 0°C. Dimethylformamide (20 mL) was added drop wise over a period of 60 minutes. CAUTION: The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0°C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After two hours, the reaction was again cooled to 0°C, and triethylamine (43.12 mmol, 6.01 mL) was added drop wise over a period of 30 minutes. Then 2-(pyrrolidin-1-yl)ethan-1-amine (KR-253) (51.81 mmol, 1.81 g) was added, and the ice bath was removed. The reaction was stirred for fourteen hours at room temperature then concentrated in vacuo yielding a red solid. Silica gel column chromatography of
the solid (15:1 dichloromethane/methanol) provided pure **KR-202253** as a yellow powder in a 46% yield.

**KR-202253**: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.20 (d, 1H, $J = 2.0$ Hz), 8.09 (dd, 1H, $J = 8.8$ Hz, $J' = 2.0$ Hz), 7.31 (d, 1H, $J = 8.8$ Hz), 3.47 (t, 2H, $J = 6.8$ Hz), 2.63 (t, 2H, $J = 6.8$ Hz), 2.50 (m, 4H), 1.68 (m, 4H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 165.8, 151.1, 147.4, 140.0, 121.4, 114.0, 104.5, 54.7, 53.7, 40.0, 23.3; HRMS mass calculated for C$_{13}$H$_{17}$N$_4$O$_3$ (M+H)$^+$ 277.1295, found 277.1297; Delta 0.7 ppm.

$N^2$-(2-(pyrrolidin-1-yl)ethyl)Benzo[d]oxazole-2,6-diamine (KR-203253)

![Figure 2.21](image)

**Figure 2.21**: $N^2$-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203253).

To a solution of 6-nitro-$N$-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazol-2-amine (**KR-202253**) (2.75 mmol, 0.76 g) in methanol (100 mL), PtO$_2$ (0.14 mmol, 0.03 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for twelve hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction
contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (60 mL). The filtrate was concentrated *in vacuo* yielding a brown oil. Silica gel column chromatography of the oil (10:1 dichloromethane/methanol) provided pure **KR-203253** as a faint purple oil in a 68% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.1-4.0% acetonitrile in water. **KR-203253** is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

**KR-203253:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.59 (br s, 1H, NH), 7.46 (d, 1H, $J$ = 2.0 Hz), 7.33 (d, 1H, $J$ = 8.4 Hz), 7.18 (dd, 1H, $J$ = 8.4 Hz, $J'$ = 2.0 Hz), 3.68 (t, 2H, $J$ = 5.6 Hz), 3.60 (br s, 2H), 3.40 (br s, 2H), 3.03 (br s, 2H), 1.97 (br s, 2H), 1.84 (t, 2H, $J$ = 5.6 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 163.3, 148.5, 143.0, 125.3, 119.6, 116.4, 105.2, 53.8, 53.1, 39.3, 23.2; HRMS mass calculated for **C$_{13}$H$_{19}$N$_4$O (M+H)$^+$** 247.1553, found 247.1552; Delta -0.4 ppm.
6-Nitro-\(N\)-(3-(pyrrolidin-1-yl)propyl)benzo[\(d\)oxazol-2-amine (KR-202353)

\[
\text{O}_2\text{N} \quad \text{O} \quad \text{NH} \\
\text{N} \quad \text{KR-202353}
\]

**Figure 2.22:** 6-nitro-\(N\)-(3-(pyrrolidin-1-yl)propyl)benzo[\(d\)oxazol-2-amine (KR-202353).

A solution of 6-nitrobenzo[\(d\)oxazole-2-thiol (KR-200) (13.36 mmol, 2.62 g) in dichloromethane (445 mL) and oxalyl chloride (16.69 mmol, 1.43 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (15 mL) was added drop wise over a period of 45 minutes. CAUTION: The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After four hours, the reaction was again cooled to 0 °C, and triethylamine (40.07 mmol, 5.59 mL) was added drop wise over a period of 25 minutes. Then 3-(pyrrolidin-1-yl)propan-1-amine (KR-353) (14.69 mmol, 1.88 g) was added, and the ice bath was removed. The reaction was stirred for fourteen hours at room temperature then concentrated *in vacuo* yielding an orange solid. Silica gel column chromatography of the solid (20:1 dichloromethane/methanol) provided pure KR-202353 as a yellow powder in an 88% yield.
KR-202353: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.00 (br s, 1H, NH), 8.17 (d, 1H, J = 1.6 Hz), 8.05 (dd, 1H, J = 8.4 Hz, J' = 1.6 Hz), 7.31 (d, 1H, J = 8.8 Hz), 3.44 (br s, 2H), 3.17 (m, 4H), 2.03 (p, 2H, J = 8.0 Hz, J' = 6.4 Hz), 1.89 (br s, 4H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 166.1, 151.1, 148.0, 141.1, 121.8, 115.0, 105.3, 53.4, 52.0, 40.5, 25.8, 23.4; HRMS mass calculated for C$_{14}$H$_{19}$N$_4$O$_3$ (M+H)$^+$ 291.1452, found 291.1457; Delta 1.7 ppm.

$N^2$-(3-(pyrrolidin-1-yl)propyl)Benzo[\textit{d}]oxazole-2,6-diamine (KR-203353)

![KR-203353](image)

Figure 2.23: $N^2$-(3-(pyrrolidin-1-yl)propyl)benzo[\textit{d}]oxazole-2,6-diamine (KR-203353).

To a solution of 6-nitro-$N$-(3-(pyrrolidin-1-yl)propyl)benzo[\textit{d}]oxazol-2-amine (KR-202353) (7.23 mmol, 2.10 g) in methanol (125 mL), PtO$_2$ (0.36 mmol, 0.08 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for twelve hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (75 mL). The filtrate was concentrated \textit{in vacuo} yielding a brown oil. Silica gel column chromatography of the oil (10:1 dichloromethane/methanol) provided pure KR-203353 as a faint brown oil in a
79% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.1-4.0% acetonitrile in water. **KR-203353** is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

**KR-203353**: ¹H NMR (400 MHz, DMSO-d₆) δ 8.37 (br s, 1H, NH), 7.40 (d, 1H, J = 2.4 Hz), 7.30 (d, 1H, J = 8.4 Hz), 7.13 (dd, 1H, J = 8.0 Hz, J' = 2.4 Hz), 3.53 (br s, 2H), 3.38 (t, 2H, J = 6.8 Hz), 3.19 (br s, 2H), 2.97 (br s, 2H), 1.96 (m, 4H), 1.84 (p, 2H, J = 6.8 Hz); ¹³C NMR (400 MHz, DMSO-d₆) δ 163.6, 148.5, 143.0, 125.2, 119.3, 116.1, 104.9, 53.8, 52.3, 40.2, 26.0, 23.3; HRMS mass calculated for C₁₄H₂₁N₄O (M+H)⁺ 261.1710, found 261.1711; Delta 0.4 ppm.

**KR-203353FB**: ¹H NMR (400 MHz, DMSO-d₆) δ 7.40 (br s, 1H, NH), 6.88 (d, 1H, J = 8.0 Hz), 6.55 (d, 1H, J = 2.0 Hz), 6.35 (dd, 1H, J = 8.0 Hz, J' = 2.0 Hz), 4.77 (br s, 1H, NH₂), 3.23 (q, 2H, J = 6.4 Hz), 2.39 (m, 6H), 1.71 (p, 2H, J = 6.8 Hz), 1.64 (m, 4H); ¹³C NMR (400 MHz, DMSO-d₆) δ 161.3, 149.6, 144.0, 134.1, 116.0, 110.5, 95.9, 54.3, 53.8, 41.5, 28.9, 23.8.
6-Nitro-N-(2-(piperidin-1-yl)ethyl)benzo[d]oxazol-2-amine (KR-202249)

![KR-202249]

**Figure 2.24:** 6-nitro-N-(2-(piperidin-1-yl)ethyl)benzo[d]oxazol-2-amine (KR-202249).

A solution of 6-nitrobenzo[d]oxazole-2-thiol (KR-200) (9.38 mmol, 1.84 g) in dichloromethane (313 mL) and oxalyl chloride (11.72 mmol, 1.01 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (12 mL) was added drop wise over a period of 37 minutes. CAUTION: The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After three hours, the reaction was again cooled to 0 °C, and triethylamine (28.14 mmol, 3.92 mL) was added drop wise over a period of 20 minutes. Then 2-(piperidin-1-yl)ethan-1-amine (KR-249) (10.32 mmol, 1.32 g) was added, and the ice bath was removed. The reaction was stirred for sixteen hours at room temperature then concentrated *in vacuo* yielding a red solid. Silica gel column chromatography of the solid (15:1 dichloromethane/methanol) provided pure KR-202249 as a yellow powder in a 69% yield.
$N^2$-(2-(piperidin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203249)

![KR-203249]

**Figure 2.25:** $N^2$-(2-(piperidin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203249).

A solution of 6-nitro-$N$-(2-(piperidin-1-yl)ethyl)benzo[d]oxazol-2-amine (KR-202249) (13.30 mmol, 3.86 g) in ethanol (95.0 mL) was placed under argon atmosphere. Then a solution of stannous chloride dihydrate (39.89 mmol, 9.00 g) in concentrated hydrochloric acid (15.22 mL) was added drop wise with constant magnetic stirring. The reaction mixture was then heated to 58 $^0$C for two hours. The reaction was cooled to 0 $^0$C, and the pH was adjusted to 10 by addition of 1N sodium hydroxide. The resulting heterogeneous mixture was filtered, and the filtrand was washed with ethanol (75 mL). The filtrate was concentrated, and silica gel column chromatography of the resulting orange solid (10:1 dichloromethane/methanol) provided pure KR-203249 as a brown oil in a 57% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.5-35.0% acetonitrile in water. KR-203249 is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.
**KR-203249:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.92 (br s, 2H, NH$_2$), 9.66 (br s, 1H, NH), 7.44 (s, 1H), 7.32 (d, 1H, $J = 8.0$ Hz), 7.17 (d, 1H, $J = 8.0$ Hz), 3.70 (t, 2H, $J = 5.6$ Hz), 3.54 (d, 2H, $J = 11.2$ Hz), 3.32 (t, 2H, $J = 5.6$ Hz), 2.91 (t, 2H, $J = 11.2$ Hz), 1.77 (m, 2H), 1.66 (m, 3H), 1.36 (m, 1H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 163.4, 148.6, 142.9, 125.3, 119.5, 116.3, 105.2, 55.4, 53.1, 37.6, 23.0, 21.7; HRMS mass calculated for C$_{14}$H$_{21}$N$_4$O (M+H)$^+$ 261.1710, found 261.1708; Delta -0.8 ppm.

**6-Nitro-N-(3-(piperidin-1-yl)propyl)benzo[d]oxazol-2-amine (KR-202349)**

![KR-202349](image)

**Figure 2.26:** 6-nitro-N-(3-(piperidin-1-yl)propyl)benzo[d]oxazol-2-amine (KR-202349).

A solution of 6-nitrobenzo[d]oxazole-2-thiol (**KR-200**) (17.53 mmol, 3.44 g) in dichloromethane (438 mL) and oxalyl chloride (21.92 mmol, 1.88 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (20 mL) was added drop wise over a period of 60 minutes. CAUTION: The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After three hours, the reaction was again cooled to 0 °C, and triethylamine (56.60 mmol, 7.34 mL)
was added drop wise over a period of 40 minutes. Then 3-(piperidin-1-yl)propan-1-amine (KR-349) (19.29 mmol, 2.74 g) was added, and the ice bath was removed. The reaction was stirred for twenty-four hours at room temperature then concentrated in vacuo yielding an orange solid. Silica gel column chromatography of the solid (20:1 dichloromethane/methanol) provided pure KR-202349 as a yellow powder in an 84% yield.

KR-202349: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.20 (d, 1H, $J = 2.4$ Hz), 8.08 (d, 1H, $J = 8.4$ Hz, $J' = 2.4$), 7.31 (d, 1H, $J = 8.4$ Hz), 3.35 (t, 3H, $J = 6.8$ Hz), 2.28 (t, 6H, $J = 6.8$ Hz), 1.71 (p, 2H, $J = 6.8$ Hz), 1.45 (p, 4H, $J = 5.6$ Hz), 1.34 (p, 1H, $J = 5.6$ Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 166.3, 151.5, 148.0, 140.8, 121.9, 114.7, 105.2, 56.6, 54.7, 41.8, 26.8, 26.3, 24.8; HRMS mass calculated for C$_{15}$H$_{21}$N$_4$O$_3$ (M+H)$^+$ 305.1608, found 305.1606; Delta -0.6 ppm.

$N^2$-(3-(piperidin-1-yl)propyl)Benzo[d]oxazole-2,6-diamine (KR-203349)

![KR-203349](image)

**Figure 2.27:** $N^2$-(3-(piperidin-1-yl)propyl)benzo[d]oxazole-2,6-diamine (KR-203349).

A solution of 6-nitro-$N$-(3-(piperidin-1-yl)propyl)benzo[d]oxazol-2-amine (KR-202349) (14.79 mmol, 4.50 g) in ethanol (106.0 mL) was placed under argon
atmosphere. Then a solution of stannous chloride dihydrate (44.36 mmol, 10.01 g) in concentrated hydrochloric acid (16.92 mL) was added drop wise with constant magnetic stirring. The reaction mixture was then heated to 54 °C for two hours. The reaction was cooled to 0 °C, and the pH was adjusted to 10 by addition of 1N sodium hydroxide. The resulting heterogeneous mixture was filtered, and the filtrand was washed with ethanol (75 mL). The filtrate was concentrated, and silica gel column chromatography of the resulting red solid (10:1 dichloromethane/methanol) provided pure KR-203349 as a brown oil in a 59% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 2.0-37.0% acetonitrile in water. KR-203349 is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

KR-203349: $^1$H NMR (400 MHz, DMSO-\textit{d$_6$}) $\delta$ 9.72 (br s, 1H, NH), 9.54 (br s, 2H, NH$_2$), 7.42 (d, 1H, J = 2.0 Hz), 7.30 (d, 1H, J = 8.0 Hz), 7.15 (dd, 1H, J = 8.0 Hz, J’ = 2.0 Hz), 3.39 (t, 4H, J = 6.4 Hz), 3.10 (t, 2H, J = 8.0 Hz), 2.83 (t, 2H, J = 10.8 Hz), 2.00 (p, 2H, J = 8.0 Hz), 1.76 (m, 2H), 1.64 (m, 3H), 1.27 (m, 1H); $^{13}$C NMR (400 MHz, DMSO-\textit{d$_6$}) $\delta$ 163.5, 148.4, 142.7, 125.4, 119.3, 116.0, 105.0, 54.3, 52.8, 40.2, 24.0, 23.1, 21.8; HRMS mass calculated for C$_{15}$H$_{23}$N$_4$O (M+H)$^+$ 275.1866, found 275.1867; Delta 0.4 ppm.
**Figure 2.28: N-(2-morpholinoethyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202247).**

A solution of 6-nitrobenzo[d]oxazole-2-thiol (**KR-200**) (18.76 mmol, 3.68 g) in dichloromethane (468 mL) and oxalyl chloride (23.45 mmol, 2.01 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (25 mL) was added drop wise over a period of 75 minutes. **CAUTION:** The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After two hours, the reaction was again cooled to 0 °C, and triethylamine (56.27 mmol, 7.85 mL) was added drop wise over a period of 30 minutes. Then 2-morpholinoethan-1-amine (**KR-247**) (20.63 mmol, 2.69 g) was added, and the ice bath was removed. The reaction was stirred for fifteen hours at room temperature then concentrated *in vacuo* yielding a red solid. Silica gel column chromatography of the solid (25:1 dichloromethane/methanol) provided pure **KR-202247** as a yellow powder in a 51% yield.
**KR-202247:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.69 (br s, 1H, NH), 8.21 (d, 1H, J = 2.4 Hz), 8.08 (dd, 1H, $J = 8.4$ Hz, $J' = 2.0$ Hz), 7.32 (d, 1H, $J = 8.4$ Hz), 3.53 (t, 4H, J = 6.4 Hz), 3.46 (q, 2H, J = 6.4 Hz), 2.51 (t, 2H, J = 6.4 Hz), 2.40 (t, 4H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 166.3, 151.2, 147.9, 140.9, 121.8, 114.8, 105.2, 66.8, 57.7, 53.9, 40.6; HRMS mass calculated for C$_{13}$H$_{17}$N$_4$O$_4$ (M+H)$^+$ 293.1247, found 293.1248; Delta 0.3 ppm.

$N^2$-(2-morpholinoethyl)benzo[d]oxazole-2,6-diamine (KR-203247)

![KR-203247](image)

**Figure 2.29:** $N^2$-(2-morpholinoethyl)benzo[d]oxazole-2,6-diamine (KR-203247).

To a solution of $N$-(2-morpholinoethyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202247) (3.39 mmol, 0.99 g) in methanol (110 mL), PtO$_2$ (0.17 mmol, 0.04 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for eighteen hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (95 mL). The filtrate was concentrated *in vacuo* yielding a brown solid. Silica gel column chromatography
of the solid (30:1 dichloromethane/methanol) provided pure **KR-203247** as a red solid in an 82% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.5-10.0% acetonitrile in water. **KR-203247** is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

**KR-203247FB:** \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.33 (br s, 1H, NH), 6.89 (d, 1H, \(J = 8.0\) Hz), 6.56 (d, 1H, \(J = 1.6\) Hz), 6.36 (dd, 1H, \(J = 8.0\) Hz, \(J' = 2.0\) Hz), 4.79 (br s, 2H, NH\(_2\)), 3.53 (t, 4H, \(J = 4.4\) Hz), 3.32 (s, 2H), 2.47 (t, 2H, \(J = 6.8\) Hz), 2.37 (br s, 4H); \(^{13}\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 161.2, 149.7, 144.0, 134.0, 116.0, 110.6, 95.9, 66.8, 57.9, 54.0, 40.2; HRMS mass calculated for C\(_{13}\)H\(_{19}\)N\(_4\)O\(_2\) (M+H\(^+\)) 263.1505, found 263.1504; Delta -0.4 ppm.


![KR-202347](image)

**Figure 2.30:** **N-(3-morpholinopropyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202347).**

A solution of 6-nitrobenzo[d]oxazole-2-thiol (**KR-200**) (13.61 mmol, 2.67 g) in dichloromethane (454 mL) and oxalyl chloride (17.01 mmol, 1.46 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (15 mL)
was added drop wise over a period of 45 minutes. CAUTION: The addition of
dimethylformamide should be conducted slowly to avoid rapid evolution of gas.
The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed
and the reaction was allowed to warm to room temperature. After two hours, the
reaction was again cooled to 0 °C, and triethylamine (40.83 mmol, 5.69 mL) was
added drop wise over a period of 25 minutes. Then 3-morpholinopropan-1-amine
(KR-347) (14.97 mmol, 2.16 g) was added, and the ice bath was removed. The
reaction was stirred for twenty hours at room temperature then concentrated in
vacuo yielding an orange solid. Silica gel column chromatography of the solid
(20:1 dichloromethane/methanol) provided pure KR-202347 as a yellow powder
in a 70% yield.

KR-202347: 1H NMR (400 MHz, DMSO-d6) δ 8.72 (br s, 1H, NH), 8.16 (d, 1H, J
= 1.2 Hz), 8.05 (dd, 1H, J = 8.8 Hz, J’ = 1.2 Hz), 7.28 (d, 1H, J = 8.8 Hz), 3.52 (t,
4H, J = 4.4 Hz), 3.35 (t, 2H), 2.31 (m, 6H), 1.72 (p, 2H, J = 6.8 Hz); 13C NMR
(400 MHz, DMSO-d6) δ 166.2, 151.3, 147.9, 140.9, 121.8, 114.8, 105.2, 66.8,
56.1, 53.9, 41.4, 26.2; HRMS mass calculated for C14H19N4O4 (M+H)+ 307.1401,
found 307.1404; Delta 1.0 ppm.
A solution of \( N^-\text{-(3-morpholinopropyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202347)} \) (4.28 mmol, 1.31 g) in ethanol (31.0 mL) was placed under argon atmosphere. Then a solution of stannous chloride dihydrate (12.83 mmol, 2.89 g) in concentrated hydrochloric acid (4.90 mL) was added drop wise with constant magnetic stirring. The reaction mixture was then heated to 54 \( ^\circ \text{C} \) for two hours. The reaction was cooled to 0 \( ^\circ \text{C} \), and the pH was adjusted to 10 by addition of 1N sodium hydroxide. The resulting heterogeneous mixture was filtered, and the filtrand was washed with ethanol (50 mL). The filtrate was concentrated, and silica gel column chromatography of the resulting brown solid (30:1 dichloromethane/methanol) provided pure \textbf{KR-203347} as a brown oil in a 64% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.2-4.0% acetonitrile in water. \textbf{KR-203347} is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

\textbf{KR-203347FB}: \textsuperscript{1}H NMR (400 MHz, DMSO-\( d_6 \)) \( \delta \) 7.41 (br s, 1H, NH), 6.89 (d, 1H, \( J = 8.0 \text{ Hz} \)), 6.56 (s, 1H), 6.35 (d, 1H, \( J = 8.0 \text{ Hz} \)), 4.78 (br s, 2H, NH\textsubscript{2}), 3.53 (t,
4H, J = 4.0 Hz), 3.23 (t, 2H, J = 6.4 Hz), 2.29 (m, 6H), 1.69 (p, 2H, J = 6.8 Hz);
13C NMR (400 MHz, DMSO-d6) δ 161.2, 149.6, 144.0, 134.0, 116.0, 110.5, 95.9, 66.9, 56.4, 54.0, 41.3, 26.5; HRMS mass calculated for C14H21N4O2 (M+H)+
277.1659, found 277.1662; Delta 1.0 ppm.

**N-(2-(4-methylpiperazin-1-yl)ethyl)-6-Nitrobenzo[d]oxazol-2-amine** (KR-202251)

![Structure](image)

**Figure 2.32:** N-(2-(4-methylpiperazin-1-yl)ethyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202251).

A solution of 6-nitrobenzo[d]oxazole-2-thiol (KR-200) (11.63 mmol, 2.28 g) in dichloromethane (465 mL) and oxalyl chloride (14.54 mmol, 1.25 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (12 mL) was added drop wise over a period of 40 minutes. CAUTION: The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After two hours, the reaction was again cooled to 0 °C, and triethylamine (34.90 mmol, 4.87 mL) was added drop wise over a period of 25 minutes. Then 2-(4-methylpiperazin-1-yl)ethan-1-amine (KR-251) (12.80 mmol, 1.83 g) was added, and the ice bath
was removed. The reaction was stirred for sixteen hours at room temperature then concentrated in vacuo yielding a red solid. Silica gel column chromatography of the solid (10:1 dichloromethane/methanol) provided pure KR-202251 as a yellow powder in a 53% yield.

**KR-202251:** $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.76 (d, 1H, J = 8.4 Hz), 7.33 (s, 1H), 6.48 (s, 1H), 3.32 (t, 2H, J = 6.8 Hz), 2.36 (t, 2H, J = 6.4 Hz), 2.27 (br s, 8H), 2.21 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 158.1, 148.3, 144.1, 134.5, 123.1, 108.2, 100.0, 60.2, 55.5, 53.7, 46.5, 40.4; HRMS mass calculated for C$_{14}$H$_{20}$N$_5$O$_3$ (M+H)$^+$ 306.1561, found 306.1559; Delta -0.7 ppm.

**$^{N^2}$-(2-(4-methylpiperazin-1-yl)ethyl)Benzo[d]oxazole-2,6-diamine (KR-203251)**

![Diagram](image)

**Figure 2.33:** $^{N^2}$-(2-(4-methylpiperazin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203251).

A solution of $N$-(2-(4-methylpiperazin-1-yl)ethyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202251) (7.60 mmol, 2.32 g) in ethanol (54.4 mL) was placed under argon atmosphere. Then a solution of stannous chloride dihydrate (22.79 mmol, 5.14 g) in concentrated hydrochloric acid (8.70 mL) was added drop wise with
constant magnetic stirring. The reaction mixture was then heated to 55 °C for three hours. The reaction was cooled to 0 °C, and the pH was adjusted to 10 by addition of 1N sodium hydroxide. The resulting heterogeneous mixture was filtered, and the filtrand was washed with ethanol (50 mL). The filtrate was concentrated, and silica gel column chromatography of the resulting brown solid (7:1 dichloromethane/methanol) provided pure **KR-203251** as a brown oil in a 53% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.2-3.0% acetonitrile in water. **KR-203251** is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

**KR-203251**: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.40 (d, 1H, J = 2.0 Hz), 7.33 (d, 1H, J = 8.0 Hz), 7.14 (dd, 1H, J = 8.0 Hz, J' = 2.0 Hz), 6.45 (br s, 3H), 3.66 (t, 2H, J = 6.0 Hz), 3.47 (br s, 8H), 3.33 (t, 2H, J = 6.0 Hz), 2.84 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 163.5, 148.7, 143.1, 125.3, 119.4, 116.5, 105.1, 54.9, 50.3, 48.9, 42.5, 38.1; HRMS mass calculated for C$_{14}$H$_{22}$N$_5$O (M+H)$^+$ 276.1819, found 276.1817; Delta -0.7 ppm.

**KR-203251FB**: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.32 (br s, 1H, NH), 6.89 (d, 1H, J = 8.0 Hz), 6.56 (s, 1H), 6.36 (d, 1H, J = 8.0 Hz), 4.79 (br s, 2H, NH$_2$), 3.31 (t, 2H, J = 6.0 Hz), 2.46 (t, 2H, J = 6.4 Hz), 2.38 (br s, 4H), 2.27 (br s, 4H), 2.10 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 161.2, 149.6, 144.0, 134.0, 116.0, 110.6, 95.9, 57.5, 55.4, 53.4, 46.4, 40.6.
**N-(3-(4-methylpiperazin-1-yl)propyl)-6-Nitrobenzo[d]oxazol-2-amine (KR-202351)**

![KR-202351](image)

**Figure 2.34:** \(N-(3-(4-methylpiperazin-1-yl)propyl)-6-nitrobenzo[d]oxazol-2-amine \) (KR-202351).

A solution of 6-nitrobenzo[d]oxazole-2-thiol (KR-200) (10.40 mmol, 2.04 g) in dichloromethane (347 mL) and oxalyl chloride (13.00 mmol, 1.12 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (10 mL) was added drop wise over a period of 30 minutes. **CAUTION:** The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After three hours, the reaction was again cooled to 0 °C, and triethylamine (31.20 mmol, 4.35 mL) was added drop wise over a period of 25 minutes. Then 3-(1-methylpiperidin-4-yl)propan-1-amine (KR-351) (11.44 mmol, 1.79 g) was added, and the ice bath was removed. The reaction was stirred for fourteen hours at room temperature then concentrated *in vacuo* yielding an orange solid. Silica gel column chromatography of the solid (15:1 dichloromethane/methanol) provided pure KR-202351 as a yellow powder in an 85% yield.
KR-202351: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.18 (d, 1H, $J = 2.0$ Hz), 8.07 (dd, 1H, $J = 8.4$ Hz, $J' = 2.0$ Hz), 7.30 (d, 1H, $J = 8.4$ Hz), 3.35 (t, 2H, $J = 6.0$ Hz), 2.31 (t, 2H, $J = 7.6$ Hz), 2.27 (br s, 8H), 2.10 (s, 3H), 1.71 (p, 2H, $J = 7.6$ Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 166.3, 151.6, 148.0, 140.7, 121.9, 114.6, 105.1, 55.8, 55.4, 53.4, 46.4, 41.8, 26.7; HRMS mass calculated for C$_{15}$H$_{22}$N$_5$O$_3$ (M+H)$^+$ 320.1717, found 320.1716; Delta -0.3 ppm.

$N^2$-(3-(4-methylpiperazin-1-yl)propyl)Benzo[d]oxazole-2,6-diamine (KR-203351)

Figure 2.35: $N^2$-(3-(4-methylpiperazin-1-yl)propyl)benzo[d]oxazole-2,6-diamine (KR-203351).

A solution of $N$-(3-(4-methylpiperazin-1-yl)propyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202351) (8.83 mmol, 2.82 g) in ethanol (63.3 mL) was placed under argon atmosphere. Then a solution of stannous chloride dihydrate (26.49 mmol, 5.98 g) in concentrated hydrochloric acid (10.11 mL) was added drop wise with constant magnetic stirring. The reaction mixture was then heated to 52 $^\circ$C for three hours. The reaction was cooled to 0 $^\circ$C, and the pH was adjusted to 10 by addition of 1N sodium hydroxide. The resulting heterogeneous mixture was filtered, and the filtrand was washed with ethanol (50 mL). The filtrate was
concentrated, and silica gel column chromatography of the resulting brown solid (10:1 dichloromethane/methanol) provided pure KR-203351 as a red oil in a 63% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.5-35% acetonitrile in water. KR-203351 is unstable in the presence of air and appropriate measures should be taken to minimize air exposure.

KR-203351: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.24 (br s, 3H), 7.42 (s, 1H, J = 8.4 Hz), 7.32 (d, 1H, J = 8.0 Hz), 7.16 (d, 1H, J = 8.0 Hz), 3.56 (br s, 8H), 3.41 (t, 2H, J = 6.0 Hz), 3.27 (t, 2H, J = 7.6 Hz), 2.86 (s, 3H), 2.01 (p, 2H, J = 7.2 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 163.4, 148.4, 142.6, 125.4, 119.3, 115.9, 104.9, 53.8, 50.0, 48.5, 42.4, 40.1, 24.0; HRMS mass calculated for C$_{15}$H$_{24}$N$_5$O (M+H)$^+$ 290.1975, found 290.1981; Delta 2.0 ppm.

6-Chlorobenzo[d]oxazole-2(3H)-thione (KR-200Cl)

Figure 2.36: 6-chlorobenzo[d]oxazole-2(3H)-thione (KR-200Cl).

Potassium hydroxide (44.51 mol, 2.50 g) was added to a solution of 2-amino-5-chlorophenol (40.47 mmol, 5.81 g) in carbon disulfide (80.94 mmol, 4.89 mL), ethanol (34.0 mL) and water (6.7 mL) with constant magnetic stirring. The
reaction was placed under argon atmosphere and refluxed for 72 hours. The reaction mixture was then cooled to room temperature and filtered. The filtrand was then rinsed with ice cold ethanol (10 mL), transferred to a separatory funnel, acidified with 1N hydrochloric acid (150 mL), and extracted with ethyl acetate (6 x 160 mL). The organic layer was then dried over sodium sulfate and concentrated under reduced pressure yielding an orange solid. Silica gel column chromatography of the solid (2:1 hexanes/chloroform) provided pure \textbf{KR-200Cl} as a white crystalline solid in an 80\% yield.

\textbf{KR-200Cl}: $^1$H NMR (400 MHz, DMSO-\textit{d}_6) $\delta$ 7.64 (d, 1H, $J = 1.6$ Hz), 7.27 (dd, 1H, $J = 8.4$ Hz, $J' = 1.2$ Hz), 7.17 (d, 1H, $J = 8.4$ Hz), 3.42 (br s, 1H, SH); $^{13}$C NMR (400 MHz, DMSO-\textit{d}_6) $\delta$ 181.0, 149.1, 131.0, 128.7, 125.8, 111.9, 111.2; HRMS mass calculated for C$_7$H$_3$NOClS (M+H)$^+$ 183.9629, found 183.9631; Delta 1.1 ppm; Crystal structure available.
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$N^{1}$-(6-chlorobenzo[d]oxazol-2-yl)-$N^{3},N^{3}$-dimethylpropane-1,3-diamine (KR-202Cl)

![KR-202Cl](image)

**Figure 2.37:** $N^{1}$-(6-chlorobenzo[d]oxazol-2-yl)-$N^{3},N^{3}$-dimethylpropane-1,3-diamine (KR-202Cl).

A solution of 6-chlorobenzo[d]oxazole-2-thiol (KR-200Cl) (16.16 mmol, 3.00 g) in dichloromethane (400 mL) and oxalyl chloride (24.24 mmol, 2.08 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (10 mL) was added drop wise over a period of 30 minutes. **CAUTION:** The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas. The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After three hours, the reaction was again cooled to 0 °C, and triethylamine (48.48 mmol, 6.76 mL) was added drop wise over a period of 25 minutes. Then $N^{1},N^{1}$-dimethylpropane-1,3-diamine (17.78 mmol, 2.24 mL) was added, and the ice bath was removed. The reaction was stirred for sixteen hours at room temperature then concentrated *in vacuo* yielding a brown solid. Silica gel column chromatography of the solid (15:1 dichloromethane/methanol) provided pure KR-202 as a faint yellow oil in a 74% yield.
KR-202Cl: $^1$H NMR (400 MHz, Methanol-$d_4$) $\delta$ 7.28 (d, 1H, $J = 2.0$ Hz), 7.18 (d, 1H, $J = 8.4$ Hz), 7.11 (dd, 1H, $J = 8.4$ Hz, $J' = 1.6$ Hz), 3.48 (t, 2H, $J = 6.8$ Hz), 3.13 (m, 2H), 2.81 (s, 6H), 2.07 (p, 2H, $J = 7.6$ Hz); $^{13}$C NMR (400 MHz, Methanol-$d_4$) $\delta$ 163.4, 148.7, 141.4, 125.9, 124.1, 115.8, 109.4, 55.5, 42.6, 39.7, 25.0; HRMS mass calculated for $C_{12}H_{17}ClN_3O$ (M+H)$^+$ 254.1055, found 254.1052; Delta -0.8 ppm.

2-Amino-5-fluorophenol (KR-200F)

![Structure](attachment:image.png)

**Figure 2.38:** 2-amino-5-fluorophenol (KR-200F).

To a of solution of 5-fluoro-2-nitrophenol (6.37 mmol, 1.00 g) in methanol (100 mL), 10% palladium on activated carbon (10 wt% of 5-fluoro-2-nitrophenol, 0.10 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for three hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered over a thin pad of celite which was then washed with methanol (70 mL). The filtrate was concentrated in vacuo yielding a brown solid. Silica gel column chromatography (10:1 hexanes/chloroform) provided a crystalline orange solid in an 82% yield. **KR-200F** is unstable in the
presence of air and appropriate measures should be taken to minimize air exposure.

**KR-200F**: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 6.52 (dd, 1H, $J = 8.8$, $J' = 6.4$ Hz), 6.46 (dd, 1H, $J = 7.2$ Hz, $J' = 2.8$ Hz), 6.34 (dt, 1H, $J = 8.8$ Hz, $J' = 2.8$ Hz), 4.40 (br s, 1H, OH), 3.41 (br s, 2H, NH$_2$); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 156.1, 153.8, 145.4, 145.3, 133.7, 133.6, 114.7, 114.6, 105.5, 105.3, 102.7, 102.4; HRMS mass calculated for C$_6$H$_7$FNO (M+H)$^+$ 128.0506, found 128.0505; Delta - 0.8 ppm.

**6-Fluorobenzo[d]oxazole-2-thiol (KR-201F)**

![Figure 2.39: 6-fluorobenzo[d]oxazole-2-thiol (KR-201F).](image)

Potassium hydroxide (23.84 mol, 1.34 g) was added to a solution of 2-amino-5-fluorophenol (**KR-200F**) (15.89 mmol, 2.02 g) in carbon disulfide (31.78 mmol, 1.92 mL), ethanol (13.2 mL) and water (2.65 mL) with constant magnetic stirring. The reaction was placed under argon atmosphere and refluxed for 72 hours. The reaction mixture was then cooled to room temperature and filtered. The filtrand is then rinsed with ice cold ethanol (10 mL), transferred to a separatory funnel, acidified with 1N hydrochloric acid (65 mL), and extracted with ethyl acetate (6 x 75 mL). The organic layer was then dried over sodium sulfate.
and concentrated under reduced pressure yielding a brown solid. Silica gel column chromatography of the solid (5:1 hexanes/ethyl acetate) provided pure KR-201F as a faint orange crystalline solid in an 86% yield.

**KR-201F:** \(^{1}\text{H NMR} (400 \text{ MHz, DMSO-}\text{d}_6)\) \(\delta 7.51 \text{ (ddd, 1H, J = 8.4 Hz, J' = 2.8 Hz, J'' = 0.8 Hz)}, 7.20 \text{ (dd, 1H, J = 8.4 Hz, J' = 4.8 Hz)}, 7.12 \text{ (m, 1H)}, 3.37 \text{ (br s, 1H)};\)

\(^{13}\text{C NMR} (400 \text{ MHz, DMSO-}\text{d}_6)\) \(\delta 181.2, 160.8, 158.4, 149.0, 148.8, 128.7, 112.8, 112.6, 111.6, 111.5, 99.8, 99.5; \) HRMS mass calculated for C\(_7\)H\(_3\)FNOS (M+H)\(^{-}\) 167.9925, found 167.9926; Delta 0.6 ppm.

**N\(^1\)-(6-fluorobenzo[d]oxazol-2-yl)-N\(^3\),N\(^3\)-Dimethylpropane-1,3-diamine (KR-202F)**

\[\text{KR-202F}\]

**Figure 2.40:** \(N^1\)-(6-fluorobenzo[d]oxazol-2-yl)-N\(^3\),N\(^3\)-dimethylpropane-1,3-diamine (KR-202F).

A solution of 6-fluorobenzo[d]oxazole-2-thiol (KR-200F) (5.91 mmol, 1.00 g) in dichloromethane (236 mL) and oxalyl chloride (7.39 mmol, 0.63 mL) was placed under argon atmosphere and cooled to 0 °C. Dimethylformamide (7 mL) was added drop wise over a period of 20 minutes. CAUTION: The addition of dimethylformamide should be conducted slowly to avoid rapid evolution of gas.
The reaction was stirred for 30 minutes at 0 °C, then the ice bath was removed and the reaction was allowed to warm to room temperature. After two hours, the reaction was again cooled to 0 °C, and triethylamine (17.73 mmol, 2.47 mL) was added drop wise over a period of 15 minutes. Then \( N^1,N^1 \)-dimethylpropane-1,3-diamine (6.50 mmol, 0.82 mL) was added, and the ice bath was removed. The reaction was stirred for eighteen hours at room temperature then concentrated in vacuo yielding a red solid. Silica gel column chromatography of the solid (15:1 dichloromethane/methanol) provided pure **KR-202F** as a faint brown oil in a 70% yield.

**KR-202F**: \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 8.18 (br s, 1H, NH), 7.33 (dd, 1H, \(J = 8.4\) Hz, \(J' = 2.0\) Hz), 7.19 (ddd, 1H, \(J = 8.4\) Hz, \(J' = 7.2\) Hz, \(J'' = 2.0\) Hz), 6.94 (m, 1H), 3.34 (q, 2H, \(J = 6.4\) Hz), 3.07 (t, 2H, \(J = 8.0\) Hz), 2.69 (s, 6H), 1.97 (p, 2H, \(J = 8.0\) Hz); \(^{13}\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 163.4, 158.8, 156.5, 148.6, 148.4, 140.2, 140.1, 115.9, 115.8, 110.9, 110.7, 98.4, 98.2, 54.9, 42.7, 40.3, 24.5; HRMS mass calculated for \(C_{12}H_{17}FN_3O\) (M+H)\(^+\) 238.1350, found 238.1353; Delta 1.3 ppm.

**General Techniques**

Commercial reagents were obtained (Sigma Aldrich, Acros, Alfa Aesar, Fisher) and used without any further purification. Non-aqueous reactions were carried out under anhydrous conditions using oven-dried glassware under an inert atmosphere in dry, freshly distilled solvents. All air-sensitive reaction
solutions were transferred via syringe or stainless steel cannula. Reactions were monitored by TLC, which was performed on pre-coated silica gel 60 F254 plates supplied by EMD, visualized utilizing UV light, and developed using ceric ammonium molybdate stain (CAM) with heat or ninhydrin stain with heat. Organic solvents were removed by rotary evaporation below 30 °C at approximately 15 mmHg. Flash column chromatography was executed with silica gel 60 (230-400 mesh) supplied by Silicycle and eluting solvents are indicated in the text. Yields refer to chromatographically and spectroscopically (\(^1\)H NMR, \(^13\)C NMR) homogeneous materials, unless otherwise stated. NMR spectra were recorded on Varian Mercury 400 MHz, Jeol Unity 500 MHz, and Agilent VNMR S 500 MHz outfitted with an XSens cold probe using deuterochloroform, deuteromethanol, deuterowater or deuterodimethyl sulfoxide (Cambridge Isotope) as solvents. The chemical shifts are given in ppm relative to the standard reference TMS or residual undeuterated solvent. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublet, dt = doublet of triplet. Low resolution mass spectra were obtained on a ThermoFinnigan LCQDECA-MS spectrometer. High resolution mass spectra (HRMS) were recorded on a VG 7070 HS or a VG ZAB-ZSE mass spectrometer. Crystals were mounted in nylon loops using Paratone oil and then placed on the diffractometer under a nitrogen stream at 100K. All crystallographic data has been deposited at the CCDC, 12 Union Road, Cambridge CB21EZ, UK.
Spectral Data

Nuclear Magnetic Resonance

**Spectrum 2.1**: 6-nitrobenzo[d]oxazol-2-amine (KR-100) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
Spectrum 2.2: 6-nitrobenzo[d]oxazol-2-amine (KR-100) $^{13}\text{C}$ NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.3: benzo[d]oxazole-2,6-diamine (KR-101) $^1$H NMR (400 MHz, MeOD-$d_4$).
Spectrum 2.4: benzo[d]oxazole-2,6-diamine (KR-101) $^{13}$C NMR (400 MHz, MeOD-$d_4$).
Spectrum 2.5: 6-nitrobenzo[d]oxazole-2-thiol (KR-200) $^1$H NMR (400 MHz, DMSO-\textit{d$_6$}).
Spectrum 2.6: 6-nitrobenzo[d]oxazole-2-thiol (KR-200) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.7: $N^1,N^1$-dimethyl-$N^2$-(6-nitrobenzo[d]oxazol-2-yl)ethane-1,2-diamine (KR-202255) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.8: $N^1,N^1$-dimethyl-$N^2$-(6-nitrobenzo[d]oxazol-2-yl)ethane-1,2-diamine (KR-202255) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.9: $N^2$-(2-(dimethylamino)ethyl)benzo[d]oxazole-2,6-diamine (KR-203255FB) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.10: $N^2$-(2-(dimethylamino)ethyl)benzo[d]oxazole-2,6-diamine (KR-203255FB) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.11: $N^1,N^1$-dimethyl-$N^3$-(6-nitrobenzo[d]oxazol-2-yl)propane-1,3-diamine (KR-202) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.12: \( N^1,N^1\)-dimethyl-\( N^3\)-(6-nitrobenzo[d]oxazol-2-yl)propane-1,3-diamine (KR-202) \(^{13}\)C NMR (400 MHz, DMSO-\(d_6\)).
Spectrum 2.13: $N^2$-(3-(dimethylamino)propyl)benzo[d]oxazole-2,6-diamine (KR-203) $^1\text{H NMR}$ (400 MHz, CDCl$_3$).
Spectrum 2.14: \( N^2-(3\text{-}(\text{dimethylamino})\text{propyl})\text{benzo[}\text{d}\\text{oxazole-2,6-diamine (KR-203)}\) \(^{13}\text{C NMR (400 MHz, CDCl}_3\text{).}
Spectrum 2.15: 6-nitro-N-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazol-2-amine (KR-202253) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.16: 6-nitro-N-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazol-2-amine (KR-202253) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 2.17:** $N^2$-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203253) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.18: $N^2$-(2-(pyrrolidin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203253) $^{13}$C NMR (400 MHz, DMSO-$d_6$).

KR-203253
**Spectrum 2.19**: 6-nitro-N-(3-(pyrrolidin-1-yl)propyl)benzo[d]oxazol-2-amine (KR-202353) \( ^1 \)H NMR (400 MHz, DMSO-\( d_6 \)).
Spectrum 2.20: 6-nitro-\textit{N}-(3-(pyrrolidin-1-yl)propyl)benzo[\textit{d}]oxazol-2-amine (KR-202353) $^{13}$C NMR (400 MHz, DMSO-\textit{d$_6$}).
**Spectrum 2.21:** $N^2$-(3-(pyrrolidin-1-yl)propyl)benzo[d]oxazole-2,6-diamine (KR-203353) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.22: $N^2$-(3-(pyrrolidin-1-yl)propyl)benzo[d]oxazole-2,6-diamine (KR-203353) $^{13}$C NMR (400 MHz, DMSO-$d_6$).

KR-203353
**Spectrum 2.23:** $N^2$-(3-(pyrrolidin-1-yl)propyl)benzo[d]oxazole-2,6-diamine (KR-203353FB) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.24: $N^2$-(3-(pyrrolidin-1-yl)propyl)benzo[d]oxazole-2,6-diamine (KR-203353FB) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.25: $N^2$-(2-(piperidin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203249) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.26: $N^2$-(2-(piperidin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203249) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 2.27**: 6-nitro-N-(3-(piperidin-1-yl)propyl)benzo[d]oxazol-2-amine (KR-202349) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.28: 6-nitro-N-(3-(piperidin-1-yl)propyl)benzo[d]oxazol-2-amine (KR-202349) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 2.29:** \(N^2-(3-(\text{piperidin-1-yl})\text{propyl})\text{benzo}[d]\text{oxazole-2,6-diamine} \) (KR-203349) \(^1\text{H NMR (400 MHz, DMSO-}\delta\)).
Spectrum 2.30: \( N^2-(3\text{-}(\text{piperidin}-1\text{-yl})\text{propyl})\text{benzo}[d]\text{oxazole-2,6-diamine (KR-203349)\text{ \(^{13}\)C NMR (400 MHz, DMSO-}d_6).} \)
Spectrum 2.31: \(N\)-(2-morpholinoethyl)-6-nitrobenzo[\(d\)]oxazol-2-amine (KR-202247) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
Spectrum 2.32: \( \text{N-(2-morpholinoethyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202247)} \) \(^{13}\text{C NMR (400 MHz, DMSO-d}_6\).
Spectrum 2.33: \( N^2-(2\text{-morpholinoethyl})\text{benzo}[d]\text{oxazole-2,6-diamine} \) \((\text{KR-203247FB})\) \(^1\text{H} \text{NMR} \) (400 MHz, DMSO-\(d_6\)).
Spectrum 2.34: $N^2$-(2-morpholinoethyl)benzo[d]oxazole-2,6-diamine (KR-203247FB) $^{13}$C NMR (400 MHz, DMSO-$d_6$).

KR-203247FB
Spectrum 2.35: \( N-(3\text{-morpholinopropyl})-6\text{-nitrobenzo}[d]\text{oxazol}-2\text{-amine} \) (KR-202347) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
**Spectrum 2.36:** \( N-(3\text{-morpholinopropyl})\text{-6-nitrobenzo}[d]\text{oxazol-2-amine (KR-202347)} \) \(^{13}\text{C NMR (400 MHz, DMSO-}d_6)\).
Spectrum 2.37: $N^2$-(3-morpholinopropyl)benzo[d]oxazole-2,6-diamine (KR-203347FB) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.38: \( N^2-(3\text{-morpholinopropyl})\text{benzo}[d]\text{oxazole-2,6-diamine} \) (KR-203347FB) \(^{13}\text{C} \) NMR (400 MHz, DMSO-\( d_6 \)).
Spectrum 2.39: \( N-(2-(4\text{-methylpiperazin-1-yl})\text{ethyl})\text{-6-nitrobenzo[d]oxazol-2-amine (KR-202251)} \) \(^1\text{H NMR (400 MHz, DMSO-}d_6\)).
Spectrum 2.40: \( N-(2-(4\text{-methylpiperazin-1-yl})\text{ethyl}-6\text{-nitrobenzo[d]oxazol-2-amine (KR-202251)}} \) \( ^{13} \text{C NMR (400 MHz, DMSO-}d_6\).}
**Spectrum 2.41:** $N^2$-(2-(4-methylpiperazin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203251) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.42: \(N^2-(2-(4\text{-methylpiperazin}-1\text{-yl})\text{ethyl})\text{benzo[d]oxazole-2,6-diamine (KR-203251)}\) \(^{13}\text{C NMR (400 MHz, DMSO-}\text{d}_6\text{).}
Spectrum 2.43: $N^2$-(2-(4-methylpiperazin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203251FB) $^1$H NMR (400 MHz, DMSO-$d_6$).
**Spectrum 2.44:** $N^2$-(2-(4-methylpiperazin-1-yl)ethyl)benzo[d]oxazole-2,6-diamine (KR-203251FB) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.45: \(N\)-(3-(4-methylpiperazin-1-yl)propyl)-6-nitrobenzo[d]oxazol-2-amine (KR-202351) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
**Spectrum 2.46:** \( N-(3-(4\text{-methylpiperazin-1-yl})\text{propyl})-6\text{-nitrobenzo}[d]\text{oxazol-2-amine (KR-202351)} \) \(^{13}\text{C NMR (400 MHz, DMSO-}\text{d}_6)\).
Spectrum 2.47: \( N^2-(3-(4\text{-}methylpiperazin\text{-}1\text{-}yl})\text{propyl})\text{benzo}[d]\text{oxazole\text{-}2,6\text{-}diamine (KR\text{-}203351) }^1\text{H NMR (400 MHz, DMSO\text{-}d}_6\).
Spectrum 2.48: $N^2$-(3-(4-methylpiperazin-1-yl)propyl)benzo[d]oxazole-2,6-diamine (KR-203351) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 2.49:** 6-chlorobenzo[d]oxazole-2(3H)-thione (KR-200Cl) $^1$H NMR (400 MHz, DMSO-$d_6$).

![Spectrum Image]
Spectrum 2.50: 6-chlorobenzo[d]oxazole-2(3H)-thione (KR-200Cl) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 2.51:** $N^1$-(6-chlorobenzo[d]oxazol-2-yl)-$N^2,N^3$-dimethylpropane-1,3-diamine (KR-202Cl) $^1$H NMR (400 MHz, CD$_3$OD).
Spectrum 2.52: N¹-(6-chlorobenzo[d]oxazol-2-yl)-N²,N²-dimethylpropane-1,3-diamine (KR-202Cl) $^{13}$C NMR (400 MHz, CD$_3$OD).
Spectrum 2.53: 2-amino-5-fluorophenol (KR-200F) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.54: 2-amino-5-fluorophenol (KR-200F) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.55: 6-fluorobenzo[d]oxazole-2-thiol (KR-201F) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.56: 6-fluorobenzo[d]oxazole-2-thiol (KR-201F) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.57: $N^1$-(6-fluorobenzo[d]oxazol-2-yl)-$N^3,N^3$-dimethylpropane-1,3-diamine (KR-202F) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 2.58: $N^1$-(6-fluorobenzo[d]oxazol-2-yl)-$N^2,N^2$-dimethylpropane-1,3-diamine (KR-202F) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Crystal Structures

**Spectrum 2.59:** Crystal structure of benzo[d]oxazole-2,6-diamine (KR-101).

**Spectrum 2.60:** Hydrogen bonding pattern observed in crystal structure of benzo[d]oxazole-2,6-diamine (KR-101).
**Spectrum 2.61:** Crystal packing observed in crystal structure of benzo[d]oxazole-2,6-diamine (KR-101).

**Spectrum 2.62:** Alternative view of crystal packing observed in crystal structure of benzo[d]oxazole-2,6-diamine (KR-101).

Spectrum 2.64: Hydrogen bonding pattern observed in crystal structure of 6-chlorobenzo[d]oxazole-2(3H)-thione (KR-200Cl).
Spectrum 2.65: Crystal packing observed in crystal structure of (6-chlorobenzo[d]oxazole-2(3H)-thione (KR-200Cl)).

Spectrum 2.66: Alternative view of crystal packing observed in crystal structure of 6-chlorobenzo[d]oxazole-2(3H)-thione (KR-200Cl)).
References


Acknowledgements

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Chapter 3

6-Substituted Aminobenzoxazoles Targeting the HCV IRES
Introduction

The internal ribosomal entry site (IRES) is a highly structured and highly conserved regulatory element within the hepatitis C virus (HCV) responsible for initiating translation in a cap-independent fashion.\cite{1-7} Subdomain Ila of the HCV IRES has been shown to be critical to proper IRES function. Small molecule ligands which bind to subdomain Ila have been previously identified that act as viral translation inhibitors by interfering with conformation adopted by the dynamic RNA.\cite{8-11} The preparation of novel ligands which are capable of interacting with this flexible region of RNA could lead to the development of viral translation inhibitors.

Design of Ligands for Targeting IRES Subdomain Ila

Small molecule ligand design will be based on previously discovered compounds which interact with RNA and modulate function. Ligand recognition of the RNA is provided by the dense arrangement of hydrogen bond donors and acceptors around a rigid planar scaffold. Small molecules which present edges of hydrogen bond donors and acceptors allow for the recognition of unpaired nucleobases within the dynamic folds of RNA while the planarity permits access to spaces between the bases. The presence of basic motifs within the ligand foster additional non-specific electrostatic interactions with the anionic RNA. The
assembly of small molecule ligands exemplifying this paradigm should demonstrate an increased affinity for RNA targets.

Inspired by benzimidazole 1, a set of benzoxazoles was designed with features biased toward interaction with RNA to explore the intrinsic flexibility of subdomain IIa of the HCV IRES. Expanding upon the success of the 2-position substituted aminobenzoxazoles, a series of 6-position substituted aminobenzoxazoles was designed aimed at facilitating new interactions with subdomain IIa in order to enhance target affinity (Figure 3.1). The co-crystal structure of translation inhibitor 1 and subdomain IIa shows the chroman ring adjacent to the more dynamic RNA strand which is responsible for the formation of a deep binding cavity (Figure 2.6). Ligands which interact with this adaptable region of RNA could lead to the discovery of additional binding modes which also inhibit viral translation. The 6-substituted series was constructed around a core aminobenzoxazoles designed to behave as a recognition element which anchors the ligand to subdomain IIa in the same fashion as inhibitor 1 ensuring proper orientation of the various substituents toward the RNA region of interest. The preparation of aminobenzoxazoles substituted at the 6-position will maximize the potential of interacting with the highly dynamic segment of subdomain IIa RNA responsible for the binding pocket.
Figure 3.1: Comparison of the known HCV translation inhibitor 1 and novel benzoxazole inhibitor 2 with designed 6-substituted benzoxazole scaffold 3. The retained core scaffold is highlighted in red. The various head groups are listed below.

The synthetically demanding chroman scaffold (Figure 3.1, ring A) is not amenable to facile derivatization. As a result, the chroman ring was replaced by various amines directly attached to the 6-position of the aminobenzoxazole core allowing straightforward access to substituted ligands of various composition with reduced synthetic complexity. By directing substituents of various sizes and composition toward the capricious RNA, the flexibility of subdomain IIa will be examined. The substituents will be comprised of moieties capable of forming additional hydrogen bonding, electrostatic and stacking interactions intended to enhance both specific and non-specific affinity for subdomain IIa while allowing insight into the overall character of this region of RNA. Furthermore, this modular approach will facilitate the efficient synthesis of diverse ligands allowing the
creation of a structure activity relationship (SAR) which will shed light on the specific features that enable favorable RNA-ligand interactions.

Complementing the previously synthesized 2-substituted aminobenzoxazoles, the set of 6-substituted aminobenzoxazoles can be regarded as a series of fragments meant to evaluate the binding potential of one side of the RNA fold. The identification of 6-substituted aminobenzoxazoles which bind with modest affinity to subdomain IIa can then be combined with complementary fragments exploring other areas of the RNA binding site resulting in ligands with superior affinity and selectivity for the target. The amalgamation of diverse ligands around a core scaffold has proven to be a valuable tool toward the preparation of selective biologically active agents.

**Retrosynthesis of 6-Substituted Benzoxazole Ligands**

The general synthesis of 6-substitued benzoxazole 3 was envisaged from the cyclization of corresponding 6-substituted ortho-aminophenol 4 (Scheme 3.1). The reduction of the 6-substituted ortho-nitrophenol 5 via hydrogenation would permit straightforward access intermediate 4. The substituted ortho-nitrophenol precursor 5 would be synthesized by aromatic substitution of commercially available 5-fluoro-2-nitrophenol (6).
Synthesis of 6-Substituted Benzoxazole Ligands

Synthesis of the desired 6-substituted benzoazoles began with the aromatic substitution of 5-fluoro-2-nitrophenol (6) with various amines (Scheme 3.2). The activating nitro group within the substrate facilitates the substitution which provided nearly quantitative yields of the desired 6-substituted nitrophenol precursor 5 despite the varying composition of the nucleophiles used.\textsuperscript{[13]} Nitrophenol 5 was then reduced using palladium hydrogenation conditions. The resulting 6-substituted aminophenol 4 was extremely sensitive to oxidation.\textsuperscript{[14]} Since the electron rich 6-substituted aminophenol scaffold readily decomposes when exposed to air, the aminophenol was immediately cyclized using di(imidazole-1-yl)methanimine providing expedient access to the desired 6-substituted aminobenzoxazoles (3).\textsuperscript{[15]}
Scheme 3.2: Synthetic route to the family of 6-substituted benzoxazoles 3.

Reagents and conditions: a) NHR$_2$, ACN, reflux, 80-100%; b) 10% Pd/C, H$_2$, MeOH; c) di(imidazole-1-yl)methanimine, ACN, reflux, 41-74% 2 steps.

The 6-substituted aminobenzoxazoles proved to be difficult to isolate. Initially this was attributed to the polar nature of this family of molecules. However, as observed with the series of 2-substituted aminobenzoxazoles, the 6-substituted aminobenzoxazoles are also unstable when exposed to air for prolonged periods of time. The ligands which contained tertiary amines at the 6-position were considerably more stable than the ligands which possessed secondary amines at the same position. This observation is likely the result of the additional alkyl group stabilizing the electron rich scaffold. The addition of electron withdrawing halogens at the 6-position result in ligands with no appreciable sensitivity to air suggesting that electronic factors play a significant role in the oxidative sensitivity of these ligands. Additionally, the incorporation of electron withdrawing groups could be used as a strategy to attenuate the air sensitivity of these ligands.
Evaluation of Subdomain IIa Binding of 6-substituted Aminobenzoxazoles

The affinity of the synthesized 6-substituted aminobenzoxazoles for subdomain IIa of the HCV IRES was evaluated by the FRET assay.[8,16] The FRET measurements investigate the effect of ligand binding on the interhelical angle adopted by subdomain IIa (see Chapter 2). Ligands 7, 8, 9 and 10 reduced the FRET signal (Table 3.1) indicating a binding event which captures the inactive elongated form of subdomain IIa (Figure 2.5). All of the active ligands in the FRET assay contain a secondary amine at the 6-position of the aminobenzoxazole indicating that steric bulk is not tolerated within the binding cavity. Furthermore, the proton of the secondary amine could be engaging in a favorable hydrogen bonding interaction with the RNA, fostering increased target affinity. The most potent interactions with subdomain IIa were observed for aminobenzoxazoles 7 and 10 with EC$_{50}$ values of 25 μM and 27 μM respectively. The similar affinity of ligand 7 and 10 belies the unique nature of the attached substituents. The two-fold improvement in target affinity over simplified ligand 8 suggests that the dimethyl amino group of ligand 7 and the benzyl group of ligand 10 play a role in stabilizing the functionally impaired linear form of subdomain IIa. The increased affinity of benzoxazole 7 stems from electrostatic interactions facilitated by the basic dimethyl amino side chain, whereas benzoxazole 10 likely forms additional stacking interactions which lead to stronger binding.
Table 3.1: Ligand affinity measured by FRET and IVT assays.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID (Notebook ID)</th>
<th>FRET (EC$_{50}$)</th>
<th>IVT (% inhibition at 100 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure 7" /></td>
<td>7 (Kr-102303)</td>
<td>25 µM</td>
<td>11±2%</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure 8" /></td>
<td>8 (Kr-102001)</td>
<td>80 µM</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure 9" /></td>
<td>9 (Kr-102061)</td>
<td>90 µM</td>
<td>49±5%</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure 10" /></td>
<td>10 (Kr-102059)</td>
<td>27 µM</td>
<td>41±9%</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure 11" /></td>
<td>11 (Kr-102053)</td>
<td>n.a.</td>
<td>55±8%</td>
</tr>
<tr>
<td><img src="image6.png" alt="Structure 12" /></td>
<td>12 (Kr-102047)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image7.png" alt="Structure 13" /></td>
<td>13 (Kr-102003)</td>
<td>n.a.</td>
<td>39±8%</td>
</tr>
<tr>
<td><img src="image8.png" alt="Structure 14" /></td>
<td>14 (Kr-101F)</td>
<td>n.a.</td>
<td>41±14%</td>
</tr>
<tr>
<td><img src="image9.png" alt="Structure 15" /></td>
<td>15 (Kr-203303)</td>
<td>63 µM</td>
<td>24±3%</td>
</tr>
<tr>
<td><img src="image10.png" alt="Structure 16" /></td>
<td>16 (Kr-101)</td>
<td>n.a.</td>
<td>31±6%</td>
</tr>
<tr>
<td><img src="image11.png" alt="Structure 17" /></td>
<td>17 (Kr-101Cl)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image12.png" alt="Structure 18" /></td>
<td>18 (Kr-102049)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>
Table 3.1, Continued: Ligand affinity measured by FRET and IVT assays.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID (Notebook ID)</th>
<th>FRET (EC$_{50}$)</th>
<th>IVT (% inhibition at 100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure" /></td>
<td><strong>19</strong> <em>(Kr-102051)</em></td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

EC$_{50}$ is the concentration required for a fifty percent reduction in the observed FRET signal. IVT measurements were made in the presence of 100 μM of the ligand under study. Percent inhibition is normalized to the relative signal of the luciferase reporter in the absence of ligand. n.a. = no activity.

Many of the other ligands from this series reduced FRET signal only at high concentrations indicating a very weak affinity for the target. However, the reduction in FRET observed with these weak binding ligands could be the result of non-specific binding to subdomain IIa resulting in precipitation of the RNA. Based on the FRET data, aminobenzoxazoles with bulky tertiary amines at the 6-position are not capable of binding with subdomain IIa of the HCV IRES. Despite the observed intrinsic flexibility of subdomain IIa, the large substituents likely destabilize RNA-RNA interactions across the binding pocket, disfavoring the capture of the elongated subdomain IIa. Likewise, a halogen at the 6-position resulted in ligands that lack affinity for subdomain IIa according to the FRET assay. However, the smaller halogenated ligands appear to lack the specific interactions which enable the stabilization of the binding pocket formed by the extended RNA.
The 6-substituted aminobenzoxazoles were also evaluated in the IVT assay which allows a general assessment of the selectivity of the ligand. The IVT assay uses a bicistronic reporter system which assesses the ability of the ligand to discriminate between cap-driven translation and IRES-driven translation by measuring the levels of fluorescence from downstream reporters (see Chapter 2). Ligands 7, 9, and 10 (Figure 3.5) showed weak to modest inhibition of IRES-driven translation at 100 \( \mu \text{M} \) (Table 3.1). The affinity of ligands 7, 9 and 10 for subdomain IIa was confirmed by the FRET assay indicating that these compounds are active inhibitors of translation by interacting with subdomain IIa of the IRES. The reduction of IRES-driven translation in the IVT assay for compounds 9 and 10 are commensurate with the affinity assessed by the FRET assay, indicating that these ligands are selective for IRES inhibition via interaction with subdomain IIa. However, benzoxazole 7 displays promiscuity. Inspection of the levels of inhibition between the two assays shows that ligand 7 is considerably more active in the FRET assay which lacks a source of competitor RNA. The exposure of ligand 7 to foreign RNA in the IVT assay
sequesters the ligand through non-specific interactions away from its target resulting in the observed meager reduction of IRES-driven translation.

Ligands which exhibit activity in the IVT assay, but not in the FRET assay, are not acting upon subdomain IIa of the IRES. Aminobenozaxoles 11, 13 and 14 are modest translation inhibitors in the IVT assay, but showed no interaction with the subdomain IIa in the FRET assay (Table 3.1). Since these ligands were prepared to be biased toward RNA interaction, aminobenzoxazoles 11, 13 and 14 are likely binding directly with the ribosome and acting as general translation inhibitors. Furthermore, the relatively small size and planarity of these ligands could facilitate non-specific intercalation resulting in the observed suppression of translation. Finally, ligands 11, 13 and 14 could be acting upon the IRES outside of subdomain IIa and inhibiting translation. However, the conclusive determination of the mode of action of these ligands is not possible based on data provided by the IVT assay. The thorough examination of ligand binding of the 6-substituted aminobenzoxazoles using the IVT assay has revealed general translation inhibitors and two novel ligands which selectively inhibit IRES-driven translation through subdomain IIa interaction.
In an effort to develop more potent ligands targeting subdomain IIa of the IRES, the binding data for the 6-sustituted aminobenzoxazole was evaluated in conjunction with the binding data for the 2-aminobenzoxazoles. By amalgamating the structural motifs which confer activity around a common benzoxazole core,
novel ligands can be synthesized with enhanced affinity for subdomain IIa. To evaluate the potential of this synergistic effect, moderately FRET-active 6-sustituted aminobenzoxazole 7 was combined with modestly active 2-substitued aminobenzoxazole 2 giving disubstituted oxazole 15 (Scheme 3.3). Direct access to ligand 15 was provided from aminobenzoxazole 7 by nucleophilic substitution of dimethylaminopropyl chloride. The affinity for subdomain IIa of the novel ligand was then evaluated in both the FRET and IVT assays. Surprisingly, disubstituted oxazole 15 exhibited weaker binding for subdomain IIa than the parental ligand 7. The additional side chain results in unfavorable interactions with the binding pocket suggesting the binding cavity may be too narrow to accommodate the slightly wider benzoxazole 15. Furthermore, this reduced affinity could be rooted in the entropic penalty associated with preorganization of many rotatable bonds of the side chains disfavoring interaction with the RNA.

Scheme 3.3: Synthetic route to 2,6-disubstituted benzoxazole 15 from previously synthesized 6-substituted aminobenzoxazole 7.

Reagents and conditions: a) 3-dimethylaminopropyl chloride, K₂CO₃, ACN, 75 °C, 41%.
Conclusions

The series of 6-substituted aminobenzoxazoles revealed the dynamic region of subdomain IIa to be less flexible than originally proposed. The intrinsic lack of affinity of bulky 6-substituted aminobenzoxazoles for subdomain IIa indicates a lack of space in this region of the binding pocket, as well as, limited flexibility of the RNA to reorganize around the bigger ligands. The large substituents at the 6-position likely destabilize the formation of the binding pocket observed in the elongated form of the RNA by interrupting the formation of hydrogen bonds and base triples across the RNA helix. However, smaller and more flexible secondary amines at the 6-postion readily bind to the extended form of subdomain IIa resulting in compromised translation, illustrating spatial constraints of the binding cavity. Additionally, the proton of the secondary amine may be involved in a weak interaction with the RNA target fostering affinity. Hybrid ligand 15, which was designed to be a potent ligand for subdomain IIa, showed weak affinity for the target further demonstrating the restricted space within the rigid RNA cavity. The series of 6-substituted aminobenzoxazoles identified three novel ligands (7, 9, and 10) which inhibit translation through interaction with subdomain IIa of the IRES.

The synthesized aminobenzoxazoles have revealed many of the fundamental interactions which govern small molecule binding to subdomain IIa of the HCV IRES. However, continued development of benzoxazoles as ligands targeting subdomain IIa requires a re-evaluation of the scaffold in order to
address the poor stability of the ligands. Furthermore, a shift in the pattern of substitution of these ligands is needed to assess the spatial constraints of the binding pocket formed by the RNA.
Materials and Methods

6-Nitrobenzo[d]oxazol-2-amine (KR-100)

![KR-100](image)

**Figure 3.4:** 6-nitrobenzo[d]oxazol-2-amine (KR-100).

Di(imidazole-1-yl)methanimine (12.98 mmol, 1.51 g) was added to a solution of 2-amino-5-nitrophenol (6.49 mmol, 1.00 g) in acetonitrile (40 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for fifteen hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) of the resulting brown solid provided pure **Kr-100** as a yellow solid in an 81% yield. See Chapter 2 for spectra.

**KR-100:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.17 (br s, 2H, NH$_2$), 8.16 (d, 1H, J = 2.4 Hz), 8.04 (dd, 1H, J = 8.8 Hz, J' = 2.4 Hz), 7.26 (d, 1H, J = 8.8 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 167.0, 151.6, 147.8, 140.9, 121.6, 114.7, 105.2; HRMS mass calculated for C$_7$H$_6$N$_3$O$_3$ (M+H)$^+$ 180.0404, found 180.0406; Delta 1.1 ppm.
Benzo[d]oxazole-2,6-diamine (KR-101)

Figure 3.5: benzo[d]oxazole-2,6-diamine (KR-101).

To a of solution of 6-nitrobenzo[d]oxazol-2-amine (KR-100) (6.20 mmol, 1.11 g) in methanol (65 mL), 10% palladium on activated carbon (10 wt% of KR-100, 0.11 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for three hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (20 mL). The filtrate was concentrated in vacuo yielding an off-white crystalline solid in a 96% yield. Benzo[d]oxazole-2,6-diamine (KR-101) is air unstable and appropriate measures should be taken to minimize air exposure. Silica gel column chromatography (1:1 hexanes/ethyl acetate) can be utilized to re-isolate KR-101 from a decomposed mixture. See Chapter 2 for spectra.

KR-101: $^1$H NMR (400 MHz, MeOD-$d_4$) δ 6.98 (d, 1H, J = 8.0 Hz), 6.73 (d, 1H, J = 2.0 Hz), 6.59 (dd, 1H, J = 8.0 Hz, J' = 2.0 Hz), 4.91 (s, 4H, NH$_2$); $^{13}$C NMR (400 MHz, MeOD-$d_4$) δ 162.3, 149.2, 142.2, 134.5, 115.1, 112.1, 97.3; HRMS mass
calculated for C₇H₆N₂OCl (M+H)⁺ 150.0662, found 150.0665; Delta 2.0 ppm; Crystal structure available.

**6-Chlorobenzo[d]oxazol-2-amine (KR-101Cl)**

![KR-101Cl](image)

**Figure 3.6:** 6-chlorobenzo[d]oxazol-2-amine (KR-101Cl).

Di(2-imidazole-1-yl)methanimine (10.49 mmol, 1.69 g) was added to a solution of 2-amino-5-chlorophenol (3.50 mmol, 0.50 g) in acetonitrile (50 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for eight hours. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (3:1 hexanes/ethyl acetate) provided **Kr-101Cl** as a crystalline white solid in an 86% yield.

**KR-101Cl:** $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.55 (br s, 2H, NH$_2$), 7.45 (d, 1H, J = 2.0 Hz), 7.15 (d, 1H, J = 8.8 Hz), 7.09 (dd, 1H, J = 8.8 Hz, J' = 2.0 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 164.0, 148.9, 143.4, 124.4, 124.2, 116.5, 109.8; HRMS mass calculated for C$_7$H$_6$N$_2$OCl (M+H)$^+$ 169.0163, found 169.0162; Delta -0.6 ppm; Crystal structure available.
2-Amino-5-fluorophenol (KR-200F)

![Figure 3.7: 2-amino-5-fluorophenol (KR-200F).](image)

To a solution of 5-fluoro-2-nitrophenol (6.37 mmol, 1.00 g) in methanol (100 mL), 10% palladium on activated carbon (10 wt% of 5-fluoro-2-nitrophenol, 0.10 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for three hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered over a thin pad of celite which was then washed with methanol (70 mL). The filtrate was concentrated *in vacuo* yielding a brown solid. Silica gel column chromatography (10:1 hexanes/chloroform) provided a crystalline orange solid in an 82% yield. **KR-200F** is unstable in the presence of air and appropriate measures should be taken to minimize air exposure. See Chapter 2 for spectra.

**KR-200F**: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 6.52 (dd, 1H, $J = 8.8$, $J' = 6.4$ Hz), 6.46 (dd, 1H, $J = 7.2$ Hz, $J' = 2.8$ Hz), 6.34 (dt, 1H, $J = 8.8$ Hz, $J' = 2.8$ Hz), 4.40 (br s, 1H, OH), 3.41 (br s, 2H, NH$_2$); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 156.1, 153.8, 145.4, 145.3, 133.7, 133.6, 114.7, 114.6, 105.5, 105.3, 102.7, 102.4;
KR-101F: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.85 (br s, 2H, NH$_2$), 7.40 (d, 1H, J = 8.4 Hz), 7.24 (dd, 1H, J = 8.8 Hz, J = 4.0 Hz), 6.99 (t, 1H, J = 8.8 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 162.4, 159.6, 157.2, 147.0, 146.9, 133.7, 114.4, 114.3, 112.0, 111.7, 99.4, 99.1; HRMS mass calculated for C$_7$H$_6$FN$_2$O (M+H)$^+$ 153.0459, found 153.0460; Delta 0.7 ppm.
5-(methylamino)-2-Nitrophenol (KR-100001)

![Structure of 5-(methylamino)-2-Nitrophenol (KR-100001)](image)

**Figure 3.9:** 5-(methylamino)-2-nitrophenol (KR-100001).

Methylamine hydrochloride (12.73 mmol, 0.86 g) was added to a solution of 5-fluoro-2-nitrophenol (6.37 mmol, 1.00 g) in acetonitrile (75 mL) and triethylamine (15.92 mmol, 2.22 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for four hours. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (1:1 hexanes/ethyl acetate) provided a crystalline yellow solid in an 89% yield.

**KR-100001:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.35 (br s, 1H, OH), 7.71 (d, 1H, $J$ = 9.6 Hz), 7.52 (d, 1H, $J$ = 4.4 Hz), 6.21 (dd, 1H, $J = 9.6$ Hz, $J'$ = 2.4 Hz), 5.94 (br s, 1H, NH); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 158.8, 158.1, 127.5, 123.6, 109.0, 95.7, 29.8; HRMS mass calculated for C$_7$H$_9$N$_2$O$_3$ (M+H)$^+$ 169.0608, found 169.0604; Delta -2.4 ppm.
To a solution of 5-(methylamino)-2-nitrophenol (KR-100001) (6.72 mmol, 1.13 g) in methanol (125 mL), PtO₂ (0.34 mmol, 0.08 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for fifteen hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (40 mL). The filtrate was concentrated in vacuo yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (60 mL) and di(imidazole-1-yl)methanimine (16.81 mmol, 2.71 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) provided a crystalline white solid in a 41% yield. The product of the reduction (2-amino-5-(methylamino)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical
sample of **Kr-102001** was isolated by reverse-phase HPLC utilizing a gradient from 2-30% acetonitrile in water.

**KR-102001:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.09 (br s, 2H, NH$_2$), 8.95 (br s, 1H, NH), 7.38 (s, 1H), 7.28 (d, 1H, J = 8.2 Hz), 7.09 (d, 1H, J = 8.2 Hz), 2.86 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 162.2, 147.3, 136.7, 134.1, 116.8, 114.5, 101.8, 35.6; HRMS mass calculated for C$_8$H$_{10}$N$_3$O (M+H)$^+$ 164.0818, found 164.0819; Delta 0.8 ppm.

5-(dimethylamino)-2-Nitrophenol (KR-100003)

![Figure 3.11: 5-(dimethylamino)-2-nitrophenol (KR-100003).](image)

Dimethylamine (19.10 mmol, 9.55 mL, 2M in MeOH) was added to 5-fluoro-2-nitrophenol (6.37 mmol, 1.00 g). The reaction was then placed under argon atmosphere and refluxed for three hours with constant magnetic stirring. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (3:1 hexanes/ethyl acetate) provided a crystalline yellow solid in a 100% yield.
**KR-100003:** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 11.37 (br s, 1H, OH), 7.91 (d, 1H, J = 9.6 Hz), 6.26 (dd, 1H, J = 9.6 Hz, J' = 2.4 Hz), 6.09 (d, 1H, J = 2.4 Hz); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 158.1, 156.7, 127.5, 124.1, 106.0, 97.6, 40.48; HRMS mass calculated for C$_8$H$_{11}$N$_2$O$_3$ (M+H)$^+$ 183.0764, found 183.0764; Delta 0.0 ppm.

$N^6,N^6$-Dimethylbenzo[d]oxazole-2,6-diaminediamine (KR-102003)

![KR-102003](image)

**Figure 3.12:** $N^6,N^6$-dimethylbenzo[d]oxazole-2,6-diaminediamine (KR-102003).

To a solution of 5-(dimethylamino)-2-nitrophenol (KR-100003) (6.37 mmol, 1.15 g) in methanol (125 mL), 10% palladium on activated carbon (10 wt% of KR-100003, 0.12 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for four hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (50 mL). The filtrate was concentrated *in vacuo* yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (30 mL) and di(imidazole-1-yl)methanimine (15.91 mmol, 2.56 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The
reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) provided a crystalline white solid in a 68% yield. The product of the reduction (2-amino-5-(dimethylamino)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical sample of **Kr-102003** was isolated by reverse-phase HPLC utilizing a gradient from 2-55% acetonitrile in water.

**KR-102003:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.09 (br s, 2H, NH$_2$), 7.32 (s, 1H), 7.20 (d, 1H, $J = 8.0$ Hz), 7.00 (d, 1H, $J = 8.0$ Hz), 2.99 (s, 6H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 161.9, 147.4, 143.0, 131.0, 114.0, 113.9, 99.2, 43.9; HRMS mass calculated for C$_9$H$_{12}$N$_3$O (M+H)$^+$ 178.0975, found 178.0977; Delta 1.1 ppm.

**2-Nitro-5-(pyrrolidin-1-yl)phenol (KR-100053)**

![Figure 3.13: 2-nitro-5-(pyrrolidin-1-yl)phenol (KR-100053).](image)

Pyrrolidine (7.96 mmol, 0.66 mL) was added to a solution of 5-fluoro-2-nitrophenol (3.18 mmol, 0.50 g) in acetonitrile (60 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for two hours. The reaction was then cooled to room temperature, concentrated
under reduced pressure and silica gel column chromatography (1:1 hexanes/ethyl acetate) provided a crystalline yellow solid in a 95% yield.

**KR-100053:** \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 11.47 (br s, 1H, OH), 7.85 (d, 1H, J = 9.6 Hz), 6.13 (dd, 1H, J = 9.6 Hz, J' = 2.4 Hz), 5.93 (d, 1H, J = 2.4 Hz), 3.38 (t, 4H, J = 6.0 Hz), 2.04 (m, 4H); \(^{13}\)C NMR (400 MHz, CDCl\(_3\)) \(\delta\) 158.3, 154.3, 127.7, 123.9, 106.8, 97.5, 48.3, 25.5; HRMS mass calculated for C\(_{10}\)H\(_{13}\)N\(_2\)O\(_3\) (M+H)\(^+\) 209.0921, found 209.0922; Delta 0.4 ppm.

**6-(pyrrolidin-1-yl)Benzo[d]oxazol-2-amine (KR-102053)**

![KR-102053](image)

**Figure 3.14:** 6-(pyrrolidin-1-yl)benzo[d]oxazol-2-amine (KR-102053).

To a solution of 2-nitro-5-(pyrrolidin-1-yl)phenol (KR-100053) (3.03 mmol, 0.63 g) in methanol (80 mL), 10% palladium on activated carbon (10 wt% of KR-100053, 0.06 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for four hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (25 mL). The filtrate was
concentrated *in vacuo* yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (20 mL) and di(imidazole-1-yl)methanimine (7.57 mmol, 1.22 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (3:1 hexanes/ethyl acetate) provided a crystalline white solid in a 72% yield. The product of the reduction (2-amino-5-(pyrrolidin-1-yl)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical sample of **Kr-102053** was isolated by reverse-phase HPLC utilizing a gradient from 7-65% acetonitrile in water.

**KR-102053:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.48 (br s, 2H, NH), 7.17 (d, 1H, J = 8.0 Hz), 6.77 (s, 1H), 6.49 (d, 1H, J = 8.0 Hz), 3.19 (s, 4H), 1.93 (s, 4H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 159.8, 147.5, 145.4, 121.7, 113.5, 110.0, 94.9, 49.1, 25.5; HRMS mass calculated for C$_{11}$H$_{14}$N$_3$O (M+H)$^+$ 204.1131, found 204.1132; Delta 0.5 ppm; Crystal structure available.
**2-Nitro-5-(piperidin-1-yl)phenol (KR-100049)**

![KR-100049](image)

**Figure 3.15:** 2-nitro-5-(piperidin-1-yl)phenol (**KR-100049**).

Piperidine (7.96 mmol, 0.79 mL) was added to a solution of 5-fluoro-2-nitrophenol (3.18 mmol, 0.50 g) in acetonitrile (25 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for two hours. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (7:1 hexanes/ethyl acetate) provided a crystalline yellow solid in a 100% yield.

**KR-100049:** $^1$H NMR (400 MHz, CDCl$_3$) δ 11.33 (br s, 1H, OH), 7.90 (d, 1H, $J = 10.0$ Hz), 6.40 (dd, 1H, $J = 10.0$ Hz, $J' = 2.8$ Hz), 6.26 (d, 1H, $J = 3.2$ Hz), 3.46 (t, 4H, $J = 5.2$ Hz), 1.67 (m, 6H); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 158.5, 156.7, 127.6, 124.2, 107.2, 99.0, 48.5, 25.6, 24.5; HRMS mass calculated for C$_{11}$H$_{15}$N$_2$O$_3$ (M+H)$^+$ 223.1077, found 223.1080; Delta 1.3 ppm.
To a solution of 2-nitro-5-(piperidin-1-yl)phenol (KR-100049) (3.18 mmol, 0.71 g) in methanol (70 mL), 10% palladium on activated carbon (10 wt% of KR-100049, 0.07 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for four hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (25 mL). The filtrate was concentrated in vacuo yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (25 mL) and di(imidazole-1-yl)methanimine (7.96 mmol, 1.28 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) provided a crystalline white solid in a 74% yield. The product of the reduction (2-amino-5-(piperidin-1-yl)phenol) is air unstable and appropriate measures should be taken.
to minimize air exposure. An analytical sample of **Kr-102049** was isolated by reverse-phase HPLC utilizing a gradient from 4-60% acetonitrile in water.

**Kr-102049**: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.25 (br s, 2H, NH$_2$), 7.82 (d, 1H, J = 2.0 Hz), 7.44 (dd, 1H, J = 8.4 Hz, J = 2.0 Hz), 7.32 (d, 1H, J = 8.4 Hz), 3.51 (t, 4H, J = 5.6 Hz), 1.88 (p, 4H, J = 5.6 Hz), 1.63 (br s, 2H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 164.1, 147.9, 141.8, 137.5, 117.7, 115.3, 103.3, 56.8, 24.1, 21.5; HRMS mass calculated for C$_{12}$H$_{16}$N$_3$O (M+H)$^+$ 218.1288, found 218.1290; Delta 0.9 ppm; Crystal structure available.

**5-Morpholino-2-nitrophenol (KR-100047)**

![Structure](image)

**Figure 3.17**: 5-morpholino-2-nitrophenol (KR-100047).

Morpholine (7.96 mmol, 0.69 mL) was added to a solution of 5-fluoro-2-nitrophenol (3.18 mmol, 0.50 g) in acetonitrile (25 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for two hours. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) provided a crystalline yellow solid in a 97% yield.
KR-100047: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 11.15 (br s, 1H, OH), 7.91 (d, 1H, J = 9.6 Hz), 6.40 (dd, 1H, J = 9.6 Hz, $J' = 2.8$ Hz), 6.27 (d, 1H, J = 2.8 Hz), 3.81 (t, 4H, J = 5.0 Hz), 3.38 (t, 4H, J = 5.0 Hz); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 158.1, 157.1, 127.4, 125.3, 106.8, 99.8, 66.5, 46.9; HRMS mass calculated for C$_{12}$H$_{11}$N$_2$O$_4$ (M-H)$^-$ 223.0724, found 223.0725; Delta 0.4 ppm.

6-Morpholinobenzo[d]oxazol-2-amine (KR-102047)

![KR-102047](image)

Figure 3.18: 6-morpholinobenzo[d]oxazol-2-amine (KR-102047).

To a solution of 5-morpholino-2-nitrophenol (KR-100047) (3.07 mmol, 0.69 g) in methanol (90 mL), 10% palladium on activated carbon (10 wt% of KR-100047, 0.07 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for four hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (25 mL). The filtrate was concentrated in vacuo yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (25 mL) and di(imidazole-1-yl)methanimine (7.70 mmol, 1.24 g) was added with constant magnetic stirring. The reaction was
then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (1:2 hexanes/ethyl acetate) provided a crystalline white solid in a 66% yield. The product of the reduction (2-amino-5-morpholinophenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical sample of **Kr-102047** was isolated by reverse-phase HPLC utilizing a gradient from 7-55% acetonitrile in water.

**Kr-102047**: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.11 (br s, 2H, NH$_2$), 7.03 (d, 1H, J = 8.4 Hz), 6.98 (d, 1H, J = 2.0 Hz), 6.70 (d, 1H, J = 8.4 Hz), 3.71 (t, 4H, J = 4.0 Hz), 2.99 (t, 4H, J = 4.0 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 162.6, 149.5, 147.0, 137.4, 115.6, 112.5, 98.4, 66.9, 50.9; HRMS mass calculated for C$_{11}$H$_{14}$N$_3$O$_2$ (M+H)$^+$ 220.1081, found 220.1083; Delta 0.9 ppm; Crystal structure available.

**5-(4-methylpiperazin-1-yl)-2-nitrophenol (KR-100051)**

![Figure 3.19](image)

**Figure 3.19**: 5-(4-methylpiperazin-1-yl)-2-nitrophenol (**KR-100051**).

$N$-Methylpiperazine (7.96 mmol, 0.88 mL) was added to a solution of 5-fluoro-2-nitrophenol (3.18 mmol, 0.50 g) in acetonitrile (30 mL) with constant
magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for one hour. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (1:5 hexanes/ethyl acetate) yielded a crystalline yellow solid in a 100% yield.

**KR-100051:** $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 11.21 (br s, 1H, OH), 7.90 (d, 1H, $J = 9.6$ Hz), 6.41 (dd, 1H, $J = 9.6$ Hz, $J' = 2.8$ Hz), 6.27 (d, 1H, $J = 2.8$ Hz), 3.45 (t, 4H, $J = 5.0$ Hz), 2.51 (t, 4H, $J = 5.0$ Hz), 2.33 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 157.6, 156.8, 127.6, 124.8, 107.5, 99.7, 54.8, 46.7, 46.2; HRMS mass calculated for C$_{11}$H$_{16}$N$_3$O$_3$ (M+H)$^+$ 238.1186, found 238.1190; Delta 1.7 ppm.

6-(4-methylpiperazin-1-yl)Benzo[d]oxazol-2-amine (KR-102051)

![KR-102051](image)

**Figure 3.20:** 6-(4-methylpiperazin-1-yl)benzo[d]oxazol-2-amine (KR-102051).

To a solution of 5-(4-methylpiperazin-1-yl)-2-nitrophenol (KR-100051) (3.18 mmol, 0.75 g) in methanol (100 mL), 10% palladium on activated carbon (10 wt% of KR-100051, 0.07 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary
pressure (15 psi) for four hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (30 mL). The filtrate was concentrated in vacuo yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (45 mL) and di(imidazole-1-yl)methanimine (7.96 mmol, 1.28 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (15:1 dichloromethane/methanol) provided a crystalline white solid in a 73% yield. The product of the reduction (2-amino-5-(4-methylpiperazin-1-yl)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical sample of Kr-102051 was isolated by reverse-phase HPLC utilizing a gradient from 0.5-40.0% acetonitrile in water.

**Kr-102051:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.64 (br s, 2H, NH$_2$), 7.29 (d, 1H, J = 2.0 Hz), 7.25 (d, 1H, J = 8.8 Hz), 6.94 (dd, 1H, J = 8.8 Hz, J$'$ = 2.0 Hz), 3.76 (d, 2H, J = 11.8 Hz), 3.52 (d, 2H, J = 11.8), 3.16 (t, 2H, J = 11.2 Hz), 3.00 (t, 2H, J = 11.2 Hz), 2.84 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 161.2, 147.7, 146.9, 129.0, 114.2, 114.0, 100.0, 52.9, 47.4, 42.7; HRMS mass calculated for C$_{12}$H$_{17}$N$_4$O (M+H)$^+$ 233.1397, found 233.1398; Delta 0.5 ppm; Crystal structure available.
5-((3-(dimethylamino)propyl)amino)-2-Nitrophenol (KR-100303)

\[
\begin{align*}
&\text{N}^1,\text{N}^1-\text{Dimethylpropane-1,3-diamine (19.10 mmol, 2.40 mL)} \text{ was added to} \\
&\text{5-fluoro-2-nitrophenol (6.37 mmol, 1.00 g) with constant magnetic stirring. The} \\
&\text{reaction was then placed under argon atmosphere and refluxed for eight hours.} \\
&\text{The reaction was then cooled to room temperature concentrated under reduced} \\
&\text{pressure and silica gel column chromatography (15:1 ethyl acetate/methanol) provided a yellow oil in an 80\% yield.}
\end{align*}
\]

\textbf{KR-100303:} $^1\text{H NMR (400 MHz, DMSO-}$d$_6$\text{)} \delta 7.75 (d, 1H, J = 9.6 Hz), 7.61 (d, 1H, J = 2.4 Hz), 6.28 (dd, 1H, J = 9.6 Hz, J' = 1.6 Hz), 6.03 (br s, 1H, NH), 3.13 (q, 2H, J = 6.4 Hz), 2.30 (t, 2H, J = 6.8 Hz), 2.14 (s, 6H), 1.66 (p, 2H, J = 6.8 Hz); $^{13}\text{C NMR (400 MHz, DMSO-}$d$_6$\text{)} \delta 158.8, 157.4, 127.7, 123.7, 108.6, 96.1, 56.9, 45.5, 41.1, 26.7; HRMS mass calculated for C$_{11}$H$_{18}$N$_3$O$_3$ (M+H)$^+$ 240.1343, found 240.1342; Delta -0.4 ppm.
Figure 3.22: $N^6$-(3-(dimethylamino)propyl)benzo[d]oxazole-2,6-diamine (KR-102303).

To a solution of 5-((3-(dimethylamino)propyl)amino)-2-nitrophenol (KR-100303) (5.10 mmol, 1.22 g) in methanol (75 mL), 10% palladium on activated carbon (10 wt% of KR-100303, 0.12 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for four hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (25 mL). The filtrate was concentrated in vacuo yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (25 mL) and di(imidazole-1-yl)methanimine (12.75 mmol, 2.05 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (10:1 dichloromethane/methanol) provided a crystalline white solid in a 61% yield. The
product of the reduction (2-amino-5-((3-(dimethylamino)propyl)amino)phenol) is air unstable and appropriate measures should be taken to minimize air exposure.

An analytical sample of **Kr-102303** was isolated by reverse-phase HPLC utilizing a gradient from 0.2-16.0\% acetonitrile in water.

**KR-102303:** $^1$H NMR (400 MHz, DMSO-$d_6$ and TFA) $\delta$ 9.15 (bs, 2H, NH$_2$), 7.18 (d, 1H, J = 8.4 Hz), 6.99 (s, 1H), 6.75 (d, 1H, J = 8.4 Hz), 3.14 (dt, 4H, J = 7.6 Hz), 2.76 (s, 6H), 1.92 (p, 2H, J = 7.6 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$ and TFA) $\delta$ 160.8, 147.6, 142.3, 127.0, 114.2, 113.2, 97.1, 55.2, 43.1, 42.8, 23.3; HRMS mass calculated for C$_{12}$H$_{19}$N$_4$O (M+H)$^+$ 235.1553, found 235.1534; Delta 0.5 ppm.

**N$_1^\prime$,N$_1$-(benzo[\textit{d}]oxazole-2,6-diyl)Bis(N$_3^\prime$,N$_3$-dimethylpropane-1,3-diamine)**

(KR-203303)

Figure 3.23: $N_1^\prime$,N$_1$-(benzo[\textit{d}]oxazole-2,6-diyl)bis(N$_3^\prime$,N$_3$-dimethylpropane-1,3-diamine) (KR-203303).

To a solution of $N^6$-(3-(dimethylamino)propyl)benzo[\textit{d}]oxazole-2,6-diamine (KR-102303) (1.96 mmol, 0.46 g) in acetonitrile (50 mL), K$_2$CO$_3$ (4.91 mmol, 0.68
g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere. 3-Chloro-N,N-dimethylpropan-1-amine (2.06 mmol, 0.27 mL) was added and the reaction was heated to 75 °C for three days. Then the reaction was cooled to room temperature, filtered and the filtrand was washed with methanol (20 mL). The filtrate was concentrated in vacuo yielding a brown oil. Silica gel column chromatography (5:1 chloroform/methanol) provided a faint brown oil in a 41% yield. An analytical sample of **Kr-203303** was isolated by reverse-phase HPLC utilizing a gradient from 0.2-7.0% acetonitrile in water.

**Kr-203303**: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.21 (br s, 2H, NH), 7.15 (d, 1H, J = 8.0 Hz), 6.91 (s, 1H), 6.67 (d, 1H, J = 8.0 Hz), 3.39 (t, 2H, J = 7.2 Hz), 3.30 (t, 2H, J = 7.2 Hz), 3.13 (t, 2H, J = 7.2 Hz), 3.06 (t, 2H, J = 7.2 Hz), 3.03 (s, 6H), 2.78 (s, 6H), 2.09 (m, 2H), 1.97 (m, 2H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 168.9, 160.2, 147.6, 144.2, 114.1, 112.1, 96.0, 62.3, 60.7, 53.9, 50.9, 42.8, 42.2, 21.9, 18.3.
2-Nitro-5-(phenylamino)phenol (KR-100061)

![image of KR-100061]

**Figure 3.24:** 2-nitro-5-(phenylamino)phenol (KR-100061).

Aniline (19.10 mmol, 1.74 mL) was added to a solution of 5-fluoro-2-nitrophenol (9.55 mmol, 1.50 g) in triethylamine (19.10 mmol, 2.66 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for 18 hours. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (5:1 hexanes/ethyl acetate) provided a crystalline orange solid in a 91% yield.

**KR-100061:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.94 (br s, 1H, OH), 9.34 (br s, 1H, NH), 7.89 (d, 1H, J = 9.2 Hz), 7.37 (t, 2H, J = 7.6 Hz), 7.21 (d, 2H, J = 8.4 Hz), 7.11 (t, 1H, J = 8.0 Hz), 6.55 (dd, 1H, J = 8.8 Hz, J’ = 1.6 Hz), 6.50 (d, 1H, J = 1.6 Hz) ; $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 157.5, 153.2, 140.3, 130.2, 128.4, 126.4, 124.6, 122.2, 108.8, 100.0; HRMS mass calculated for C$_{12}$H$_9$N$_2$O$_3$ (M+H)$^+$ 229.0613, found 229.0620; Delta 3.0 ppm.
$N^6$-Phenylbenzo[d]oxazole-2,6-diamine (KR-102061)

\[
\text{Figure 3.25: } N^6\text{-phenylbenzo[d]oxazole-2,6-diamine (KR-102061).}
\]

To a solution of 2-nitro-5-(phenylamino)phenol (KR-100061) (12.03 mmol, 2.77 g) in methanol (120 mL), PtO$_2$ (0.60 mmol, 0.14 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for fifteen hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (45 mL). The filtrate was concentrated in vacuo yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (60 mL) and di(imidazole-1-yl)methanimine (30.08 mmol, 4.85 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature concentrated under reduced pressure and silica gel column chromatography (3:1 hexanes/ethyl acetate) provided a crystalline white solid in a 69% yield. The product of the reduction (2-amino-5-(phenylamino)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical
sample of **Kr-102061** was isolated by reverse-phase HPLC utilizing a gradient from 4-44% acetonitrile in water.

**KR-102061**: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.23 (br s, 4H), 7.07 (br s, 3H), 6.89 (bs, 1H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 161.0, 147.6, 144.5, 144.0, 139.8, 139.2, 129.9, 129.1, 120.2, 116.7, 115.7, 114.4, 100.3; HRMS mass calculated for $\text{C}_{13}\text{H}_{12}\text{N}_3\text{O}$ (M+H)$^+$ 226.0975, found 226.0978; Delta 1.3 ppm.

**5-(benzylamino)-2-Nitrophenol (KR-100059)**

![Structure of 5-(benzylamino)-2-Nitrophenol (KR-100059)](image)

**Figure 3.26**: 5-(benzylamino)-2-nitrophenol (**KR-100059**).

Benzyamine (19.10 mmol, 2.09 mL) was added to 5-fluoro-2-nitrophenol (6.37 mmol, 1.00 g) with constant magnetic stirring. The reaction was then placed under argon atmosphere and heated to 65 °C for six hours. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (5:1 hexanes/ethyl acetate) provided a crystalline yellow solid in a 94% yield.
KR-100059: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.14 (br s, 1H, OH), 8.05 (t, 1H, NH, $J = 5.6$ Hz), 7.78 (d, 1H, $J = 9.6$ Hz), 7.33 (m, 4H), 7.26 (m, 1H), 6.36 (dd, 1H, $J = 9.6$ Hz, $J' = 2.4$ Hz), 6.06 (s, 1H), 4.39 (d, 2H, $J = 6.0$ Hz); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 160.0, 155.9, 137.1, 129.2, 128.2, 127.7, 127.6, 125.1, 107.9, 97.6, 47.8; HRMS mass calculated for C$_{13}$H$_{13}$N$_2$O$_3$ (M+H)$^+$ 245.0921, found 245.0923; Delta 0.8 ppm; Crystal structure available.

$N^6$-Benzylbenzo[d]oxazole-2,6-diamine (KR-102059)

![KR-102059](image)

Figure 3.27: $N^6$-benzylbenzo[d]oxazole-2,6-diamine (KR-102059).

To a solution of 5-(benzylamino)-2-nitrophenol (KR-100059) (7.98 mmol, 1.95 g) in methanol (120 mL), PtO$_2$ (0.40 mmol, 0.09 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for fifteen hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (40 mL). The filtrate was concentrated in vacuo yielding an intense purple residue. The residue was quickly re-dissolved in acetonitrile (60 mL) and
di(imidazole-1-yl)methanimine (19.96 mmol, 3.22 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and gently refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (3:1 hexanes/ethyl acetate) provided a crystalline white solid in a 63% yield. The product of the reduction (2-amino-5-(benzylamino)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical sample of Kr-102059 was isolated by reverse-phase HPLC utilizing a gradient from 4-48% acetonitrile in water.

**KR-102059:** \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 9.42 (br s, 2H, NH\(_2\)), 7.36 (d, 2H, J = 7.2 Hz), 7.29 (t, 2H, J = 7.2 Hz), 7.21 (d, 1H, J = 6.8 Hz), 7.16 (d, 1H, J = 8.4 Hz), 6.95 (s, 1H), 6.78 (d, 1H, J = 8.4 Hz), 4.33 (s, 2H); \(^{13}\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 160.5, 147.2, 142.9, 138.2, 128.9, 128.7, 127.9, 125.3, 113.7, 113.3, 97.3, 49.4; HRMS mass calculated for C\(_{14}\)H\(_{14}\)N\(_3\)O (M+H)\(^+\) 240.1131, found 240.1135; Delta 1.7 ppm; Crystal structure available.

**General Techniques**

Commercial reagents were obtained (Sigma Aldrich, Acros, Alfa Aesar, Fisher) and used without any further purification. Non-aqueous reactions were carried out under anhydrous conditions using oven-dried glassware under an inert atmosphere in dry, freshly distilled solvents. All air-sensitive reaction solutions were transferred via syringe or stainless steel cannula. Reactions were
monitored by TLC, which was performed on pre-coated silica gel 60 F254 plates supplied by EMD, visualized utilizing UV light, and developed using ceric ammonium molybdate stain (CAM) with heat or ninhydrin stain with heat. Organic solvents were removed by rotary evaporation below 30 °C at approximately 15 mmHg. Flash column chromatography was executed with silica gel 60 (230-400 mesh) supplied by Silicycle and eluting solvents are indicated in the text. Yields refer to chromatographically and spectroscopically (¹H NMR, ¹³C NMR) homogeneous materials, unless otherwise stated. NMR spectra were recorded on Varian Mercury 400 MHz, Jeol Unity 500 MHz, and Agilent VNMR S 500 MHz outfitted with an XSens cold probe using deuterochloroform, deuteromethanol, deuterowater or deuterodimethyl sulfoxide (Cambridge Isotope) as solvents. The chemical shifts are given in ppm relative to the standard reference TMS or residual undeuterated solvent. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublet, dt = doublet of triplet. Low resolution mass spectra were obtained on a ThermoFinnigan LCQDECA-MS spectrometer. High resolution mass spectra (HRMS) were recorded on a VG 7070 HS or a VG ZAB-ZSE mass spectrometer. Crystals were mounted in nylon loops using Paratone oil and then placed on the diffractometer under a nitrogen stream at 100K. All crystallographic data has been deposited at the CCDC, 12 Union Road, Cambridge CB21EZ, UK.
Spectral Data

Nuclear Magnetic Resonance

Spectrum 3.1: 6-chlorobenzo[d]oxazol-2-amine (KR-101Cl) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.2: 6-chlorobenzo[d]oxazol-2-amine (KR-101Cl) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 3.3:** 6-fluorobenzo[d]oxazol-2-amine (KR-101F) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
Spectrum 3.4: 6-fluorobenzo[d]oxazol-2-amine (KR-101F) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 3.5:** 5-(methylamino)-2-nitrophenol (KR-100001) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.6: 5-(methylamino)-2-nitrophenol (KR-100001) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.7: \(N^6\)-methylbenzo[d]oxazole-2,6-diamine (KR-102001) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
**Spectrum 3.8**: $N^6$-methylbenzo[d]oxazole-2,6-diamine (KR-102001) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 3.9:** 5-(dimethylamino)-2-nitrophenol (KR-100003) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 3.10: 5-(dimethylamino)-2-nitrophenol (KR-100003) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 3.11: $N^6,N^6$-dimethylbenzo[d]oxazole-2,6-diaminediamine (KR-102003) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.12: $N^6,N^6$-dimethylbenzo[d]oxazole-2,6-diaminediamine (KR-102003) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.13: 2-nitro-5-(pyrrolidin-1-yl)phenol (KR-100053) $^1$H NMR (400 MHz, CDCl$_3$).
**Spectrum 3.14:** 2-nitro-5-(pyrrolidin-1-yl)phenol (KR-100053) $^{13}$C NMR (400 MHz, CDCl$_3$).

![NMR spectrum of 2-nitro-5-(pyrrolidin-1-yl)phenol (KR-100053)](image)
Spectrum 3.15: 6-(pyrrolidin-1-yl)benzoxazol-2-amine (KR-102053) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.16: 6-(pyrrolidin-1-yl)benzo[d]oxazol-2-amine (KR-102053) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.17: 2-nitro-5-(piperidin-1-yl)phenol (KR-100049) $^1$H NMR (400 MHz, CDCl₃).
Spectrum 3.18: 2-nitro-5-(piperidin-1-yl)phenol (KR-100049) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 3.19: 6-(piperidin-1-yl)benzo[d]oxazol-2-amine (KR-102049) (\(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
Spectrum 3.20: 6-(piperidin-1-yl)benzo[d]oxazol-2-amine (KR-102049) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 3.21**: 5-morpholino-2-nitrophenol (KR-100047) $^1$H NMR (400 MHz, CDCl$_3$).
**Spectrum 3.22:** 5-morpholino-2-nitrophenol (KR-100047) $^{13}$C NMR (400 MHz, CDCl$_3$).
**Spectrum 3.23:** 6-morpholinobenzo[d]oxazol-2-amine (KR-102047) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).

KR-102047
Spectrum 3.25: 5-(4-methylpiperazin-1-yl)-2-nitrophenol (KR-100051) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 3.26: 5-(4-methylpiperazin-1-yl)-2-nitrophenol (KR-100051) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.27: 6-(4-methylpiperazin-1-yl)benzo[d]oxazol-2-amine (KR-102051)
$^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.28: 6-(4-methylpiperazin-1-yl)benzo[d]oxazol-2-amine (KR-102051)

$^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.29: 5-((3-(dimethylamino)propyl)amino)-2-nitrophenol (KR-100303)
$^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.30: 5-((3-(dimethylamino)propyl)amino)-2-nitrophenol (KR-100303) 
$^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.31: $N^6$-(3-(dimethylamino)propyl)benzo[d]oxazole-2,6-diamine (KR-102303) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.32: $N^6$-(3-(dimethylamino)propyl)benzo[d]oxazole-2,6-diamine (KR-102303) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.33: $N^1,N^{1'}$-(benzo[$d$]oxazole-2,6-diyl)bis($N^2,N^2$-dimethylpropane-1,3-diamine) (KR-203303) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.34: $N^1,N^1'$-(benzo[d]oxazole-2,6-diyl)bis($N^2,N^2'$-dimethylpropane-1,3-diamine) (KR-102303) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.35: 2-nitro-5-(phenylamino)phenol (KR-100061) $^1$H NMR (400 MHz, DMSO-$d_6$).

[Image of an NMR spectrum with peak assignments]
Spectrum 3.36: 2-nitro-5-(phenylamino)phenol (KR-100061) $^{13}$C NMR (400 MHz, DMSO-d$_6$).
Spectrum 3.37: $N^6$-phenylbenzo[d]oxazole-2,6-diamine (KR-102061) $^1$H NMR (400 MHz, CD$_3$OD).
Spectrum 3.38: \(N^6\)-phenylbenzo[d]oxazole-2,6-diamine (KR-102061) \(^{13}\)C NMR (400 MHz, DMSO-\(d_6\)).
**Spectrum 3.39**: 5-(benzylamino)-2-nitrophenol (KR-100059) $^1$H NMR (400 MHz, DMSO-$d_6$).
**Spectrum 3.40:** 5-(benzylamino)-2-nitrophenol (KR-100059) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 3.41: $N^6$-benzylbenzo[d]oxazole-2,6-diamine (KR-102059) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 3.42: $N^6$-benzylbenzo[d]oxazole-2,6-diamine (KR-102059) $^{13}$C NMR (400 MHz, DMSO-$d_6$).

KR-102059
Crystal Structures


**Spectrum 3.45:** Alternative view of crystal packing observed in crystal structure of 6-chlorobenzod[8]oxazol-2-amine (KR-101Cl).
Spectrum 3.46: Crystal structure of 6-(pyrrolidin-1-yl)benzo[d]oxazol-2-amine (KR-102053) in the presence of TFA.

Spectrum 3.47: Hydrogen bonding pattern observed in crystal structure of 6-(pyrrolidin-1-yl)benzo[d]oxazol-2-amine (KR-102053) in the presence of TFA.
**Spectrum 3.48:** Crystal packing observed in crystal structure of 6-(pyrrolidin-1-yl)benzo[d]oxazol-2-amine (KR-102053) in the presence of TFA.

**Spectrum 3.49:** Alternative view of crystal packing observed in crystal structure of 6-(pyrrolidin-1-yl)benzo[d]oxazol-2-amine (KR-102053) in the presence of TFA.
**Spectrum 3.50:** Crystal structure of 6-(piperidin-1-yl)benzo[d]oxazol-2-amine (KR-102049).

**Spectrum 3.51:** Hydrogen bonding pattern observed in crystal structure of 6-(piperidin-1-yl)benzo[d]oxazol-2-amine (KR-102049) in the presence of water.
**Spectrum 3.52:** Crystal packing observed in crystal structure of 6-(piperidin-1-y)l)benzo[d]oxazol-2-amine (KR-102049) in the presence of water.

**Spectrum 3.53:** Alternative view of crystal packing observed in crystal structure of 6-(piperidin-1-y)l)benzo[d]oxazol-2-amine (KR-102049) in the presence of water.

**Spectrum 3.55**: Hydrogen bonding pattern observed in crystal structure of 6-morpholinobenzo[d]oxazol-2-amine (KR-102047).
Spectrum 3.56: Crystal packing observed in crystal structure of 6-morpholinobenzo[d]oxazol-2-amine (KR-102047).

**Spectrum 3.58:** Crystal structure of 6-(4-methylpiperazin-1-yl)benzo[d]oxazol-2-amine (KR-102051) in the presence of TFA.

**Spectrum 3.59:** Hydrogen bonding pattern observed in crystal structure of 6-(4-methylpiperazin-1-yl)benzo[d]oxazol-2-amine (KR-102051) in the presence of TFA.
**Spectrum 3.60:** Crystal packing observed in crystal structure of 6-(4-methylpiperazin-1-yl)benzo[d]oxazol-2-amine (KR-102051) in the presence of TFA.

![Crystal Packing](image1)

**Spectrum 3.61:** Alternative view of crystal packing observed in crystal structure of 6-(4-methylpiperazin-1-yl)benzo[d]oxazol-2-amine (KR-102051) in the presence of TFA.

![Alternative View](image2)
**Spectrum 3.62:** Crystal structure of $N^6$-phenylbenzo[d]oxazole-2,6-diamine (KR-102061).

**Spectrum 3.63:** Hydrogen bonding pattern observed in crystal structure of $N^6$-phenylbenzo[d]oxazole-2,6-diamine (KR-102061).
**Spectrum 3.64:** Crystal packing observed in crystal structure of $N^6$-phenylbenzo[\textit{d}]oxazole-2,6-diamine (KR-102061).

**Spectrum 3.65:** Alternative view of crystal packing observed in crystal structure of $N^6$-phenylbenzo[\textit{d}]oxazole-2,6-diamine (KR-102061).
**Spectrum 3.66:** Crystal structure of $N^6$-benzylbenzo[d]oxazole-2,6-diamine (KR-102059) in the presence of TFA.

**Spectrum 3.67:** Hydrogen bonding pattern observed in crystal structure of $N^6$-benzylbenzo[d]oxazole-2,6-diamine (KR-102059) in the presence of TFA.
Spectrum 3.68: Crystal packing observed in crystal structure of $N^6$-benzylbenzo[\textit{d}]oxazole-2,6-diamine (KR-102059) in the presence of TFA.

Spectrum 3.69: Alternative view of crystal packing observed in crystal structure of $N^6$-benzylbenzo[\textit{d}]oxazole-2,6-diamine (KR-102059) in the presence of TFA.
References


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Chapter 4

7-Substituted Aminobenzoxazoles Targeting the HCV IRES
Introduction

Subdomain IIa of the HCV IRES has been shown to exhibit exquisite flexibility necessary to induce conformational changes in the 40S subunit which culminate in the formation of translation active 80S ribosomes.\(^{[1-4]}\) Benzimidazole 1 binds to subdomain IIa by capturing an extended conformation of the dynamic RNA inhibiting IRES function. An RNA-ligand co-crystal structure shows the disruption of local structure caused by ligand binding, resulting in a conformation that prevents the proper positioning of the distal hairpin thereby blocking translation (Figure 2.6).\(^{[5-7]}\) Additionally, benzimidazole 1 arrests the articulation of subdomain IIa that is speculated to facilitate IRES release from the ribosome.\(^{[8-9]}\) Despite the challenges associated with developing ligands which interact with RNA, subdomain IIa of the HCV IRES represents a validated target for development of novel small molecule inhibitors which arrest viral translation.

The previously synthesized aminobenzoxazoles have been demonstrated to bind to the conformationally inactive elongated form of subdomain IIa of the HCV IRES resulting in translation inhibition. The structure based design of the substituted aminobenzoxazoles focused on simplified ligands to allow straightforward access to diverse structures in order to investigate the interactions which govern ligand binding toward the development of a structure activity relationship (SAR). Evaluation of the ligands showed that the benzoxazole core alone was not large enough to capture the elongated form of the RNA. However, the addition of a dimethyl amino side chain attached by a
three carbon tether to the 2- or 6-position of the aminobenzoxazole results in moderate ligand affinity for subdomain IIa, revealing sites of favorable interaction at the edge of the binding pocket (Figure 4.1). Replacement of the amine at the 6-position by a halogen abrogates target binding, showing the significance of the electronic character of the core for ligand affinity. Additionally, the preparation of 2,6-disubstituted aminobenzoxazoles revealed the overall deep, conical shape of the binding cavity; ligands which are wider near the core benzoxazole display reduced affinity for the target RNA stemming from unfavorable interaction with sides of the pocket. Despite the preparation of a series of aminobenzoxazoles with moderate affinity for subdomain IIa of the IRES, their general air sensitivity renders these compounds poor candidates for further development as potential therapeutic agents.

Figure 4.1: Structure of the HCV translation inhibitors that bind to subdomain IIa. Aminobenzimidazole inhibitor 1 was initially identified by Isis Pharmaceuticals. Aminobenzoxazoles 2 and 3 were previously designed and synthesized for interaction with subdomain IIa. The retained core scaffold is highlighted in red.

Utilizing the previous ligand studies, a series of 7-substituted benzoxazoles was conceived to address the inherent short-comings of the
synthesized aminobenzoxazoles with the goal of developing a selective and potent inhibitor of subdomain IIa.

**Design of 7-Substituted Benzoxazoles for Targeting IRES Subdomain IIa**

Molecules which target structured regions of biologically important RNA and abrogate function share fundamental traits despite the complex functional diversity displayed by these ligands. Reduction of the functional complexity of these molecules to specific interactions which govern ligand recognition of RNA should allow the development of simplified ligands which modulate function through discrete interactions with an RNA target. Molecules developed around a rigid planar core with networks of hydrogen bond donors and acceptors facilitate the recognition of unpaired bases in RNA folds. Furthermore the flat nature of the core permits access to tight spaces between nucleobases. Additionally, the presence of aromatic functionalities is beneficial, resulting in the formation of stabilizing stacking interactions with the RNA target. Ligand-RNA affinity can also be influenced by the presence of basic functionalities which enable non-specific electrostatic interactions with the phosphate backbone.

The ligands in the series of 7-substitued benzoxazoles were designed with intrinsic properties in order to facilitate interactions with subdomain IIa of the HCV IRES. The benzoxazole core of this family of ligands exemplifies the paradigm that biases molecules for RNA interaction. The rigid benzoxazole serves as a recognition element which selectively binds to the target RNA
through precisely directed hydrogen bonds. The aromatic character of the core facilitates stacking interactions that further stabilize binding. The benzoazolone core additionally serves as an anchor that is responsible for orienting the auxiliary functionality. The substituents attached to the core benzoazolone probe for distal interactions within the binding pocket with the aim of facilitating fortuitous interactions that improve ligand affinity. The varying nature of the substituents serves to elucidate the general characteristics which aid and abet ligand-target interaction.

In order to improve the stability of the 7-substituted benzoazoles, the substituents were to be attached to the core through a methylene unit rather than directly through an amine as in aminobenzoazole 2 (Figure 4.2). The reduction in electron density at the core by connecting substituents through a carbon atom should increase ligand stability. The substituents for this series all contain a tertiary amine proximally connected to the benzoazolone in order to explore the potential for favorable ligand interactions near the core. Likewise, all of the head groups are uniformly linked to the tertiary amine through an ethyl linker. The length of the tether was selected to be of comparable length to the previously active aminobenzoazoles, aiming at accessing the favorable interactions near the edge of the binding pocket. The head groups attached to the tether consist of functionalities of varying basicity to explore the electrostatic environment at the entrance to the cavity. Finally, attaching various substituents through the 7-postion will result in a narrower ligand which is not likely to clash with the pocket
walls. The similarities built into the series of 7-substituted benzoxazoles will allow the unambiguous assessment of a multitude of RNA-ligand interactions.

![Figure 4.2: Comparison of previously synthesized novel benzoxazole inhibitors 2 and 3 with designed 7-substituted benzoxazole scaffold 4. Benzoxazoles 2 and 3 display EC_{50} values of 25 μM and 31 μM respectively in the FRET assay. The retained core scaffold is highlighted in red. The various head groups are listed below.](image)

**Figure 4.2**: Comparison of previously synthesized novel benzoxazole inhibitors 2 and 3 with designed 7-substituted benzoxazole scaffold 4. Benzoxazoles 2 and 3 display EC_{50} values of 25 μM and 31 μM respectively in the FRET assay. The retained core scaffold is highlighted in red. The various head groups are listed below.

**Retrosynthesis of 7-Substituted Benzoxazole Ligands**

Similar to prior synthetic efforts, the route to the series of 7-substituted benzoxazoles was conceived to facilitate the assembly of functionally diverse ligands around a retained core scaffold. The synthesis of 7-substituted benzoxazoles 4 was envisaged from the cyclization of the corresponding 7-substituted ortho-aminophenol 5. The reduction of ortho-nitrophenol 6 via hydrogenation would permit straightforward access to precursor 5. The ortho-nitro phenol precursor 6 would be accessed directly from 3-nitrosalicylaldehyde by reductive amination utilizing the necessary primary or secondary amine.
Scheme 4.1: Retrosynthetic analysis of 7-aminobenzoxazole 4. R = primary or secondary amine.

Synthesis of 7-Substituted Benzoxazole Ligands

Synthesis of the desired 7-substituted benzoxazoles commenced with the reductive amination of 3-nitrosalicylaldehyde with various amines (Scheme 3.2). The mild reducing agent allowed near quantitative yields of nitrophenol 10.\(^{[10-12]}\) The use of primary amines in the reductive amination step resulted in secondary amines which required the installation of a methyl group to reach the desired tertiary amines. A second reductive amination using formaldehyde allowed the selective installation of the requisite methyl group upon the secondary amines, providing straightforward access to substituted nitrophenol 6.\(^{[13]}\) However, much longer reaction times were necessary for the full conversion of sterically hindered secondary amines. Nitrophenol 6 was then reduced in using palladium hydrogenation conditions. The resulting 7-substituted aminophenol 5 was sensitive to oxidation due to the electron rich character of the scaffold. Immediate cyclization was necessary as the result of the instability of the precursor.
Aminophenol 5 was cyclized using di(imidazole-1-yl)methanimine, providing expedient access to the desired 7-substituted benzoazole 4.\(^{[14]}\)

![Chemical structure image]

**Scheme 4.2:** Synthetic route to the family of 7-substituted benzoazoles 4.

Reagents and conditions: a) NH\(_2\)R, NaBH(OAc)\(_3\), THF, 92-100%; b) Pd/C, H\(_2\), MeOH; c) di(imidazole-1-yl)methanimine, ACN, reflux, 51-77% 2 steps.

In addition to the designed 7-substituted benzoazoles, a set of functionalized benzoazoles was synthesized to evaluate the stability of the core, as well as, test for RNA binding (Figure 4.3). The straightforward preparation of the ligand cores utilized the same general chemistry as the 7-substituted benzoazoles, starting from the appropriate nitrophenol. Facile reduction of the nitrophenol using hydrogenation conditions followed by cyclization with di(imidazole-1-yl)methanimine provided direct access to the desired cores.

![Additional 7-substituted benzoazoles image]

**Figure 4.3:** Additional 7-substituted benzoazoles 8 investigating the stability of the core scaffold.
The synthesized cores reveal that the benzoxazole scaffold is stabilized by the presence of electron withdrawing groups. The benzoxazoles which contain a halogen or nitro group at the 7-position show no sensitivity to air suggesting that reducing the electron density at the core of the ligand is beneficial to compound stability. Conversely, the incorporation of electron donating groups, such as an amine, destabilizes the core and results in ligands that degrade when exposed to oxygen. As anticipated, the 7-substituted benzoxazoles functionalized through a methylene unit as opposed to an amine are air stable compounds. The weak electron donating character of the substituent is tolerated by the core scaffold resulting in a series of stable benzoxazole ligands.

Evaluation of Subdomain IIa Binding of 7-Substituted Benzoxazoles

The ability of the 7-substituted benzoxazoles to capture the extended form of subdomain IIa was evaluated using a FRET assay. A fluorescently labeled oligonucleotide representing subdomain IIa examines the effect of the ligand on the conformation adopted by the RNA (see Chapter 2). Capture of the compromised elongated form of the RNA results in a dose-dependent quenching of the FRET signal due to the dyes being moved outside of the Förster radius, whereas ligands that have no effect on the RNA do not perturb the FRET signal.[15] Consistent with previous data, the core structures alone (ligands 13, 14, 15, and 16) do not affect the FRET signal indicating that these ligands are not capable at capturing the extended form of subdomain IIa RNA (Table 4.1).
cores are likely too small and lack the substituents necessary to stabilize the
binding pocket formed by subdomain IIa in the extended conformation. Ligand 9,
which represents the stabilized analog of 6-substituted aminobenzoxazole 2,
displayed surprisingly weak affinity for subdomain IIa with an EC$_{50}$ of 192 μM.
The previously developed series of 6-substituted aminobenzoxazoles provided
evidence suggesting that narrower ligands should access the binding pocket
more easily; however, the necessary modification of the side chain seems to
have a profound impact on the affinity for the target RNA. The weak interaction of
benzoxazole 9 with subdomain IIa could be the result of the methyl attached to
the embedded amine resulting in a clash with the binding pocket. Additionally,
the longer tether may be detrimental to the proper positioning of the terminal
tertiary amine at the edge of the binding cavity resulting in diminished binding
affinity. Compositionally similar benzoxazole 12 shows no affinity for subdomain
IIa, suggesting that the embedded amine in ligand 9 does not facilitate favorable
RNA-ligand interaction. Furthermore the bulk of the embedded tertiary amine
could be a source of potential incompatible contacts within the binding pocket,
resulting in the reduced affinity for subdomain IIa when compared to analog 2
(Figure 4.2). Conversely, the distal tertiary amine of benzoxazole 12 further
suggests the presence of favorable interactions near the edge of the binding
cavity which is consistent with the activity of previously synthesized compounds.
The FRET measurements also identified benzoxazole 11 as weak ligand for
subdomain IIa. The presence of the amide head group in this ligand suggests
that the distal interaction can accommodate larger functional groups than initially
expected. However, the poor affinity displayed by ligand 11 in comparison to ligand 9 reveals the character of the head group interaction to favor the smaller basic dimethyl amino unit, suggesting that electrostatic interactions may play a significant role in stabilizing the binding pocket.

**Table 4.1:** Ligand affinity measured by FRET and IVT assays.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID (Notebook ID)</th>
<th>FRET ((\text{EC}_{50}))</th>
<th>IVT (% inhibition at 100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Structure 9" /></td>
<td>9 (Kr-402424)</td>
<td>192 μM</td>
<td>28±3%</td>
</tr>
<tr>
<td><img src="image" alt="Structure 10" /></td>
<td>10 (Kr-403424)</td>
<td>310 μM</td>
<td>22±5%</td>
</tr>
<tr>
<td><img src="image" alt="Structure 11" /></td>
<td>11 (Kr-402150)</td>
<td>600 μM</td>
<td>n.a</td>
</tr>
<tr>
<td><img src="image" alt="Structure 12" /></td>
<td>12 (Kr-402103)</td>
<td>n.a.</td>
<td>18±3%</td>
</tr>
<tr>
<td><img src="image" alt="Structure 13" /></td>
<td>13 (Kr-402)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>
Table 4.1, Continued: Ligand affinity measured by FRET and IVT assays.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Compound ID (Notebook ID)</th>
<th>FRET (EC&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>IVT (% inhibition at 100 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Structure" /></td>
<td>14 (Kr-415)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image2.png" alt="Structure" /></td>
<td>15 (Kr-401F)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Structure" /></td>
<td>16 (Kr-402Cl)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure" /></td>
<td>17 (Kr-402151)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure" /></td>
<td>18 (Kr-402428)</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

EC<sub>50</sub> is the concentration required for a fifty percent reduction in the observed FRET signal. IVT measurements were made in the presence of 100 μM of the ligand under study. Percent inhibition is normalized to the relative signal of the luciferase reporters in the absence of ligand. n.a. = no activity.

The 7-substituted benzoazoles were examined by an IVT assay which assesses the ligands' selectivity for the subdomain IIa target. The IVT assay is composed of a dual reporter system which measures the ligands' ability to discriminate between cap-driven and IRES-driven translation by measuring the levels of fluorescence from downstream reporters (see Chapter 2).<sup>[16]</sup> The weak
activity of benzoxazole 9 was confirmed by the IVT assay, suggesting that this ligand is weakly interacting with subdomain IIa and inhibiting IRES-driven translation. The decrease in translation caused by ligand 9 in the IVT assay is consistent with the observed activity in the FRET assay, indicating that this compound is likely a selective inhibitor of the IRES subdomain IIa. Ligand 12 also showed weak activity in the IVT assay, but not in the FRET assay, indicating that this ligand is inhibiting IRES-driven translation outside of subdomain IIa.
Figure 4.4: Graph displaying relative luciferase activity measured in the IVT assay with 100 μM of the ligand present. The cap-driven signal is shown in navy blue. The IRES-driven translation is shown in light blue. The control measures the relative luciferase signals in the absence of ligand. Ligands 9, 10 and 12 are weakly active in the IVT assay as shown by the depression in the relative renilla signal. Compound 11 illustrates a ligand which is inactive in the IVT assay.

Based on the observed structure activity relationship of previously synthesized ligands, a disubstituted benzoazole was conceived to examine the synergistic effect of combining two active ligands. The substitution pattern of the weak translation inhibitor 9 was combined with that of the moderately active ligand 3, resulting in the 2,7-disubstituted benzoazole 10 (Scheme 4.3). Synthetic access to desired ligand 10 was accomplished from benzoazole 9 via a nucleophilic substitution of dimethylaminopropyl chloride. The binding affinity of
disubstituted benzoxazole 10 for subdomain IIa was evaluated in the FRET and IVT assays. Ligand 10 displayed weaker than anticipated affinity for subdomain IIa in the FRET assay with an EC$_{50}$ of 310 μM. The side chain at the 2-position likely results in an unfavorable clash with binding pocket reducing the ligands affinity for the subdomain IIa target when compared to parental ligand 9. Additionally, this observation is consistent with a previously synthesized 2,6-disubstituted ligand which showed reduced binding affinity which further demonstrates that alkylation at the 2-position interferes with the RNA-ligand interaction. The addition of a second basic head group could also disfavor binding through direct electrostatic repulsion of the two moieties resulting in a ligand that requires significant preorganization to gain access to the binding pocket of the RNA. As a result of the entropic penalty, the ligand may not favor a conformation that is conducive to interaction with the RNA.

Scheme 4.3: Synthetic route to 2,7-disubstituted benzoxazole 10 from previously synthesized 7-substituted benzoxazole 9.

Reagents and conditions: a) 3-chloro-N,N-dimethylpropan-1-amine, Cs$_2$CO$_3$, ACN, 60 °C, 62%.
Conclusions

A series of 7-substituted benzoxazoles was designed to access the tight binding pocket of subdomain IIa of the HCV IRES. The ligands were designed around a core benzoxazole which is anticipated to act as a recognition element that is responsible for orienting the various substituents within the deep solvent excluded binding cavity formed by the RNA in similar fashion to benzimidazole 1. The various prepared 7-substituted benzoxazole cores were not capable of capturing the extended form of the RNA resulting in translation inhibition. The smaller cores likely lack the additional functionality needed to stabilize the binding cavity formed by the subdomain IIa. As predicted, the 7-substituted benzoxazoles were stable in the presence of air; however, the ligands exhibited poor affinity for subdomain IIa. Only the 7-substituted benzoxazole 9 showed affinity for the subdomain IIa. The weak binding suggests the presence of both favorable and unfavorable interactions by the side chain. The embedded tertiary amine of ligand 9 engages in interactions which seem to disfavor binding, whereas the terminal tertiary amine facilitates favorable contacts with subdomain IIa. Additionally, the various substituents within this series indicate a preference for head groups that contain a basic functionality which can engage in electrostatic interactions with the RNA. The synthesis of hybrid benzoxazole 10, revealed that alkylation at the 2-position of an active ligand will result in a compound with diminished affinity for subdomain IIa likely due to RNA-ligand clashes within the binding pocket. The series of 7-substituted benzoxazoles
reveals that the modifications which result in more stable and narrower ligands are not beneficial to targeting subdomain Ila of the HCV IRES.
Materials and Methods

2-Amino-6-nitrophenol (KR-401)

Figure 4.5: 2-amino-6-nitrophenol (KR-401).

To a of solution of 2,6-dinitrophenol (5.43 mmol, 1.00 g) in methanol (100 mL), 10% palladium on activated carbon (10 wt% of 2,6-dinitrophenol, 0.10 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for one hour. The reaction was monitored closely by thin layer chromatography to avoid over reduction. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered over a thin pad of celite which was then washed with methanol (50 mL). The filtrate was concentrated in vacuo yielding a brown solid. Silica gel column chromatography (10:1 hexanes/ethyl acetate) provided a crystalline red solid in a 92% yield.

KR-401: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.70 (br s, 1H, OH), 7.44 (d, 1H, J = 8.8 Hz), 6.94 (d, 1H, J = 8.4 Hz), 6.77 (t, 1H, J = 8.8 Hz), 4.12 (br s, 2H, NH$_2$); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 143.5, 138.1, 133.8, 120.7, 120.0, 113.6; HRMS mass calculated for C$_6$H$_5$N$_2$O$_3$ (M+H)$^+$ 153.0306, found 153.0307; Delta 0.7 ppm.
7-Nitrobenzo[d]oxazol-2-amine (KR-402)

![KR-402](image)

Figure 4.6: 7-nitrobenzo[d]oxazol-2-amine (KR-402).

To a solution of 2-amino-6-nitrophenol (KR-401) (6.49 mmol, 1.00 g) in acetonitrile (50 mL), di(imidazole-1-yl)methanimine (16.22 mmol, 2.61 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (10:1 hexanes/ethyl acetate) provided a crystalline white solid in a 79% yield.

**KR-402:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.99 (br s, 2H, NH$_2$), 7.67 (d, 1H, $J$ = 8.4 Hz), 7.55 (d, 1H, $J$ = 8.4 Hz), 7.25 (t, 1H, $J$ = 8.4 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 164.6, 147.7, 141.8, 132.1, 124.4, 122.2, 115.8.
Benzo[d]oxazole-2,7-diamine (KR-403)

![KR-403](image)

**Figure 4.7**: benzo[d]oxazole-2,7-diamine (KR-403).

To a solution of 2,6-dinitrophenol (5.17 mmol, 0.95 g) in methanol (75 mL), 10% palladium on activated carbon (10 wt% of 2,6-dinitrophenol, 0.10 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for three hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was immediately washed with methanol (25 mL). The filtrate was concentrated *in vacuo* yielding a brown solid. The solid was quickly re-dissolved in acetonitrile (50 mL) and di(imidazole-1-yl)methanimine (12.91 mmol, 2.08 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for eight hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (1:1 hexanes/ethyl acetate) provided a crystalline white solid in a 79% yield. The product of the reduction (2,6-diaminophenol) is air unstable and appropriate measures should be taken to minimize air exposure. Likewise, exposure of
benzo[d]oxazole-2,7-diamine (KR-403) to air should be minimized to avoid degradation.

**KR-403:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.14 (br s, 2H, NH$_2$), 6.78 (t, 1H, J = 7.6 Hz), 6.46 (d, 1H, J = 7.6 Hz), 6.32 (d, 1H, J = 8.0 Hz), 4.99 (br s, 2H, NH$_2$); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 162.7, 144.6, 136.3, 132.2, 124.5, 108.3, 104.9; HRMS mass calculated for C$_7$H$_8$N$_3$O (M+H)$^+$ 150.0662, found 150.0669; Delta 4.7 ppm.

2-Amino-6-fluorophenol (KR-400F)

Figure 4.8: 2-amino-6-fluorophenol (KR-400F).

To a of solution of 2-fluoro-6-nitrophenol (6.37 mmol, 1.00 g) in methanol (100 mL), 10% palladium on activated carbon (10 wt% of 2-fluoro-6-nitrophenol, 0.10 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for one hour. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered over a thin pad of celite which was then washed with methanol (50 mL). The filtrate was concentrated *in vacuo* yielding a
red solid. Silica gel column chromatography (1:1 hexanes/chloroform) provided a crystalline white solid in an 87% yield.

**KR-400F:** $\textsuperscript{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.88 (br s, 1H, OH), 6.50 (m, 1H), 6.38 (dd, 1H, $J = 7.2$ Hz, $J' = 1.2$ Hz), 6.30 (dt, 1H, $J = 7.2$ Hz, $J' = 2.0$ Hz), 4.82 (br s, 2H, NH$_2$); $\textsuperscript{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 154.0, 151.6, 140.6, 140.5, 131.5, 131.3, 120.0, 119.9, 110.7, 110.6, 103.9, 103.7; HRMS mass calculated for C$_6$H$_7$FNO (M+H)$^+$ 128.0506, found 128.0508; Delta 1.5 ppm.

7-Fluorobenzo[d]oxazol-2-amine (KR-401F)

![KR-401F](image)

**Figure 4.9:** 7-fluorobenzo[d]oxazol-2-amine (KR-401F).

To a of solution 6-fluoro-2-aminophenol (7.87 mmol, 1.00 g) in acetonitrile (60 mL), di(imidazole-1-yl)methanimine (19.67 mmol, 3.17 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for four hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) provided a crystalline white solid in a 79% yield.
KR-401F: $^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.24 (br s, 2H, NH$_2$), 7.09 (m, 2H), 6.90 (t, 1H, J = 8.4 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 163.0, 147.2, 144.7, 134.0, 125.4, 111.6, 108.8; HRMS mass calculated for C$_7$H$_6$FN$_2$O (M+H)$^+$ 153.0459, found 153.0464; Delta 3.3 ppm.

2-Chloro-6-nitrophenol (KR-400Cl)

![Structure of 2-Chloro-6-nitrophenol](image)

Figure 4.10: 2-chloro-6-nitrophenol (KR-400Cl).

To a of solution of 2-chlorophenol (0.155 mol, 15.8 mL) in glacial acetic acid (42 mL) chilled to 5 °C, nitric acid (0.163 mol, 6.8 mL) was added dropwise over a period of 45 minutes with constant magnetic stirring. The reaction was maintained at 5 °C for thirty minutes then poured over ice (~250 mL). The black solid which forms during the reaction is removed by filtration. The resulting dark brown liquid was steam distilled providing a yellow solid which was recrystallized from water to give KR-400Cl in an 18% yield.

KR-400Cl: $^1$H NMR (400 MHz, CDCl$_3$) δ 11.01 (br s, 1H, OH), 8.04 (dd, 1H, J = 8.0 Hz, J’ = 1.6 Hz), 7.69 (dd, 1H, J = 8.0 Hz, J’ = 1.6 Hz), 6.96 (t, 1H, J = 8.4 Hz); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 151.7, 137.9, 134.8, 124.8, 123.9, 119.9;
HRMS mass calculated for C₆H₃ClNO₃ (M+H)⁻ 171.9807, found 171.9808; Delta 0.6 ppm.

2-Amino-6-chlorophenol (KR-401Cl)

\[
\text{Cl} \quad \text{OH} \\
\text{NH}_2 \\
\text{KR-401Cl}
\]

**Figure 4.11:** 2-amino-6-chlorophenol (KR-401Cl).

Stannous chloride (74.76 mmol, 16.87 g) in concentrated HCl (28.5 mL) was added to a solution of 2-chloro-6-nitrophenol (KR-400Cl) (29.90 mmol, 5.19 g) in ethanol (215 mL) with constant magnetic stirring under argon atmosphere. After 24 hours the reaction was placed on ice and the pH was adjusted to 10 with 1N NaOH. The white precipitate was removed by filtration and washed with methanol (40 mL). The filtrate was concentrated *in vacuo*, redissolved in dichloromethane (75 mL), washed over water (70 mL) and brine (70 mL) respectively, and dried over sodium sulfate. The dichloromethane layer was concentrated under reduced pressure, and silica gel column chromatography of the brown solid (10:1 hexanes/ethyl acetate) provided a crystalline brown solid in a 59% yield.

**KR-401Cl:** $^1$H NMR (400 MHz, CDCl₃) δ 6.74 (dd, 1H, J = 8.0 Hz, J’ = 1.2 Hz), 6.69 (t, 1H, J = 8.0 Hz), 6.62 (dd, 1H, J = 8.0 Hz, J’ = 1.2 Hz), 4.03 (br s, 2H,
NH$_2$); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 139.6, 135.8, 121.4, 120.1, 118.6, 114.7; HRMS mass calculated for C$_6$H$_5$NOCl (M+H)$^-$ 142.0065, found 142.0064; Delta - 0.7 ppm.

7-Chlorobenzo[d]oxazol-2-amine (KR-402Cl)

![KR-402Cl](image)

Figure 4.12: 7-chlorobenzo[d]oxazol-2-amine (KR-402Cl).

Di(imidazole-1-yl)methanimine (17.59 mmol, 2.83 g) was added to a solution of 2-amino-6-chlorophenol (7.03 mmol, 1.01 g) in acetonitrile (40 mL) with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for ten hours. The reaction was then cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (2:1 hexanes/ethyl acetate) provided a crystalline white solid in a 92% yield.

**KR-402Cl:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.32 (br s, 2H, NH$_2$), 7.17 (d, 1H, J = 7.6 Hz), 7.11 (t, 1H, J = 8.0 Hz), 7.04 (d, 1H, J = 8.0 Hz); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 162.7, 144.1, 142.6, 125.8, 121.6, 114.1, 113.6; HRMS mass calculated for C$_7$H$_6$ClN$_2$O (M+H)$^+$ 169.0163, found 169.0168; Delta 3.0 ppm.
2-((4-methylpiperazin-1-yl)methyl)-6-Nitrophenol (KR-400151)

![Molecule Structure](attachment:KR-400151.png)

**Figure 4.13**: 2-((4-methylpiperazin-1-yl)methyl)-6-nitrophenol (**KR-400151**).

1-Methylpiperazine (12.57 mmol, 1.39 mL) was added to a solution of 3-nitrosalicylaldehyde (5.98 mmol, 1.00 g) in tetrahydrofuran (40 mL) and 1,2-dichloroethane (20 mL) with constant magnetic stirring. After 15 minutes, sodium triacetoxyborohydride (6.58 mmol, 1.40 g) was added and the resulting mixture was stirred at room temperature for four hours. The reaction was filtered and the filtrand was washed with dichloromethane (50 mL). The filtrate was concentrated *in vacuo* yielding an orange solid. Silica gel column chromatography (15:1 chloroform/methanol) provided a crystalline orange solid in a 98% yield.

**KR-400151**: $^1$H NMR (400 MHz, CDCl$_3$) δ 11.55 (br s, 1H, OH), 7.80 (d, 1H, J = 7.6 Hz), 7.26 (d, 1H, J = 7.2 Hz), 6.79 (t, 1H, J = 7.6 Hz), 3.73 (s, 2H), 2.58 (br s, 4H), 2.46 (br s, 4H), 2.23 (s, 3H); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 154.0, 136.8, 134.7, 125.3, 125.0, 118.5, 59.9, 54.7, 52.6, 46.0; HRMS mass calculated for C$_{12}$H$_{18}$N$_3$O$_3$ (M+H)$^+$ 252.1343, found 252.1344; Delta 0.4 ppm.
To a solution of 2-((4-methylpiperazin-1-yl)methyl)-6-nitrophenol (KR-400151) (6.13 mmol, 1.54 g) in methanol (110 mL), PtO₂ (0.31 mmol, 0.07 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then maintained under hydrogen atmosphere at ordinary pressure (15 psi) for fifteen hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (40 mL). The filtrate was concentrated in vacuo yielding a brown solid. The solid was quickly re-dissolved in acetonitrile (70 mL) and di(imidazole-1-yl)methanimine (15.32 mmol, 2.47 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (10:1 chloroform/methanol) provided a crystalline white solid in an 84% yield. The product of the reduction (2-amino-6-((4-methylpiperazin-1-yl)methyl)phenol) is air unstable and appropriate measures should be taken to
minimize air exposure. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.5-13% acetonitrile in water.

**KR-402151**: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.42 (br s, 2H, NH$_2$), 7.38 (d, 1H, J $= 5.6$ Hz), 7.28 (m, 2H), 4.32 (s, 2H), 3.44 (br s, 4H), 3.33 (br s, 4H), 2.83 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 161.3, 146.1, 135.2, 125.8, 114.8, 114.4, 53.5, 50.9, 48.8, 42.5; HRMS mass calculated for C$_{13}$H$_{19}$N$_4$O (M+H)$^+$ 247.1553, found 247.1557; Delta 1.6 ppm.

2-((dimethylamino)methyl)-6-Nitrophenol (KR-400103)

![Image](kr-400103.png)

**Figure 4.15**: 2-((dimethylamino)methyl)-6-nitrophenol (**KR-400103**).

Dimethylamine (12.57 mmol, 6.28 mL, 2M in MeOH) was added to a solution of 3-nitrosalicylaldehyde (5.98 mmol, 1.00 g) in tetrahydrofuran (40 mL) and 1,2-dichloroethane (20 mL) with constant magnetic stirring. After 15 minutes, sodium triacetoxyborohydride (6.58 mmol, 1.40 g) was added and the resulting mixture was stirred at room temperature for four hours. The reaction was filtered and the filtrand was washed with dichloromethane (50 mL). The filtrate was concentrated *in vacuo* yielding an orange solid. Silica gel column
chromatography (10:1 chloroform/methanol) provided a crystalline orange solid in a 96% yield.

**KR-400103:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 7.73 (dd, 1H, $J = 6.8$ Hz, $J' = 1.6$ Hz), 7.21 (dd, 1H, $J = 6.4$ Hz, $J' = 1.6$ Hz), 7.10 (br s, 1H, OH), 6.38 (t, 1H, $J = 6.8$ Hz), 3.95 (s, 2H), 2.52; $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 161.8, 137.3, 135.0, 126.7, 126.1, 112.1, 61.0, 43.1; HRMS mass calculated for C$_9$H$_{11}$N$_2$O$_3$ (M+H)$^-$ 195.0775, found 195.0778; Delta 1.5 ppm.

7-((dimethylamino)methyl)Benzo[d]oxazol-2-amine (KR-402103)

![KR-402103](image)

**Figure 4.16:** 7-((dimethylamino)methyl)benzo[d]oxazol-2-amine (KR-402103).

To a solution of 2-((dimethylamino)methyl)-6-nitrophenol (KR-400103) (5.74 mmol, 1.13 g) in methanol (120 mL), PtO$_2$ (0.29 mmol, 0.07 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then stirred under hydrogen atmosphere at ordinary pressure (15 psi) for ten hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then
immediately washed with methanol (50 mL). The filtrate was concentrated in vacuo yielding a brown solid. The solid was quickly re-dissolved in acetonitrile (120 mL) and di(imidazole-1-yl)methanimine (14.35 mmol, 2.31 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (10:1 chloroform/methanol) provided a colorless oil in an 81% yield. The product of the reduction (2-amino-6-((dimethylamino)methyl)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.5-7% acetonitrile in water.

**KR-402103**: $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 8.28 (br s, 2H, NH$_2$), 7.34 (d, 1H, J = 7.2 Hz), 7.22 (t, 1H, J = 7.6 Hz), 7.16 (d, 1H, J = 7.6 Hz), 4.41 (s, 2H), 2.77 (s, 6H); $^{13}$C NMR (400 MHz, DMSO-d$_6$) $\delta$ 162.7, 147.6, 141.2, 125.1, 124.4, 116.6, 112.2, 54.6, 42.7; HRMS mass calculated for C$_{10}$H$_{14}$N$_3$O (M+H)$^+$ 192.1131, found 192.1132; Delta 0.5 ppm.
2-(((2-(dimethylamino)ethyl)amino)methyl)-6-Nitrophenol (KR-400423)

![KR-400423]

Figure 4.17: 2-(((2-(dimethylamino)ethyl)amino)methyl)-6-nitrophenol (KR-400423).

\[ N^1,N^1-Dimethylethane-1,2-diamine \ (12.57 \text{ mmol}, \ 1.37 \text{ mL}) \]

was added to a solution of 3-nitrosalicylaldehyde (5.98 mmol, 1.00 g) in tetrahydrofuran (40 mL) and 1,2-dichloroethane (20 mL) with constant magnetic stirring. After 15 minutes, sodium triacetoxyborohydride (6.58 mmol, 1.40 g) was added and the resulting mixture was stirred at room temperature for twenty hours. The reaction was filtered and the filtrand was washed with dichloromethane (50 mL). The filtrate was concentrated in vacuo yielding an orange solid. Silica gel column chromatography (10:1 chloroform/methanol) provided a yellow solid in an 88% yield.

**KR-400423:** \(^1\text{H NMR} (500 \text{ MHz, MeOD-}d_4/\text{CDCl}_3) \delta 7.89 \ (\text{dd}, \ 1\text{H}, \ J = 7.2 \text{ Hz}, \ J' = 2.0 \text{ Hz}), \ 7.28 \ (\text{dd}, \ 1\text{H}, \ J = 7.1 \text{ Hz}, \ J' = 2.0 \text{ Hz}), \ 6.51 \ (\text{t}, \ 1\text{H}, \ J = 7.2 \text{ Hz}), \ 3.98 \ (\text{s}, \ 2\text{H}), \ 2.89 \ (\text{t}, \ 2\text{H}, \ J = 6.3 \text{ Hz}), \ 2.59 \ (\text{t}, \ 2\text{H}, \ J = 6.3 \text{ Hz}), \ 2.28 \ (\text{s}, \ 6\text{H}); \ ^{13}\text{C NMR} (500 \text{ MHz, MeOD-}d_4/\text{CDCl}_3) \delta 160.4, \ 136.6, \ 135.6, \ 126.5, \ 126.3, \ 113.9, \ 55.7, \ 40.6,
44.4, 43.9; HRMS mass calculated for C_{11}H_{18}N_{3}O_{3} (M+H)^+ 240.1343, found 240.1344; Delta 0.4 ppm.

**2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-6-Nitrophenol** (KR-400424)

![Chemical Structure](attachment:image.png)

**Figure 4.18:** 2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-6-nitrophenol (KR-400424).

Formaldehyde (8.02 mmol, 0.60 mL, 37 wt. % in H_2O) was added to a solution of 2-(((2-(dimethylamino)ethyl)amino)methyl)-6-nitrophenol (KR-400423) (4.01 mmol, 0.96 g) in tetrahydrofuran (150 mL) and methanol (25 mL) with constant magnetic stirring. After 15 minutes, sodium triacetoxyborohydride (6.02 mmol, 1.28 g) was added and the resulting mixture was stirred at room temperature for twenty hours. The reaction was filtered and the filtrand was washed with dichloromethane (50 mL). The filtrate was concentrated *in vacuo* yielding an orange oil. Silica gel column chromatography (10:1 chloroform/methanol) provided a yellow solid in a 90% yield.
**KR-400424**: $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 7.59 (dd, 1H, $J = 7.2$ Hz, $J' = 1.2$ Hz), 7.09 (d, 1H, $J = 6.8$ Hz), 6.27 (t, 1H, $J = 7.2$ Hz), 3.38 (s, 2H), 3.02 (t, 2H, $J = 6.0$ Hz), 2.64 (t, 2H, $J = 6.0$ Hz), 2.51 (s, 6H), 2.15 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 162.2, 138.7, 134.9, 130.8, 125.7, 111.0, 55.9, 53.8, 51.2, 43.3, 42.3; HRMS mass calculated for C$_{12}$H$_{20}$N$_3$O$_3$ (M+H)$^+$ 254.1499, found 254.1500; Delta 0.4 ppm.

$N^1$-((2-aminobenzo[d]oxazol-7-yl)methyl)-$N^1,N^2,N^2$-Trimethylethane-1,2-diamine (KR-402424)

![Figure 4.19: $N^1$-((2-aminobenzo[d]oxazol-7-yl)methyl)-$N^1,N^2,N^2$-trimethylethane-1,2-diamine (KR-402424).](image)

To a solution of 2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-6-nitrophenol (KR-400424) (3.91 mmol, 0.99 g) in methanol (125 mL), PtO$_2$ (0.20 mmol, 0.04 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then stirred under hydrogen atmosphere at ordinary pressure (15 psi) for two hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was
then immediately washed with methanol (50 mL). The filtrate was concentrated in vacuo yielding a brown oil. The oil was quickly re-dissolved in acetonitrile (150 mL) and di(imidazole-1-yl)methanimine (7.82 mmol, 1.26 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was concentrated under reduced pressure and silica gel column chromatography (15:1 chloroform/methanol) provided a faint yellow oil in a 62% yield. The product of the reduction (2-amino-6-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)phenol) is air unstable and appropriate measures should be taken to minimize air exposure. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.5-15% acetonitrile in water.

**KR-402424:** $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.68 (br s, 2H, NH), 7.38 (d, 1H, J = 7.6 Hz), 7.34 (d, 1H, J = 7.6 Hz), 7.26 (t, 1H, J = 7.6 Hz), 4.53 (s, 2H), 3.62 (m, 4H), 2.86 (s, 6H), 2.78 (s, 3H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$ 162.0, 147.2, 139.2, 125.4, 119.9, 116.3, 112.0, 53.6, 51.1, 49.5, 43.4; HRMS mass calculated for C$_{13}$H$_{21}$N$_4$O (M+H)$^+$ 249.1710, found 249.1709; Delta -0.4 ppm.
Figure 4.20: $\text{N}^1$-(7-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)benzo[\text{d}]oxazol-2-yl)-$\text{N}^3,\text{N}^3$-dimethylpropane-1,3-diamine (KR-403424).

To a solution of $\text{N}^1$-((2-aminobenzo[\text{d}]oxazol-7-yl)methyl)-$\text{N}^1,\text{N}^2,\text{N}^2$-trimethylethane-1,2-diamine (KR-402424) (2.94 mmol, 0.73 g) in acetonitrile (200 mL), Cs$_2$CO$_3$ (3.23 mmol, 1.05 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere. 3-Chloro-$\text{N},\text{N}$-dimethylpropan-1-amine (2.96 mmol, 0.39 mL) was added and the reaction was heated to 55 °C for two days. Then the reaction was cooled to room temperature, filtered, and the filtrand was washed with methanol (50 mL). The filtrate was concentrated in vacuo yielding a brown oil. Silica gel column chromatography (5:1 chloroform/methanol) provided a faint brown oil in a 59% yield. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 0.2-10% acetonitrile in water.

KR-403424: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.47 (br s, 2H, NH$_2$), 7.09 (t, 1H, J = 8.0 Hz), 7.04 (t, 1H, J = 8.0 Hz), 6.90 (dd, 1H, J = 8.0 Hz), 3.66 (s, 3H), 3.50 (t,
2H), 3.27 (t, 2H), 3.08 (s, 6H), 2.76 (t, 2H), 2.72(s, 2H), 2.53 (t, 2H), 2.39 (s, 6H),
1.91 (m, 2H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 163.4, 147.5, 144.3, 124.0, 122.0,
119.7, 115.0, 61.9, 60.2, 56.2, 55.1, 52.0, 51.2, 44.2, 43.3, 42.0; HRMS mass
calculated for C$_{18}$H$_{32}$N$_5$O (M+H)$^+$ 334.2601, found 334.2599; Delta -0.6 ppm.

3-((2-hydroxy-3-nitrobenzyl)amino)Propanenitrile (KR-400428)

![Chemical structure of KR-400428](image)

**Figure 4.21:** 3-((2-hydroxy-3-nitrobenzyl)amino)propanenitrile (KR-400428).

3-(methylamino)Propanenitrile (6.28 mmol, 0.60 mL) was added to a
solution of 3-nitrosalicylaldehyde (5.98 mmol, 1.00 g) in tetrahydrofuran (120 mL)
with constant magnetic stirring. After 15 minutes, sodium triacetoxyborohydride
(8.98 mmol, 1.90 g) was added and the resulting mixture was stirred at room
temperature for sixteen hours. The reaction was filtered and the filtrand was
washed with dichloromethane (100 mL). The filtrate was concentrated *in vacuo*
yielding an orange solid. Silica gel column chromatography (20:1 hexanes/ethyl
acetate) provided a yellow solid in a 92% yield.
KR-400428: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 10.91 (br s, 1H, OH), 7.80 (d, 1H, J = 8.0 Hz), 7.46 (t, 1H, J = 8.0 Hz), 6.82 (d, 1H, J = 8.0 Hz), 3.66 (s, 2H), 2.75 (t, 2H, J = 6.8 Hz), 2.54 (t, 2H, J = 6.8 Hz), 2.26 (s, 3H); $^{13}$C NMR (400 MHz, CDCl$_3$) $\delta$ 153.5, 136.6, 135.3, 127.5, 124.5, 119.4, 118.9, 56.7, 52.4, 41.5, 16.2.

3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)propanenitrile (KR-402428)

![Diagram](image)

Figure 4.22: 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)propanenitrile (KR-402428).

To a solution of 3-((2-hydroxy-3-nitrobenzyl)amino)propanenitrile (KR-400428) (3.87 mmol, 0.91 g) in methanol (125 mL), PtO$_2$ (0.19 mmol, 0.04 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then stirred under hydrogen atmosphere at ordinary pressure (15 psi) for two hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (75 mL). The filtrate was concentrated in
vacuo yielding a brown oil. The oil was quickly re-dissolved in acetonitrile (175 mL) and di(imidazole-1-yl)methanimine (7.74 mmol, 1.25 g) was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (20:1 ethyl acetate/methanol) provided faint brown crystals in a 67% yield. The product of the reduction (3-((3-amino-2-hydroxybenzyl)(methyl)amino)propanenitrile) is air sensitive and appropriate measures should be taken to minimize air exposure.

**KR-402428:** \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 7.38 (br s, 2H, NH\(_2\)), 7.08 (d, 1H, J = 8.0 Hz), 7.03 (t, 1H, J = 8.0 Hz), 6.92 (d, 1H, J = 8.0 Hz), 3.66 (s, 2H), 2.66 (t, 2H, J = 7.6 Hz), 2.60 (t, 2H, J = 7.6 Hz), 2.17 (s, 3H); \(^{13}\)C NMR (400 MHz, CDCl\(_3\)) \(\delta\) 163.3, 147.5, 144.1, 123.9, 121.7, 120.6, 119.8, 114.7, 60.4, 52.3, 42.0, 16.0; Crystal structure available.
3-((2-hydroxy-3-nitrobenzyl)(methyl)amino)-N,N-Dimethylpropanamide (KR-400150)

![Chemical Structure](image)

**Figure 4.23:** 3-((2-hydroxy-3-nitrobenzyl)(methyl)amino)-N,N-dimethylpropanamide (KR-400150).

\[ N,N\text{-Dimethyl}-3\text{-(methylamino)propanamide} \text{ (6.28 mmol, 0.82 g) was added to a solution of 3-nitrosalicylaldehyde} \text{ (5.98 mmol, 1.00 g) in tetrahydrofuran (100 mL) with constant magnetic stirring. After 15 minutes, sodium triacetoxyborohydride} \text{ (8.98 mmol, 1.90 g) was added and the resulting mixture was stirred at room temperature for twenty-two hours. The reaction was filtered and the filtrand was washed with dichloromethane (100 mL). The filtrate was concentrated} \text{ in vacuo yielding a yellow solid. Silica gel column chromatography} \text{ (15:1 chloroform/methanol) provided a yellow solid in a 90% yield.}

KR-400150: \( ^1\text{H NMR (400 MHz, CDCl}_3) \delta 9.94 \text{ (br s, 1H, OH), 7.73 (dd, 1H, J = 7.2 Hz, J' = 1.6 Hz), 7.27 (dd, 1H, J = 7.2 Hz, J' = 1.6 Hz), 6.56 (t, 1H, J = 7.2 Hz), 3.97 (s, 2H), 2.78 (s, 6H), 2.68 (t, 2H, J = 7.2 Hz), 2.39 (s, 2H);} ^{13}\text{C NMR (400 MHz, CDCl}_3) \delta 161.77, 157.03, 147.04, 139.83, 130.48, 128.73, 128.01, 125.42, 124.63, 122.82, 116.49, 51.96, 47.56, 43.98, 33.84, 27.12, 23.23, 21.89, 21.57 \text{ ppm.} \]
MHz, CDCl₃) δ 170.5, 158.7, 137.5, 134.8, 126.0, 125.8, 114.7, 59.6, 52.3, 37.2, 35.4, 29.4.

3-(((2-aminobenzodioxazol-7-yl)methyl)(methyl)amino)-N,N-Dimethylpropanamide (KR-402150)

\[ \text{Figure 4.24: } 3-(((2-aminobenzodioxazol-7-yl)methyl)(methyl)amino)-N,N-di \text{-methylpropanamide (KR-402150).} \]

To a solution of 3-((2-hydroxy-3-nitrobenzyl)(methyl)amino)-N,N-dimethylpropanamide (KR-400450) (3.70 mmol, 1.04 g) in methanol (125 mL), PtO₂ (0.18 mmol, 0.04 g) was added. The reaction was flushed with argon followed by hydrogen for fifteen minutes each with constant magnetic stirring. The reaction was then stirred under hydrogen atmosphere at ordinary pressure (15 psi) for three hours. Argon was again flushed through the reaction vessel for 15 minutes. Then the reaction contents were filtered quickly over a thin pad of celite which was then immediately washed with methanol (90 mL). The filtrate was concentrated \textit{in vacuo} yielding a brown oil. The oil was quickly re-dissolved in acetonitrile (175 mL) and di(imidazole-1-yl)methanimine (7.39 mmol, 1.19 g)
was added with constant magnetic stirring. The reaction was then placed under argon atmosphere and refluxed for six hours. The reaction was cooled to room temperature, concentrated under reduced pressure and silica gel column chromatography (10:1 chloroform/methanol) provided a faint orange oil in a 64% yield. The product of the reduction (3-((3-amino-2-hydroxybenzyl)(methyl)amino)-N,N-dimethylpropanamide) is air sensitive and appropriate measures should be taken to minimize air exposure. An analytical sample of the title compound was isolated by reverse-phase HPLC utilizing a gradient from 3.0-20% acetonitrile in water.

**KR-402150:** $^1$H NMR (400 MHz, DMSO-$d_6$) δ 7.87 (br s, 2H, NH$_2$), 7.31 (d, 1H, J = 7.6 Hz), 7.21 (t, 1H, J = 8.0 Hz), 7.16 (d, 1H, J = 7.6 Hz), 4.45 (d, 2H, J = 6.8 Hz), 2.95 (s, 3H), 2.84 (t, 2H, J = 6.8 Hz), 2.82 (s, 3H), 2.76 (s, 3H), 2.48 (m, 2H); $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ 169.9, 163.4, 148.0, 143.1, 124.8, 124.0, 117.0, 111.6, 54.0, 51.0, 37.2, 35.5, 27.7.

**General Techniques**

Commercial reagents were obtained (Sigma Aldrich, Acros, Alfa Aesar, Fisher) and used without any further purification. Non-aqueous reactions were carried out under anhydrous conditions using oven-dried glassware under an inert atmosphere in dry, freshly distilled solvents. All air-sensitive reaction solutions were transferred via syringe or stainless steel cannula. Reactions were monitored by TLC, which was performed on pre-coated silica gel 60 F254 plates
supplied by EMD, visualized utilizing UV light, and developed using ceric ammonium molybdate stain (CAM) with heat or ninhydrin stain with heat. Organic solvents were removed by rotary evaporation below 30 °C at approximately 15 mmHg. Flash column chromatography was executed with silica gel 60 (230-400 mesh) supplied by Silicycle and eluting solvents are indicated in the text. Yields refer to chromatographically and spectroscopically (¹H NMR, ¹³C NMR) homogeneous materials, unless otherwise stated. NMR spectra were recorded on Varian Mercury 400 MHz, Jeol Unity 500 MHz, and Agilent VNMR S 500 MHz outfitted with an XSens cold probe using deuterochloroform, deuteromethanol, deuterowater or deuterodimethyl sulfoxide (Cambridge Isotope) as solvents. The chemical shifts are given in ppm relative to the standard reference TMS or residual undeuterated solvent. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublet, dt = doublet of triplet. Low resolution mass spectra were obtained on a ThermoFinnigan LCQDECA-MS spectrometer. High resolution mass spectra (HRMS) were recorded on a VG 7070 HS or a VG ZAB-ZSE mass spectrometer. Crystals were mounted in nylon loops using Paratone oil and then placed on the diffractometer under a nitrogen stream at 100K. All crystallographic data has been deposited at the CCDC, 12 Union Road, Cambridge CB21EZ, UK.
Spectral Data

Nuclear Magnetic Resonance

Spectrum 4.1: 2-amino-6-nitrophenol (KR-401) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 4.2: 2-amino-6-nitrophenol (KR-401) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 4.3: 7-nitrobenzo[d]oxazol-2-amine (KR-403) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.4: 7-nitrobenzo[d]oxazol-2-amine (KR-402) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.5: benzo[d]oxazole-2,7-diamine (KR-403) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.6: benzo[d]oxazole-2,7-diamine (KR-403) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.7: 2-amino-6-fluorophenol (KR-400F) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.8: 2-amino-6-fluorophenol (KR-400F) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.9: 7-fluorobenzo[d]oxazol-2-amine (KR-401F) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.10: 7-fluorobenzo[d]oxazol-2-amine (KR-401F) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.11: 2-chloro-6-nitrophenol (KR-400Cl) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 4.12: 2-chloro-6-nitrophenol (KR-400Cl) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 4.13: 2-amino-6-chlorophenol (KR-401Cl) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 4.14: 2-amino-6-chlorophenol (KR-401Cl) $^{13}$C NMR (400 MHz, CDCl$_3$).
**Spectrum 4.15:** 7-chlorobenzo[d]oxazol-2-amine (KR-402Cl) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.16: 7-chlorobenzo[d]oxazol-2-amine (KR-402Cl) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.17: 2-((4-methylpiperazin-1-yl)methyl)-6-nitrophenol (KR-400151) 
$^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 4.18: 2-((4-methylpiperazin-1-yl)methyl)-6-nitrophenol (KR-400151)

$^{13}$C NMR (400 MHz, CDCl$_3$).
**Spectrum 4.19:** 7-((4-methylpiperazin-1-yl)methyl)benzo[d]oxazol-2-amine (KR-402151) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.20: 7-((4-methylpiperazin-1-yl)methyl)benzo[d]oxazol-2-amine (KR-402151) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
**Spectrum 4.21:** 2-((dimethylamino)methyl)-6-nitrophenol (KR-400103) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.22: 2-((dimethylamino)methyl)-6-nitrophenol (KR-400103) $^{13}$C NMR (400 MHz, DMSO-$_d$$_6$).

KR-400103
$^1$H NMR (400 MHz, DMSO-$d_6$).
$^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.25: 2-(((2-(dimethylamino)ethyl)amino)methyl)-6-nitrophenol (KR-400423) $^1$H NMR (400 MHz, MeOD-$d_4$/CDCl$_3$).
**Spectrum 4.26:** 2-(((2-(dimethylamino)ethyl)amino)methyl)-6-nitrophenol (KR-400423) $^{13}$C NMR (400 MHz, MeOD-$d_4$/CDCl$_3$).
**Spectrum 4.27**: 2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-6-nitrophenol (KR-400424) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
Spectrum 4.28: 2-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)-6-nitrophenol (KR-400424) $^{13}$C NMR (400 MHz, DMSO-d$_6$).
**Spectrum 4.29:** $N^1$-((2-aminobenzo[d]oxazol-7-yl)methyl)-$N^1,N^2,N^2$-trimethyl-ethane-1,2-diamine (KR-402424) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.30: $N^1$-((2-aminobenzo[d]oxazol-7-yl)methyl)-$N^1,N^2,N^2$-trimethyl-ethane-1,2-diamine (KR-402424) $^{13}$C NMR (400 MHz, DMSO-$d_6$)
**Spectrum 4.31:** $N^1$-(7-(((2-(dimethylamino)ethyl)(methyl)amino)methyl) benzo[\textit{d}]-oxazol-2-yl)-$N^2$,\textit{N}$_3$-dimethylpropane-1,3-diamine (KR-403424) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.32: $N^1$-(7-(((2-(dimethylamino)ethyl)(methyl)amino)methyl)benzo[$d$]-oxazol-2-yl)-$N^3,N^3$-dimethylpropane-1,3-diamine (KR-403424) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.33: 3-((2-hydroxy-3-nitrobenzyl)(methyl)amino)propanenitrile (KR-400428) $^1$H NMR (400 MHz, CDCl$_3$).
**Spectrum 4.34:** 3-((2-hydroxy-3-nitrobenzyl)(methyl)amino)propanenitrile (KR-400428) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 4.35: 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)propanenitrile (KR-402428) \(^1\)H NMR (400 MHz, DMSO-\(d_6\)).
3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)propanenitrile (KR-402428) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.37: 3-((2-hydroxy-3-nitrobenzyl)(methyl)amino)-N,N-dimethyl propanamide (KR-400150) $^1$H NMR (400 MHz, CDCl$_3$).
Spectrum 4.38: 3-((2-hydroxy-3-nitrobenzyl)(methyl)amino)-N,N-dimethyl propanamide (KR-400150) $^{13}$C NMR (400 MHz, CDCl$_3$).
Spectrum 4.39: 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)-N,N-dimethylpropanamide (KR-402150) $^1$H NMR (400 MHz, DMSO-$d_6$).
Spectrum 4.40: 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)-N,N-dimethylpropanamide (KR-402150) $^{13}$C NMR (400 MHz, DMSO-$d_6$).
Crystal Structures

**Spectrum 4.41:** Crystal structure of 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)-(methyl)amino)propane-nitrile (KR-402428).

**Spectrum 4.42:** Hydrogen bonding pattern observed in crystal structure of 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)propane-nitrile (KR-402428).
**Spectrum 4.43:** Crystal packing observed in crystal structure of 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)propane-nitrile (KR-402428).

**Spectrum 4.44:** Alternative view of crystal packing observed in crystal structure of 3-(((2-aminobenzo[d]oxazol-7-yl)methyl)(methyl)amino)propane-nitrile (KR-402428).
References


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