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2-Phenyl-4-piperazinylbenzimidazoles: Orally active inhibitors of the gonadotropin releasing hormone (GnRH) receptor

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ABSTRACT

Antagonism of the gonadotropin releasing hormone (GnRH) receptor has shown positive clinical results in numerous reproductive tissue disorders such as endometriosis, prostate cancer and others. Traditional therapy has been limited to peptide agonists and antagonists. Recently, small molecule GnRH antagonists have emerged as potentially new treatments. This article describes the discovery of 2-phenyl-4-piperazinylbenzimidazoles as small molecule GnRH antagonists with nanomolar potency in in vitro binding and functional assays, excellent bioavailability (rat $\%F > 70$) and demonstrated oral activity in a rat model having shown significant serum leuteinizing hormone (LH) suppression.

1. Introduction

Gonadotropin releasing hormone (GnRH), or leuteinizing hormone releasing hormone (LHRH), is a 10 amino acid peptide synthesized and released in a pulsatile fashion from the hypothalamus.[1] The hormone stimulates a G-protein coupled receptor (GPCR)—the GnRH receptor (GnRHR)—positively coupled to phospholipase C on cell surfaces of the anterior pituitary gland. Following stimulation of the GnRH receptor the gonadotropins, follicle stimulating hormone (FSH) and leuteinizing hormone (LH) are released into the general circulation where they act on the male and female gonads to trigger gametogenesis as well as synthesis and release of sex steroids.[2] Early structural modifications to GnRH led to agonists with enhanced activity over the native hormone. In clinical settings these 'superagonists' have been used to treat various cancers of the reproductive organs as well as primary hirsutism, endometriosis, and other hormone dependant conditions through the suppression of sex steroids.[3] The concept of hormone suppression with an agonist/superagonist is unique. Constant exposure of the GnRH receptor to a GnRH agonist leads to receptor desensitization and eventual down-regulation causing an overall antagonistic effect. During this process, however, there is a 2 week delay for the onset of the antagonist effect and during this time patients experience exacerbation of symptoms (flare effect).

In recent years more extensive modifications to GnRH have given rise to peptide antagonists such as abarelix and cetrorelix.[4] Direct blockade of the GnRHR leads to immediate and full antagonism of the receptor with no flare effect. As expected, both peptide superagonists and antagonists have poor pharmacokinetic and bioavailability properties when taken orally. Hence, these agents are only effective following parenteral administration. In addition, many patients receive extended release forms of these drugs that tend to lower hormones to castration levels. An orally active small molecule GnRH antagonist could offer additional benefits such as regimen compliance, the ability to titrate drug and hormone levels as well as the flexibility to withdraw the drug relatively quickly when adverse symptoms are seen.[5]

Recently, numerous small molecule GnRH antagonists representing various pharmacophores bonded to several template classes have been reported in the literature. Indoles,[6] quinolones,[7] furans,[8] uracils,[9] benzimidazoles,[10] and thienopyridines[11] are some of the GnRH receptor antagonists disclosed (Fig. 1). Stoichiometric association and nanomolar affinity between a broad, structurally diverse class of small molecules and a single receptor is unusual and a model has been proposed to explain this promiscuity.[12] Considering the breadth of chemical spatial diversity and the acceptable ADME descriptors associated with these series it would be expected to see good to excellent oral

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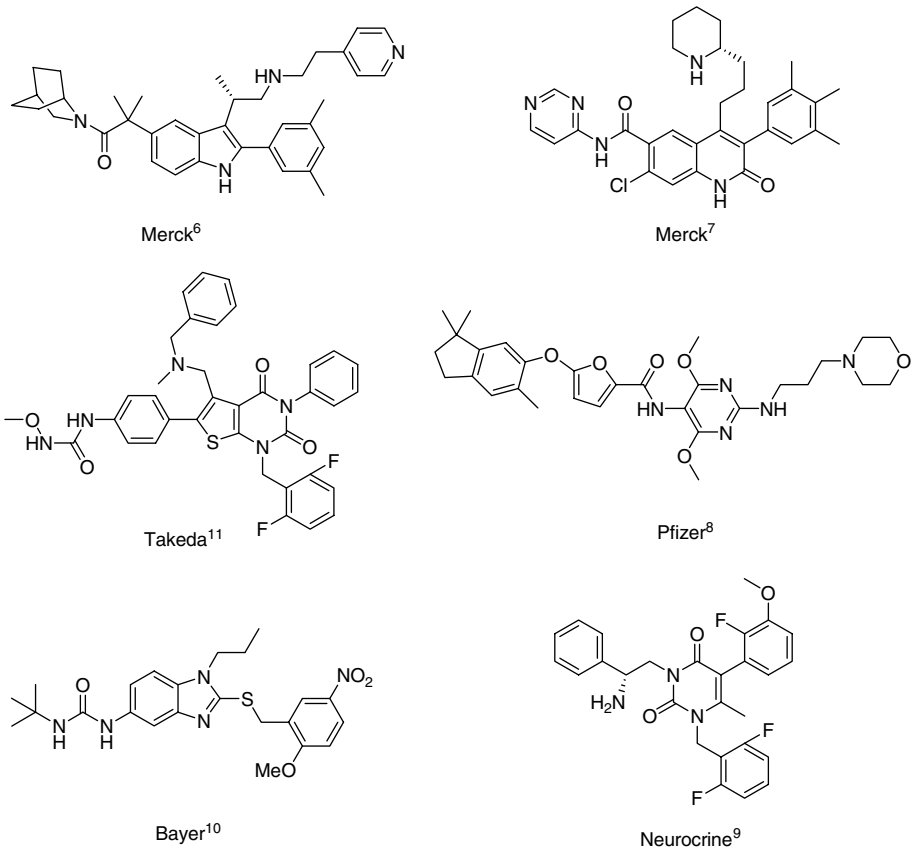
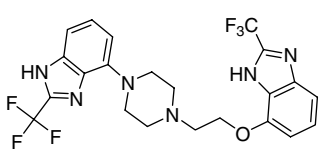


Figure 1. Representative small molecule GnRH antagonists recently reported in the literature.

bioavailability from compounds in each class. In most cases, however, oral bioavailability is poor to modest ($F < 25\%$). In addition, small molecule association with the GnRH receptor can be species selective. As a result, many compounds may require preclinical evaluation in expensive monkey models. Reported here are the lead discovery and in vitro structural optimization of a series of 2-phenyl-4-piperazinylbenzimidazole antagonists of the GnRH receptor. Optimization efforts resulted in the discovery of a compound with excellent oral bioavailability and rat plasma LH suppression properties.

Efforts to identify chemical leads were initiated through screening of a platform-based biogenic amine library rich in GPCR ligands (~2200 compounds) using a previously reported hGnRH binding assay[13] for receptor affinity followed by a functional assay[14] to ensure that compounds had antagonist properties. This led to the identification of two structurally related 4-piperazinylbenzimidazoles, **1a** and **1b** (Fig. 2), that had modest binding and antagonist properties on the human GnRH receptor as well as potent binding properties to several central 5HT-1 receptor subtypes. In addition, further screening indicated both compounds had affinity for the rat GnRH receptor and both were antagonists as measured by LH release inhibition from primary rat pituitary cells. The compounds, however, were weaker (25- to 50-fold) in this species. The possibility of in vivo evaluation in a lower species such as the rat was attractive. The leads were suitable for analogue purposes, hence an optimization effort was initiated to increase binding and functional potency at the human and rat receptors as well as selectivity over the 5HT-1 receptor subtypes.

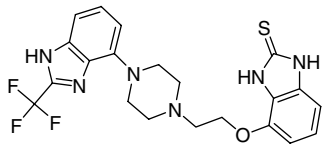


1a

hGnRH binding IC_{50} = 1.54 μM
hIP turnover inhibition IC_{50} = 9.2 μM

rGnRH binding IC_{50} = 15.0 μM
rLH release inhibition IC_{50} = 83.0 μM

h5-HT_{1A} Ki = 19 nM
h5-HT_{1B} Ki = 60 nM
h5-HT_{1D} Ki = 0.55 nM



1b

hGnRH binding IC_{50} = 0.80 μM
hIP turnover inhibition IC_{50} = 4.5 μM

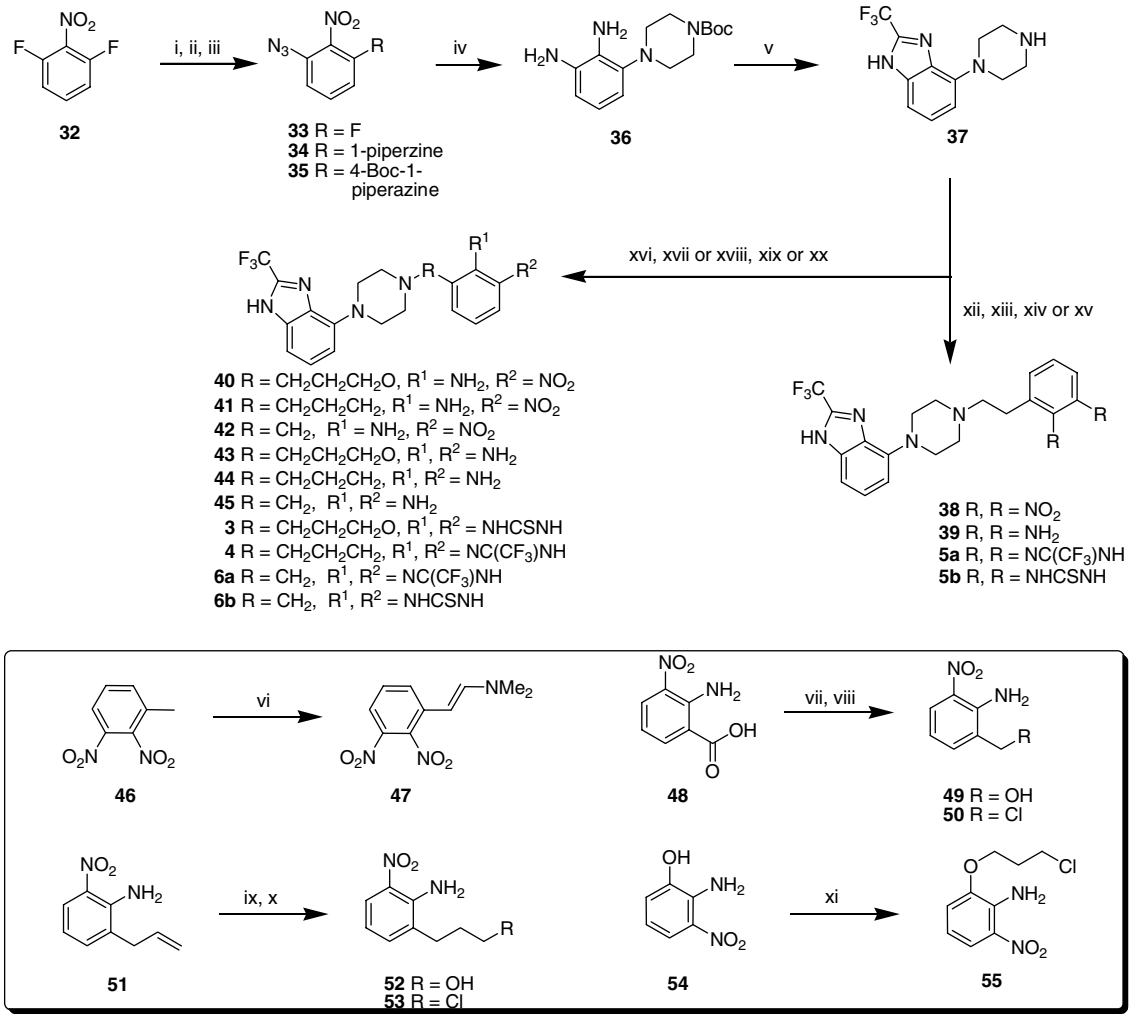
rGnRH binding IC_{50} = 21.0 μM
rLH release inhibition IC_{50} = 76.0 μM

h5-HT_{1A} Ki = 2.8 nM
h5-HT_{1B} 67% inhibition @ 1000 nM
h5-HT_{1D} = 67% inhibition @ 1000 nM

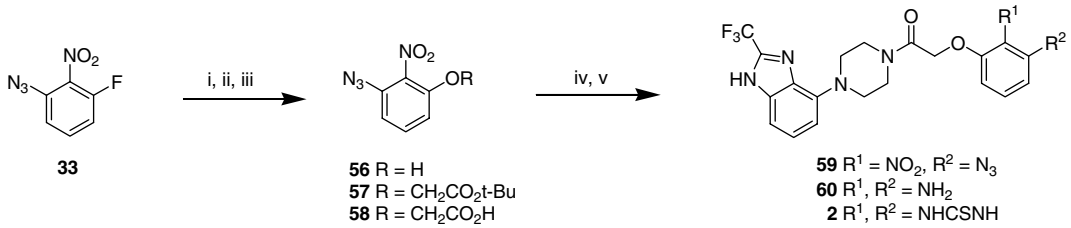
Figure 2. 4-piperazinylbenzimidazoles **1a** and **1b**, with GnRH antagonist properties, were identified from screening a GPCR privileged library.

Preparation of compounds with modified ethoxy linkers connecting the piperazine with the pendant 2-trifluoromethylbenzimidazole or the 2-thiobenzimidazolone is shown in Schemes 1 and 2. Treatment of 2,6-difluoronitrobenzene **32** with sodium azide then piperazine gave the azido-piperazinyl nitrobenzene intermediate **34** which was protected and fully reduced to the phenylenediamine **36**. Treatment of this compound with hot trifluoroacetic acid (TFA)[15] gave the key deprotected intermediate benzimidazole **37**. Reductive amination or N-alkylation with electrophiles, prepared from literature procedures shown in the Scheme 1 inset,[16–20] gave intermediates with the various linkers. Nitro groups on all of these intermediates were reduced under the standard conditions of hydrogenation or tin(II) chloride dihydrate ($SnCl_2 \cdot 2H_2O$) to provide the corresponding phenylenediamines, which were further reacted with thiocarbonyldiimidazole (thioCDI) and/or hot TFA to provide the desired products **3**, **4**, **5a**, **5b**, **6a**, and **6b**. In addition, the glycolamide **2** was prepared by converting the fluoro of 2-azido-6-fluoronitrobenzene **33** to a phenol[21] then alkylating the phenol with *tert*-butyl bromoacetate to provide **57** (Scheme 2). TFA deprotection of the acid followed by coupling with the piperazine **37** led to amide **59**. The nitro and azide groups were reduced by catalytic hydrogenation and conversion to the desired product **2** was achieved by reacting the phenylenediamine **60** with thioCDI.

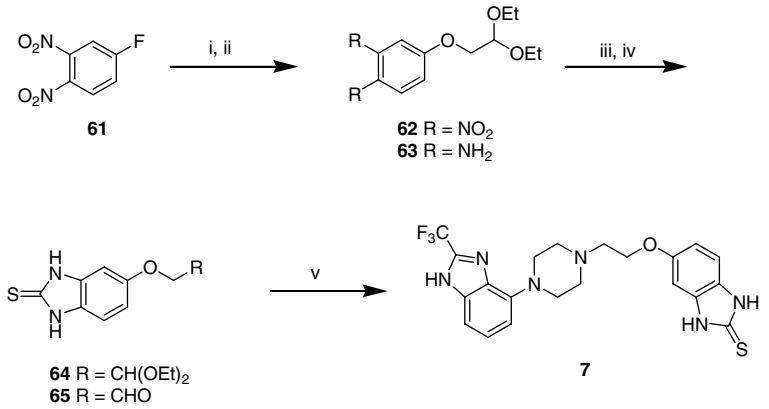
Preparation of compound **7**, with the ethoxy linker shifted one carbon on the pendant thiobenzimidazolone, is shown in Scheme 3. The fluorine of 3,4-dinitrofluorobenzene **61** was displaced with the sodium salt of glycolaldehyde diethyl acetal, and the nitro groups were reduced to provide the phenylene diamine **63**. Treatment of this intermediate with thioCDI followed by hydrolysis gave the thiobenzimidazolone glycolaldehyde **65**, which was reductively aminated with piperazine derivative **37** to provide **7**. In addition, the piperazine on the piperazinylbenzimidazole template was also shifted one carbon. The preparation of this compound is shown in Scheme 4. As in Scheme 3, the fluoro of 3,4-dinitrofluorobenzene was displaced with monoprotected piperazine, and the product was fully reduced to the phenylenediamine to provide intermediate **67**. This underwent deprotection and trifluorobenzimidazole formation upon treatment with hot TFA to give **68** which was reacted with aldehyde **70**, prepared in similar fashion to aldehyde **65** (see Scheme 3) starting from 2-azido-6-fluoronitrobenzene, to provide the nitroazide **71**. Full reduction followed by thioCDI treatment as above provided the desired product **9**.



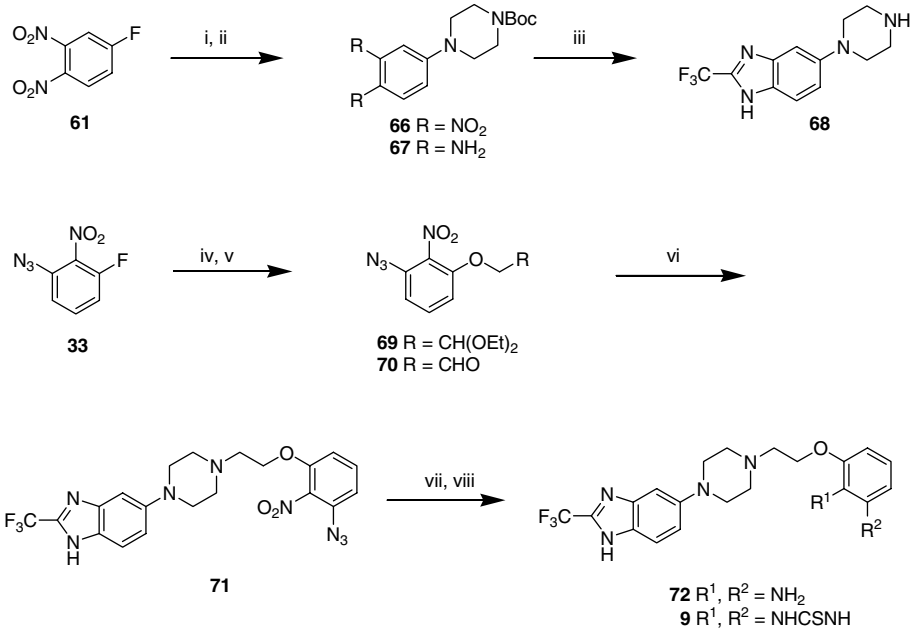
Scheme 1. Preparation of analogues with a modified linker. Reagents and conditions: (i) NaN_3, DMSO, 60 °C, 100%; (ii) piperazine, DMSO, 60 °C, 95%; (iii) $(Boc)_2O$, 100%; (iv) H_2, 10% Pd/C, MeOH, 100%; (v) TFA, 70 °C, 89%; (vi) DMF–DMA, 150 °C (Ref. 16); (vii) $SOCl_2$, PhH, Ref., then $NaBH_4$, MeOH, 46% for two steps (Refs. 17,18); (viii) $SOCl_2$, TEA, CH_2Cl_2, 82%; (ix) 1.0 M BH_3/THF, THF, 62%; (x) $SOCl_2$, TEA, CH_2Cl_2, 57%; (xi) 1-bromo-3-chloropropane, K_2CO_3, DMF, 90 °C, 46%; (xii) **47**, $NaCNBH_3$, THF, AcOH, H_2O, 44%; (xiii) H_2, 10% Pd/C, MeOH; (xiv) ThioCDI, THF, 70 °C, 23% for three steps to **5b**; (xv) TFA, 70 °C, 61% for **5a**; (xvi) **50** or **53** or **55**, DIPEA, DMF, 80 °C, yield of **42** = 41%, **41** = 22%, **40** = 85%; (xvii) **40**, H_2, 10% Pd/C, MeOH, 100%; (xviii) **41** or **42**, $SnCl_2 \cdot 2H_2O$, NMP, yield of **44** = 63%, **45** = 50%; (xix) **43** or **45**, ThioCDI, THF, 70 °C, yield of **3** = 40%, **6b** = 32%; (xx) **44** or **45**, TFA, 70 °C, yield of **4** = 42%, **6a** = 24%.



Scheme 2. Preparation of glycolamide linker analogue **2**. Reagents and conditions: (i) 2-(methanesulfonyl)ethanol, NaH, DMF, 57%; (ii) *tert*-butylbromoacetate, K_2CO_3, acetone, 87%; (iii) TFA, CH_2Cl_2, 100%; (iv) **37**, HATU, DIPEA, DMF, 56%; (v) H_2, 10% Pd/C, MeOH then ThioCDI, THF, 70 °C, 18%.



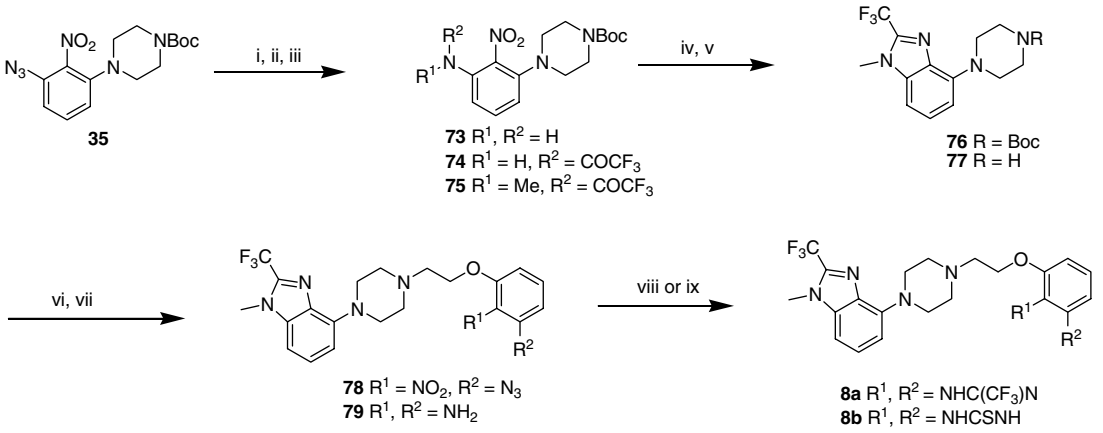
Scheme 3. Preparation of an analogue (**7**) with the ethoxy linker shifted one position on the thiobenzimidazolone. Reagents and conditions: (i) 2,2-diethoxyethanol, NaH, THF, 0 °C, 39%; (ii) H_2, 10% Pd/C, MeOH, 93%; (iii) ThioCDI, THF, 70 °C, 98%; (iv) 1 N HCl, THF, 70 °C; (v) **37**, $NaBH(OAc)_3$, NMP, 21%.



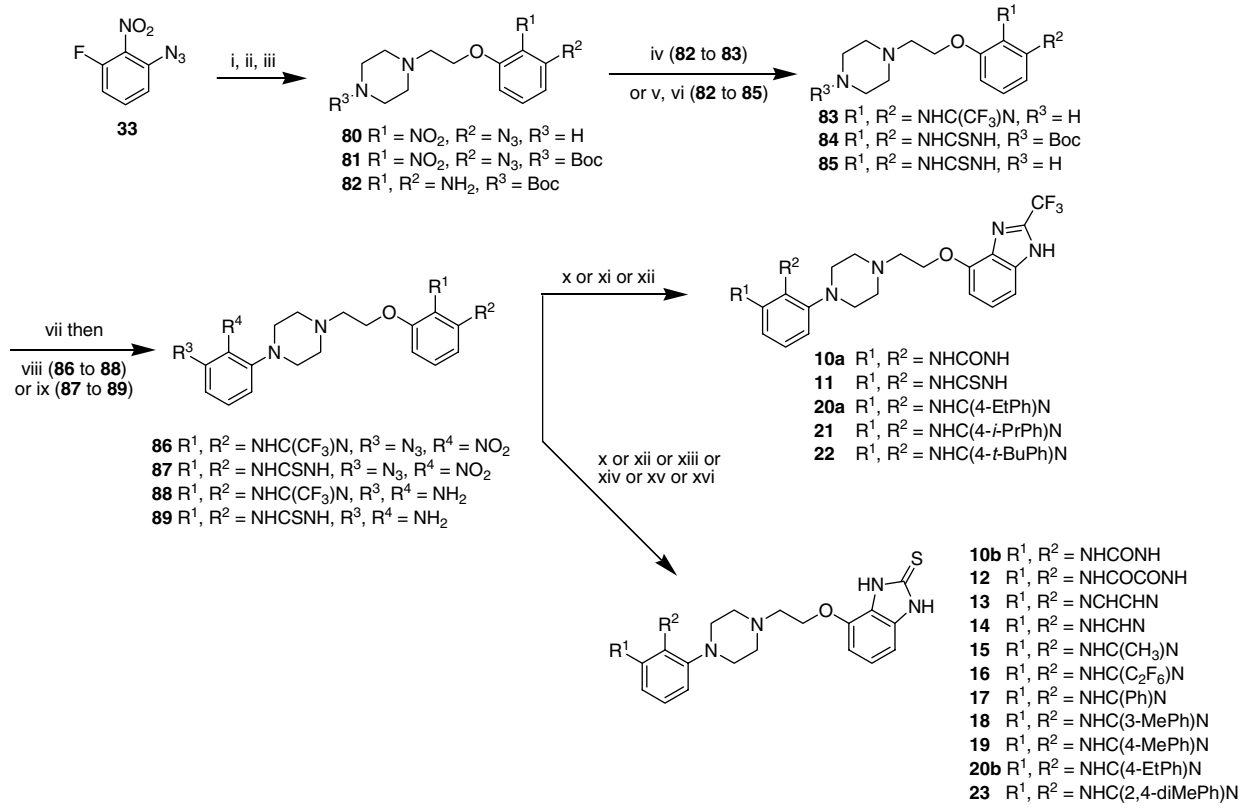
Scheme 4. Preparation of an analogue (**9**) with the piperazine shifted to the 5-position of the benzimidazole. Reagents and conditions: (i) 1-(Boc)piperazine, NMM, NMP, 100%; (ii) H_2, 10% Pd/C, MeOH, 97%; (iii) TFA, 70 °C, 66%; (iv) 2,2-diethoxyethanol, NaH, THF, 0 °C, 90%; (v) 1 N HCl, THF, 70 °C, 62%; (vi) **68**, $NaBH(OAc)_3$, DMF, 44%; (vii) H_2, 10% Pd/C, MeOH; (viii) ThioCDI, THF, 70 °C, 36% for two steps.

N-Methyl derivatives **8a** and **8b** were synthesized as shown in Scheme 5. Hence, the azide of intermediate **35**, prepared as shown in Scheme 1, was selectively reduced and trifluoroacetylated to give **74**, which was methylated under Mitsunobu conditions to provide **75**. Reduction of the nitro group on **75** followed by TFA removal of the Boc protecting group gave **77**. Reductive amination of this piperazine derivative with aldehyde **70** (see Scheme 4) followed by reduction and treatment with hot TFA or thioCDI gave the desired products **8a** and **8b**, respectively.

Synthesis of molecules with additional modifications to the template benzimidazole is shown in Scheme 6. The fluorine of intermediate **33** was displaced with the sodium salt of 2-(1-piperazinyl)ethanol, and the resulting free amine was protected with a Boc group. Full reduction of the azide and nitro groups provided



Scheme 5. Preparation of *N*-methyl analogues **8a** and **8b**. Reagents and conditions: (i) $SnCl_2{\cdot}2H_2O$, MeOH, 82%; (ii) TFAA, DIPEA, CH_2Cl_2, 99%; (iii) MeOH, TPP, DIAD, CH_2Cl_2, 89%; (iv) H_2, 10% Pd/C, MeOH, 68%; (v)TFA, CH_2Cl_2, 71%; (vi) **70**, $NaBH(OAc)_3$, AcOH, NMP, 45%; (vii) H_2, 5% Pd/C, MeOH, 90%; (viii) ThioCDI, THF, 70 °C, yield for **8b** = 36%; (ix) TFA, 70 °C, yield for **8a** = 27%.



Scheme 6. Preparation of additional analogues with template benzimidazole modifications. Reagents and conditions: (i) 2-(1-piperazinyl)ethanol, NaH, THF, −78 °C, 74%; (ii) $(Boc)_2O$, CH_2Cl_2, 100%; (iii) H_2, 5% Pd/C, MeOH, 93%; (iv) TFA, 20–70 °C; (v) ThioCDI, THF, 47%; (vi) TFA, polymer bound dimethylsilane, 69%; (vii) **33**, DIPEA, DMSO, yield of **86** = 63% for two steps, **87** = 64%; (viii) H_2, 5% Pd/C, MeOH, 51% for **88**; (ix) $SnCl_2{\cdot}2H_2O$, NMP, 20–100 °C, 43% for **89**; (x) CDI, THF, yield for **10a** = 61%, **10b** = 4%; (xi) ThioCDI, THF, yield for **11** = 66%; (xii) RCO_2H, HATU, NMP, then AcOH, 100 °C, yield for **18** = 13%, **19** = 36%, **20a** = 10%, **20b** = 15%, **21** = 38%, **22** = 34%, **23** = 10%; (xiii) 30% aq glyoxal, MeOH, yield for **13** = 38%; (xiv) RCO_2H, 90 °C, yield for **14** = 5%, **15** = 40%, **16** = 35%; (xv) PhCHO, *i*-PrOH, Ref., air, yield for **17** = 50%; (xvi) oxalyldiimidazole, THF, yield for **12** = 30%.

the phenylenediamine **82**. Treatment of the intermediate with thioCDI followed by TFA or with hot TFA alone gave the thiobenzimidazolone and trifluoromethylbenzimidazole derivatives **85** and **83**, respectively. Reacting this compound with **33** gave the azidonitro intermediates **86** and **87**. Catalytic hydrogenation of **86** and tin mediated reduction of **87** gave the phenylenediamines **88** and **89**, respectively. One or both of these intermediates were treated with several reagents including CDI, thioCDI, oxalyldiimidazole, glycol aldehyde, hot formic, acetic and perfluoropropionic acids, benzaldehyde and substituted benzoic acids to give the desired products **10**–**23**.

Finally, compounds with the 2-(4-*tert*-butylphenyl)-1*H*-benzimidazol-4-ylpiperazine core were prepared via reductive amination of appropriate aldehyde[22] precursors and intermediate **91** (Scheme 7). Final products **25**, **26**, **30**, and **31** were prepared as shown as well as the intermediate nitrofluoro compound **92**, which was carried on through a series of straight-forward steps to provide the quinoxalinedione **29**. Also shown in Scheme 7 is the prep-

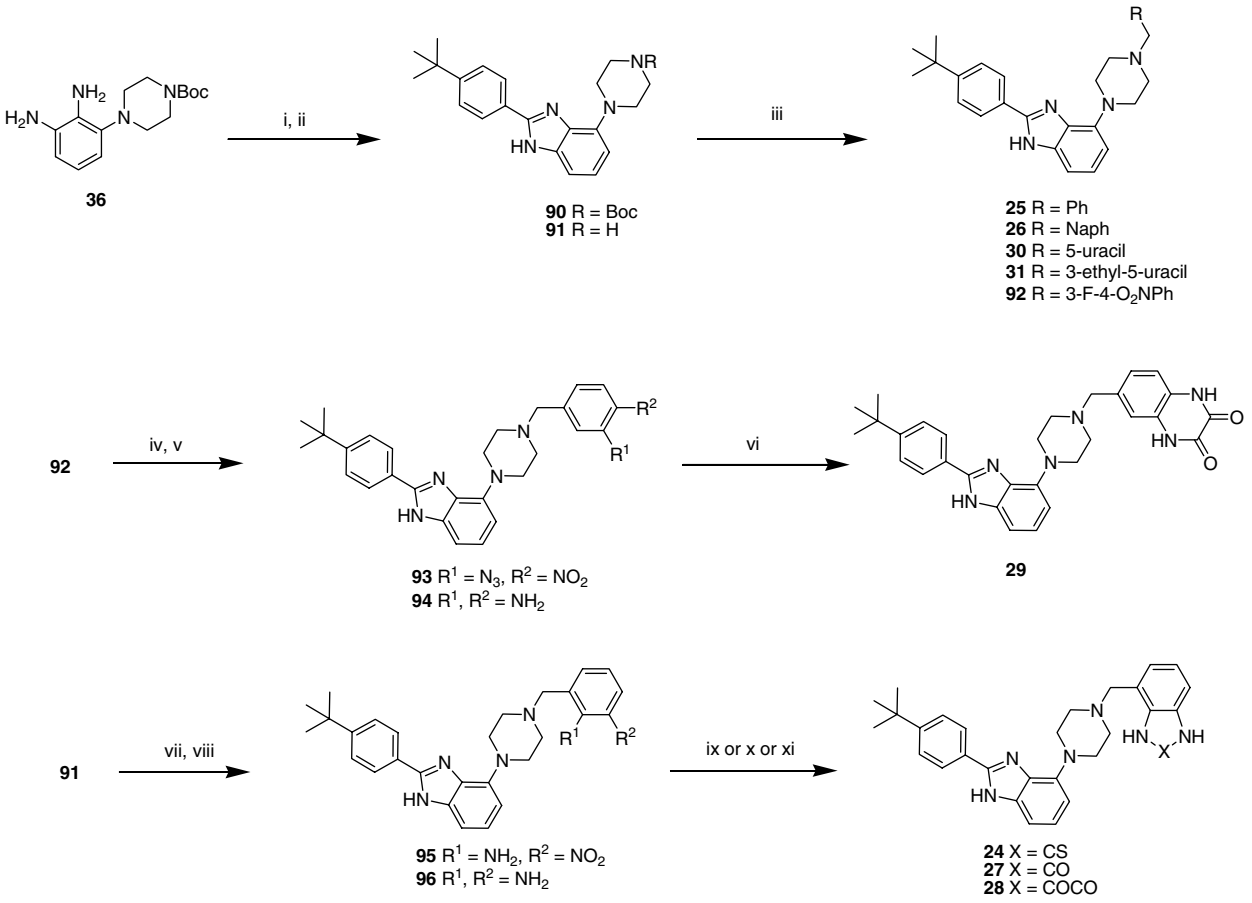
aration of **24**, **27** and **28** from **91** and the benzylchloride derivative **50**.

Human and rat binding assays were used as the primary drivers of structure–activity relationship studies and were performed on recombinant cells expressing either rat or human GnRH receptors as described in the literature.[13] Displacement of radioiodinated (D-Trp^6)-GnRH, a GnRH agonist, to these cells using test article was used to determine percent inhibition and IC_{50}'s. Human functional data were measured through the inhibition of tritiated inositols from cells overexpressing the human receptor treated with the GnRH agonist, (D-Trp6)-GnRH, or agonist combined with various concentrations of test article.[14] Finally, rat functional activity was estimated by measuring LH release from primary rat pituitary cells following treatment with the GnRH agonist, (D-Trp6)-GnRH, and test article at various concentrations (see Section 2 for details).

Initial optimization efforts focused on maximizing GnRH antagonism and selectivity through modification of the ethoxy unit linking the piperazine ring with its pendant benzimidazole or thiobenzimidazolone (Table 1). At this juncture the 2-trifluoromethylbenzimidazole and 2-thiobenzimidazolone groups pendant to the ethoxy linker were considered bioequivalent, therefore, comparison of biological properties between structurally similar compounds with either the pendant trifluoromethylbenzimidazole or the thiobenzimidazolone was considered valid. Conversion to the glycolamide **2** led to a sharp drop in activity, most likely indicating the requirement for a basic nitrogen in position 4 of the piperidine ring. Elongating to the propyloxy derivative **3** led to a modest loss in GnRH activity (human GnRH binding IC_{50} = 3300 nM) but had no effect on 5-HT_{1a} activity (K_i = 2.8 nM) when compared to the lead compound **1b**. Conversion of the ethoxy group to *n*-propyl resulted in a less active compound (Table 1, compound **4**) as did the shorter alkyl modifications of **5a**, **5b**, and **6a**. When the thiobenzimidazolone heterocycle was coupled with the piperazinylbenzimidazole via the methylene linker, however, a derivative of equal or slightly greater potency than the original lead **1b** resulted (compound **6b**). This represents an increase of nearly 20-fold in potency over the similar trifluoromethylbenzimidazole analogue **6a** and a clear separation of activities compared to the nearly equipotent leads **1a** and **1b**. In addition, human functional activity remained about equal to the lead compound, while rat binding activity weakened slightly (Table 1). Finally, transposition of the ethoxy group by one carbon unit in the aromatic region also resulted in a compound of less activity (**7**, hGnRH IC_{50} = 4.5 μM).

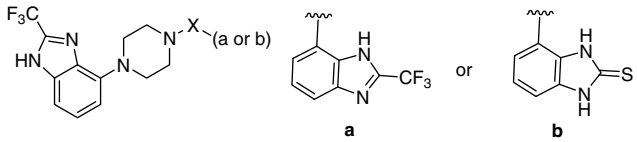
Additional structure–activity relationship (SAR) optimization focused on modification of the trifluoromethylbenzimidazole of **1a**, **1b** directly bonded to the piperazine (Table 2). Initial efforts were directed at N-alkylation of the imidazole (**8a** and **8b**), transposition of the linker-heterocycle location (**9**), and variation of the bicyclic structure to other heterocycles (**10a**–**13**). All compounds displayed relatively low activity as human GnRH binding agents. Further tests on these molecules (rat binding, functional activity) were not performed and structural focus remained on the N-unsubstituted benzimidazole.

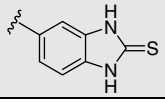
Additional optimizations on the proximal (template) benzimidazole centered on substitutions at the 2-position occupied by the



Scheme 7. Preparation of additional benzylic analogues. Reagents and conditions: (i) 4-*tert*-butylPhCHO, IPA, air, 80 °C, 95%; (ii) TFA, CH_2Cl_2, 50%; (iii) RCHO, $NaBH(OAc)_3$, NMP, yield for **25** = 100%, **26** = 84%, **30** = 57%, **31** = 61%, **92** = 68%; (iv) NaN_3, DMSO, 92%; (v) H_2, 10% Pd/C, MeOH, 100%; (vi) oxalyldiimidazole, THF, 70 °C, 24%; (vii) **50**, DIPEA, NMP, 36%; (viii) $SnCl_2 \cdot 2H_2O$, NMP, 70 °C, 20%; (ix) ThioCDI, THF, 60 °C, yield for **24** = 20%; (x) CDI, THF, 70 °C, yield for **27** = 28%; (xi) oxalyldiimidazole, THF, yield for **28** = 6%.

Table 1
In vitro activity of ethoxy linker analogues of lead compounds **1a** and **1b**



Compound	X	a or b	hGnRH binding IC_{50} ± SD[a] (μM)	hGnRH IP inhibition IC_{50} ± SD[b] (μM)	rGnRH binding IC_{50} ± SD[a] (μM)	5-HT_{1a} binding K_i IC_{50} ± SD (nM)
1a	$-(CH_2)_2O-$	a	1.5 ± 0.21	9.2 ± 3.0	15 ± 2.6	19 ± 3.2
1b	$-(CH_2)_2O-$	b	0.79 ± 0.15	4.5 ± 1.0	21 ± 3.3	2.8 ± 0.3
2	$-COCH_2O-$	b	24% @ 10 μM	—	—	—
3	$-(CH_2)_3O-$	b	3.3 ± 0.40	—	—	2.3
4	$-(CH_2)_3-$	a	42% @ 10 μM	—	—	—
5a	$-(CH_2)_2-$	a	26% @ 10 μM	—	—	—
5b	$-(CH_2)_2-$	b	51% @ 10 μM	—	—	—
6a	$-CH_2-$	a	9.5 ± 1.9	21 ± 4.5	64 ± 10	—
6b	$-CH_2-$	b	0.58 ± 0.10	3.4 ± 0.42	51 ± 3.8	—
7	$-(CH_2)_2O-$		4.5 ± 3.6	—	—	—

[a] Binding to overexpressed human or rat GnRH receptors in competiton with ^{125}I-(D-Trp^6)-GnRH (Ref. 13).
[b] Compound driven IP reduction in whole cells following stimulation with (D-Trp^6)-GnRH (Ref. 14).

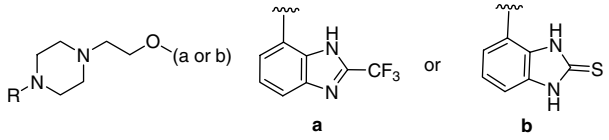
trifluoromethyl group in both lead compounds. A significant range of SAR were observed following substitution of the trifluoromethyl group with hydrogen, lower alkyl, extended perfluoro lower alkyl, and substituted phenyl groups (Table 3). Significant loss of activity was observed with small, homologous replacement units such as hydrogen (**14**), methyl (**15**), and perfluoroethyl (**16**). An investigation into phenyl ring replacement groups, however, revealed SAR patterns of interest. Simply replacing the trifluoromethyl group with phenyl resulted in a compound (**17**) with less activity (IC_{50} = 11 μM) than the leads **1a** and **1b**. However, the possibility of the phenyl group serving as additional template space was attractive. As shown in Table 3 substitution with lower alkyl groups in the 4-position on the phenyl ring led to compounds of increasing activity with an increase in steric size. Activity peaked with the 4-*tert*-butylphenyl compound (compare hGnRH binding IC_{50}: **19** = 1.3 μM, **22** = 0.050 μM), while the 3-methylphenyl was about equipotent to the unsubstituted phenyl in the human binding assay. Substitution of the 2-position of the phenyl in the 2-phenylbenzimidazoles led to inactive compounds (data not shown); however, the 2,4-dimethylphenyl derivative **23** had binding activity nearly equivalent to the 4-methylphenylbenzimidazole **19**. Additional significant trends seen in Table 3 include rat binding as well as human functional data. In nearly all examples where hGnRH binding potency increased so too did rat binding and functional human activity. Hence, the rat binding and human functional activity of the lead compound **1b** (rGnRH binding IC_{50} = 21 μM, hIP IC_{50} = 4.5 μM) were improved with the 4-*tert*-butylphenyl derivative **22** (rGnRH binding IC_{50} = 0.71 μM, hIP IC_{50} = 1.2 μM).

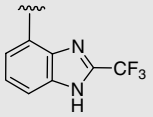
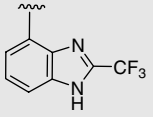
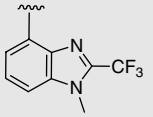
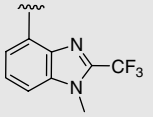
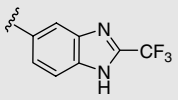
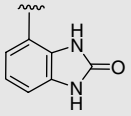
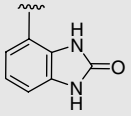
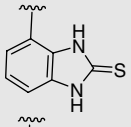
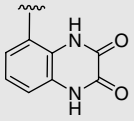
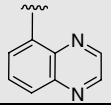
At this juncture a combination of optimized SAR pharmacophores from the studies mentioned above was investigated. This practice frequently leads to molecules with greatly enhanced desired activity. Hence, the favorable structural properties of compounds **6b** (Table 1) and **22** (Table 3) were blended into a single molecule (Scheme 8). The resulting molecule **24** possessed GnRH antagonism properties superior to all others previously tested. Human GnRH binding and functional IC_{50}'s improved to 6.2 nM and 14 nM, respectively. Rat activity also improved as the binding IC_{50} was measured at 110 nM, and LH release from rat primary pituitary cells increased to 480 nM. Also notable was the lack of binding activity of **24** at CNS GPCR's. Binding activity at the 5-HT_{1a} and alpha$_1$ adrenergic receptors dropped to undetectable levels indicating strong selectivity for GnRH over the catecholamine receptors (Scheme 8) and marked improvement over the activity of the lead compounds.

Although these improvements were noteworthy, we thought that a greater potency and functional activity at the rat receptor would be required for successful in vivo evaluation as serum LH supressors. Hence, the thiobenzimidazolone portion of the molecule was targeted for modification in an effort to address this issue. The results are summarized in Table 4. Replacement of the thiobenzimidazolone with the simple aromatic phenyl and naphthyl groups (**25** and **26**, respectively) resulted in compounds with a substantial loss of hGnRH receptor binding. Human activity remained high with benzimidazolone **27** but rat binding and functional activity diminished sixfold compared to **24**. Some of the rat activity returned with 5-substituted quinoxalinedione **28** but a dramatic increase in rat activity and human functional activity was observed with the one carbon transposed, 6-substituted quinoxalinedione **29**. Although the human and rat GnRH antagonist properties of **29** were suitable for in vivo studies the quinoxalinedione rendered the molecule highly insoluble and a poor candidate for pharmacokinetic evaluation. The phenyl group of **25** was clearly deleterious to GnRH activity and suggested that the phenyl portion of the quinoxalinedione does not add to the GnRH inhibitory activity of **29**. Replacement of the quinoxalinedione with a monocyclic heterocycle in this position may retain activity similar to this bicyclic structure. Hence, we evaluated the uracils **30** and **31** in the human and rat GnRH assays. Both compounds showed excellent binding properties at human and rat receptors. In addition they were potent antagonists in both functional assays. Hence, the uracils were attractive candidates for in vivo evaluation based on their potent, nonselective in vitro GnRH antagonist profile.

Prior to in vivo pharmacokinetic analysis uracils **30** and **31** were evaluated for their absorption–distribution–metabolism–excretion (ADME) properties. Table 5 summarizes the ADME molecular descriptors generally thought to effect bioavailability and exposure levels. All values are within recommended ranges as suggested by established models.[23,24] In addition, in vitro pharmaceutical profiling properties of solubility, PAMPA membrane diffusion and rat liver microsome half-life are also shown.[25] Both compounds

Table 2
In vitro activity results of analogues of the trifluoromethylbenzimidazole template region of **1a**, **b**



Compound	R	a or b	hGnRH binding IC_{50}[a] (μM)
1a		a	1.5 ± 0.21
1b		b	0.79 ± 0.15
8a		a	5% @ 10 μM
8b		b	8% @ 10 μM
9		b	11% @ 10 μM
10a		a	8% @ 10 μM
10b		b	6% @ 10 μM
11		a	42% @ 10 μM
12		b	9% @ 10 μM
13		b	1% @ 10 μM

[a] Binding to overexpressed human GnRH receptors in competiton with ^{125}I-(D-Trp^6)-GnRH (Ref. 13).

displayed moderate solubility and permeability properties. The unsubstituted uracil **30** was stable in the rat liver microsome assay while the *N*-ethyluracil **31** was unstable in the same assay with a half-life of 5 min. Generally speaking, compounds with a half-life less than 15 min in the rat in vitro liver microsome assay are considered unstable and will not likely survive the first-pass effect in the liver following oral absorption. These latter data suggested a relatively higher in vivo dose may overcome any first-pass elimination effects brought on by high liver clearance. In addition, because the structures differ only by an *N*-ethyl group the microsomal instability of **31** may be due to N-dealkylation of the uracil which could provide an in vivo source of compound **30** via biotransformation. This would ensure an additional GnRH active component in vivo. The data obtained from ADME molecular descriptors and in vitro pharmaceutical property analysis supported in vivo pharmacokinetic evaluation of **30** and **31**. Hence, rats were exposed to both uracils via intravenous (iv) and oral (po) administration and their pharmacokinetic parameters were established. The data are summarized in Table 6. Uracil **30** had low bioavailability (%$F \sim 5$), while the *N*-ethyl compound, **31**, was highly bioavailable (%$F = 74$). These data suggested in vivo, oral exposure for this series is absorption dependant and supported the in vivo efficacy analysis of compound **31**.

Intact animals have pulsatile patterns of serum LH making accurate measurements of the gonadotropin difficult. Orchidectomized rats, however, display elevated, stable levels of serum LH due to the removal of the testosterone mediated feedback loop that controls the central release of GnRH. Exposure of these animals to a GnRH antagonist can lower serum LH to normal levels. Hence, castrated rats were treated orally with compound **31** and their serum LH levels were monitored post-administration. As shown in Figure 3, the *N*-ethyluracil derivative successfully lowered LH levels starting at 1 h post-administration and the effect continued for at least 6 h. At the 24 h time point LH levels had returned to levels equivalent to the control group (data not shown). The in vivo experiment clearly indicated that antagonism of the GnRH receptor is occurring in vivo with compound **31**. It was also subjected to limited off-target pharmacological analysis since earlier generation leads were active at 5-HT and alpha$_1$ adrenergic receptors. Compound **31** was inactive in all assays tested with the exception of the 5-HT$_{2a}$ subtype ($K_i = 230$ nM). Functional evaluation of **31** in 5-HT$_{2a}$ assay indicated compound **31** is a full antagonist and not expected to elicit detrimental side effects or toxicity via this mechanism.

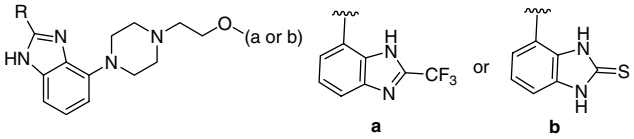
The goal of our program was to discover small molecule, orally active antagonists of the GnRH receptor from two lead compounds obtained from a GPCR directed library that focused on GnRH receptor antagonist activity. Both compounds had micromolar binding potencies to the human and rat GnRH receptors and they were shown to be antagonists in both species. Enhancement of rat activity along with human activity was critically important for in vivo evaluation. Compounds that are inactive in rat are usually evaluated in monkeys making pre-clinical discovery and development expensive and time consuming. A compartmentalized structure activity optimization process led to individual improvements to binding and efficacy properties. Combining these improvements together into a single molecule led to a nanomolar inhibitor of human GnRH and a modest antagonist of rat GnRH (compound **24**). Further refinements led to enhanced rat activity in compound **31**, which also had high bioavailability when orally administered to rats at 20 mg/kg (%$F = 74$). Suppression of serum LH levels leads to the suppression of sex hormones, which in turn has therapeutic value for diseases/conditions aggravated by these hormones. Proof of concept can be achieved when serum LH levels are suppressed to a statistically significant level. Uracil **31** dropped rat serum LH levels for several hours following administration of a single oral dose (30 mg/kg). LH levels returned to normal after 24 h. This data confirms the discovery of uracil **31** as an orally active antagonist of the GnRH receptor. Further studies on this and other compounds will be reported in due course.

2. Experimental details

2.1. General methods

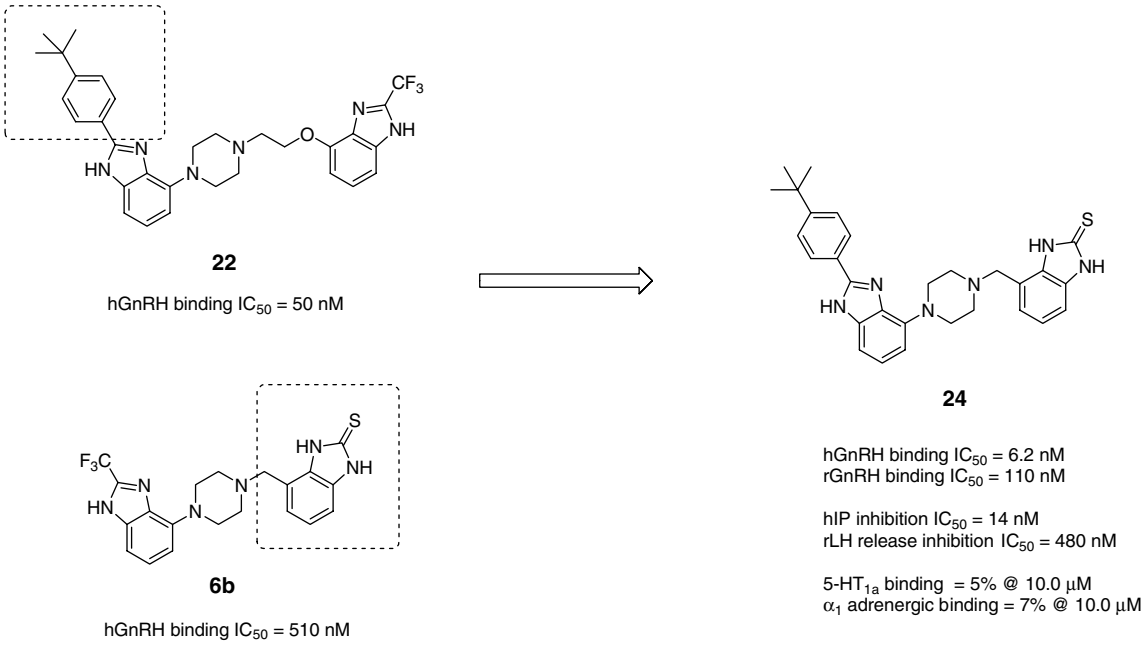
All experiments were conducted in well-ventillated fume hoods. Anhydrous solvents were purchased from Aldrich Chemical

Table 3
In vitro SAR following substitution of the trifluoromethyl group of the proximal benzimidazole region of **1a**, **b**: GnRH binding and functional data



Compound	R	a or b	hGnRH binding IC_{50} ± SD[a] (μM)	hGnRH IP inhibition IC_{50} ± SD[b] (μM)	rGnRH binding IC_{50} ± SD[a] (μM)
1a	CF_3	a	1.5 ± 0.21	9.2 ± 3.0	15 ± 2.6
1b	CF_3	b	0.79 ± 0.15	4.5 ± 1.0	21 ± 3.3
14	H	b	5% @ 10 μM	—	—
15	CH_3	b	10% @ 10 μM	—	—
16	C_2F_5	b	6.3 ± 1.1	—	>100
17	Ph	b	11	—	—
18	3-Methylphenyl	b	47% @ 10 μM	—	—
19	4-Methylphenyl	b	1.3 ± 0.12	2.8 ± 0.21	—
20a	4-Ethylphenyl	a	0.22 ± 0.05	2.3 ± 0.32	5.2 ± 0.40
20b	4-Ethylphenyl	b	0.12 ± 0.01	0.64 ± 0.055	3.9 ± 0.25
21	4-*i*-Propylphenyl	a	0.12 ± 0.002	0.45 ± 0.014	0.99 ± 0.044
22	4-*tert*-Butylphenyl	a	0.050 ± 0.0021	1.2 ± 0.070	0.71 ± 0.048
23	2,4-Dimethylphenyl	b	1.2 ± 0.055	—	—

[a] Binding to overexpressed human or rat GnRH receptors in competiton with ^{125}I-(D-Trp^6)-GnRH (Ref. 13).
[b] Compound driven IP reduction in whole cells following stimulation with (D-Trp^6)-GnRH (Ref. 14).



Scheme 8. A combination of the features of **6b** and **22** led to analogue **24** with multiple optimized features.

Co. (Milwaukee, WI) and used directly. Bulk solvents and chemicals were purchased from EMD and used directly. ^{1}H and ^{13}C NMR were recorded on Varian INOVA 400 MHz, Bruker AVANCE II 400 MHz and Bruker AVANCE II 300 MHz instruments in the indicated solvent at 20 °C. Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane (TMS). High resolution mass spectrometry was recorded on an Agilent 6210 TOF instrument. Positive and negative electrospray mass spectrometries were recorded on Waters ZQ or ZMD instruments. Elemental analysis was performed by Robertson Microlit (Madison, NJ) and agree with theoretical values within ±0.4%.

The following HPLC and LC/MS methods were used for the syntheses outlined in the examples:

Method A: Column: Xterra MS C18, 5μ, 50 × 2.1 mm. Mobile phase: 90/10–5/95 water (0.1% formic acid)/acetonitrile (0.1% formic acid), 2 min, hold 1.5 min. Flow rate: 0.8 mL/min. Detection: 210–400 nm.

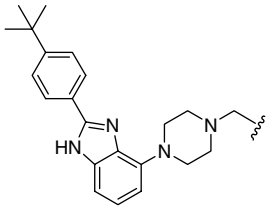
Method B: Column Xterra reverse phase 18, 3.5 μ, 150 × 4.6 mm. Mobile phase: 85/15–5/95 ammonium formate buffer (pH 3.5)/acetonitrile and methanol (1:1) for 10 min, hold 4 min, 1.2 mL/min. Detection: 210–370 nm.

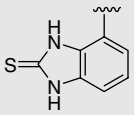
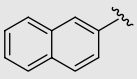
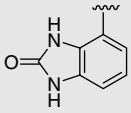
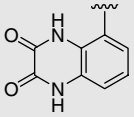
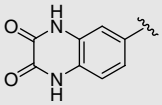
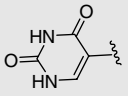
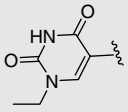
Method C: Column Xterra reverse phase C18, 3.5 μ, 150 × 2.1 mm. Mobile phase: 78/22–5/95 phosphate buffer (pH 2.1) acetonitrile and methanol (1:1) for 10 min, hold 4 min, 1.2 mL/min. Detection: 210–370 nm.

Method D: Column: Xterra reverse phase 18, 3.5 μ, 150 × 4.6 mm. Mobile phase: 85/15–5/95 phosphate buffer (pH 2.1)/acetonitrile and methanol (1:1) for 10 min, hold 4 min, 1.2 mL/min. Detection: 210–370 nm.

Method E: Column: YMC CombiPrep ProC18 50 × 20 mm I.D. S-5 μm, 12 nm. Mobile phase: 10/90 acetonitrile/water to

Table 4
In vitro results for the analogues of compound **24**



Compound	Structure	hGnRH binding $IC_{50} \pm SD$[a] (nM)	hIP $IC_{50} \pm SD$[b] (nM)	rGnRH binding $IC_{50} \pm SD$[a] (nM)	rLH release $IC_{50} \pm SD$[c] (nM)
24		6.2 ± 0.040	22 ± 3.5	110	240
25		6% @ 10 μM	–	–	–
26		7% @ 10 μM	–	–	–
27		8.7 ± 0.037	63 ± 10	680	1500
28		5.7 ± 2.4	24	72 ± 12	480
29		4.9 ± 0.20	9.4	40 ± 17	25 ± 9.0
30		5.5 ± 2.3	11 ± 3.0	32 ± 12	41 ± 11
31		1.7 ± 0.65	4.0 ± 0.64	17.5 ± 8.9	59

[a] Binding to overexpressed human or rat GnRH receptors in competiton with ^{125}I-(D-Trp^6)-GnRH (Ref. 13).
[b] Compound driven IP reduction in whole cells following stimulation with (D-Trp^6)-GnRH (Ref. 14).
[c] Compound driven reduction in LH release from primary rat pituitary cells stimulated with (D-Trp^6)-GnRH (see Section 2).

100% acetonitrile over 10 min, ramp back to 10/90 acetonitrile/water over 2 min. Detection: 210, 254 nm.
Method F: Column: YMC CombiPrep ProC18 50 × 20 mm I.D. S-5 μm, 12 nm. Mobile phase: 10/90 acetonitrile/water (0.1% TFA in both solvents) to 100% acetonitrile (0.1% TFA) over 10 min, ramp back to 10/90 acetonitrile/water (0.1% TFA in both solvents) over 2 min. Detection: 210, 254 nm.
Method G: (LC/MS slow gradient) Column: Waters Xterra MS C18, 5 μm, 50 × 2 mm. Mobile phase: 90/10 to 5/95 water (0.1% formic acid)/acetonitrile (0.1% formic acid),10 min, hold 2 min. Flow rate: 0.8 mL/min. Detection 210–400 nm.
Method H: (LC/MS very fast gradient) Column: Phenomenex C18 monolith, 3 × 100 mm. Mobile phase: 95/5–5/95 water (0.1% formic acid)/acetonitrile (0.1% formic acid), 1.5 min, hold 0.5 min. Flow rate: 1.5 mL/min. Detection 210–400 nm.
Method I: (LC/MS long gradient) Column: Phenomenex C18 monolith, 3 × 100 mm. Mobile phase: 95/5 to 5/95 water (0.1% formic acid)/acetonitrile (0.1% formic acid), 4.5 min, hold 0.4 min. Flow rate: 1.5 mL/min. Detection 210–400 nm.

2.1.1. 1-Azido-3-fluoro-2-nitrobenzene (33)

A solution of 2,6-difluoronitrobenzene (4.8 g, 30 mmol) in DMSO (35 mL) was treated with sodium azide (2.2 g, 33 mmol) and stirred for 4 h. The mixture was diluted with ethyl acetate (150 mL) and washed with water (2 × 150 mL), dried ($MgSO_4$), and evaporated to provide the title product as a semi-crystalline solid (5.4 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (td, 1H, J = 8.2 Hz, J = 6.0 Hz), 7.10 (d, 1H, J = 8.2 Hz), 7.03 (dd, 1H, J = 8.2 Hz, J = 8.2 Hz).

2.1.2. 3-Azido-2-nitrophenol (56)

A solution of 1-Azido-3-fluoro-2-nitrobenzene (**33**, 1.0 g, 5.5 mmol) and 2-(methylsulfonyl)ethanol (1.0 g, 8.2 mmol) in anhydrous dimethylformamide (10 mL) was cooled to 5 °C and treated with sodium hydride (60% in mineral oil, 0.63 g, 16.5 mmol). The reaction mixture was stirred and allowed to warm to room temperature over 1 h. The mixture was treated with 1 N HCl (100 mL) and extracted with ethyl acetate (3× 50 mL). The combined organic extracts were washed with water (3× 100 mL)

Table 5
ADME associated molecular descriptors and in vitro pharmaceutical profiling values for **30** and **31**[a]

Compound	**30**	**31**
Molecular descriptors		
Molecular weight	458	486
clog*P*	3.88	4.26
H-bond donors	3	2
H-bond acceptors	5	5
Rotatable bonds	4	5
Total polar surface area (A^2)	90	81
In vitro pharma. profiling		
Solubility @ pH 7.4 (μg/mL)	25	13
PAMPA (10^{-6} cm/s)	0.60	2.4
Rat liver microsome $t_{1/2}$ (min)	30	5

[a] For a description of the assays see Ref. 25.

Table 6
Pharmacokinetic parameters for compounds **30** and **31**

Compound	**30**		**31**	
Method (vehicle)	PO (PEG 400)	IV (DMSO)	PO (PEG 400)	IV (DMSO)
Dose (mg/kg)	10	1.0	20	1.0
Plasma $t_{1/2}$ (h)	5.8	3.5	3.1	1.1
T_{max} (h)	1.8	—	0.67	—
C_{max} (ng/mL)	19	—	480	—
$AUC_{0-\infty}$ (ng-h/mL)	200	355	2717	190
Bioavailability (%*F*)	5	—	74	—
Cl_p (mL/min/kg)	—	47	—	95
V_{ss} (L/kg)	—	3.1	—	4.8

and brine (100 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by silica chromatography (Isco instrument, 80 g normal phase column, eluted with a gradient of 15–50% ethyl acetate in hexanes) to leave the product as a yellow solid (0.56 g, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.50 (br s, 1H), 7.48 (dd, 1H, J = 7.8 Hz, J = 7.8 Hz), 6.93 (d, 1H, J = 7.8 Hz), 6.86 (d, 1H, J = 7.8 Hz).

2.1.3. *tert*-Butyl 2-(3-azido-2-nitrophenoxy)acetate (57)

A mixture of *tert*-butylbromoacetate (0.49 g, 2.5 mmol, 0.37 mL) and well-ground potassium carbonate (1.1 g, 8.3 mmol) in acetone (10 mL) was purged with nitrogen, treated with 3-azido-2-nitrophenol (**56**, 150 mg, 0.83 mmol) and stirred rapidly at 60 °C for an hour. The solvent was evaporated, and the residue was dissolved in water (25 mL) and methylene chloride (25 mL). The aqueous layer was separated, extracted with methylene chloride (25 mL) and the combined organic extracts were dried ($MgSO_4$), and evaporated. The crude material was purified by silica gel chromatography (Isco instrument, 40 g normal phase column, eluted with a gradient of 15–35% ethyl acetate in hexanes) to leave the pure product **57** as a light yellow solid (213 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.39 (dd, 1H, J = 8.3 Hz, J = 8.3 Hz), 6.90 (d, 1H, J = 8.3 Hz), 6.67 (d, 1H, J = 8.3 Hz), 4.59 (s, 2H), 1.46 (s, 2H).

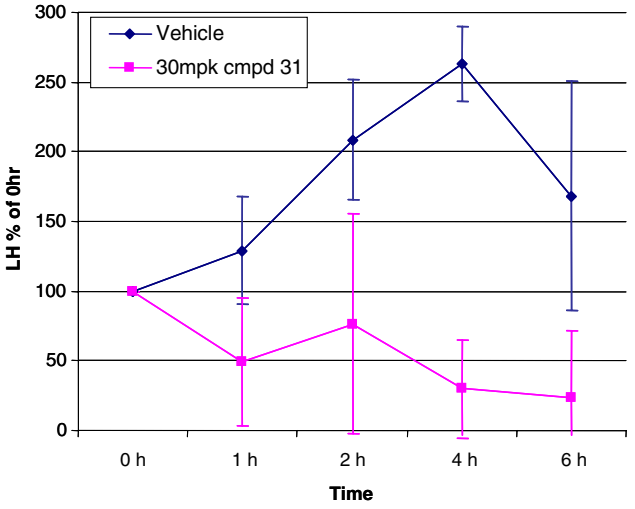


Figure 3. Serum LH levels in orchidectomized rats following oral administration of **31** (levels at t = 0 adjusted to 100). Vehicle and test article groups consisted of nine animals each. Vehicle = PEG 400.

2.1.4. 2-(3-Azido-2-nitrophenoxy)acetic acid (58)

A solution of *tert*-butyl 2-(3-azido-2-nitrophenoxy)acetate (**57**, 0.18 g, 0.61 mmol) in trifluoroacetic acid (0.5 mL) and methylene chloride (0.5 mL) was stirred for 30 min then evaporated under reduced pressure to leave the acid product as a solid (149 mg, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (dd, 1H, J = 8.5 Hz, J = 8.4 Hz), 7.00 (d, 1H, J = 8.4 Hz), 6.87 (d, 1H, J = 8.5 Hz), 4.74 (s, 2H). LC/MS (Method A), *t*R = 1.17 min (purity = 100%), $[M-H]^-$ = 237.

2.1.5. 1-(3-Azido-2-nitrophenyl)piperazine (34)

To a solution of 1-azido-3-fluoro-2-nitrobenzene (**33**, 0.20 g, 1.1 mmol) in dimethylsulfoxide (2 mL) was added piperazine (0.19 g, 2.2 mmol). The mixture was stirred for 2 h, diluted with ethyl acetate (30 mL), washed with water (2× 30 mL), dried ($MgSO_4$), and evaporated under reduced pressure to leave the product as a yellow solid (0.26 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (dd, 1H, J = 8.2 Hz, J = 8.0 Hz), 6.98 (d, 1H, J = 8.2 Hz), 6.96 (d, 1H, J = 8.0 Hz), 2.95 (br s, 8H). LC/MS (Method A), *t*R = 0.75 min (purity = 100%), $[M+H]^+$ = 249.

2.1.6. *tert*-Butyl 4-(3-azido-2-nitrophenyl)piperazine-1-carboxylate (35)

To a solution of 1-(3-azido-2-nitrophenyl)piperazine (**34**, 0.23 g, 0.93 mmol) in methylene chloride (5 mL) was added di-*tert*-butyl dicarbonate (0.26 g, 1.2 mmol). The solution stirred an hour then was treated with aminomethyl polystyrene resin (2.4 mmol/g, 0.39 g, 0.93 mmol). After an additional hour of stirring the mixture was filtered, the residue was washed with methylene chloride (3× 10 mL) and the combined organic washes were evaporated under reduced pressure to afford the product as a yellow gum (0.32 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.45 (dd, 1H, J = 8.2 Hz, J = 8.2 Hz), 7.02 (d, 1H, J = 8.2 Hz), 6.98 (d, 1H, J = 8.2 Hz), 3.50 (m, 4H), 2.93 (m, 4H), 1.48 (s, 9H). LC/MS (Method A), *t*R = 1.93 min (purity = 90.9%), $[M+H]^+$ = 349.

2.1.7. *tert*-Butyl 4-(2,3-diaminophenyl)piperazine-1-carboxylate (36)

tert-Butyl 4-(3-azido-2-nitrophenyl)piperazine-1-carboxylate (**35**, 0.30 g, 0.86 mmol) was hydrogenated over 10% Pd/C (40 mg) at one atmosphere hydrogen pressure in methanol (10 mL) for 4 h. The catalyst was filtered, washed (methanol, 2× 10 mL), and the filtrate was evaporated under reduced pressure to afford the product **36** as a dark brown gum (0.25 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.69 (dd, 1H, J = 7.7 Hz, J = 7.8 Hz), 6.59 (d, 1H, J = 7.8 Hz), 6.55 (d, 1H, J = 7.7 Hz), 3.79 (br s, 2H), 3.60 (m, 4H), 3.40 (br s, 2H), 2.82 (br s, 4H), 1.49 (s, 9H). LC/MS (Method A), *t*R = 1.06 min (purity = 93.8%), $[M+H]^+$ = 293.

2.1.8. 4-(Piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (37)

A solution of *tert*-butyl 4-(2,3-diaminophenyl)piperazine-1-carboxylate (**36**, 0.23 g, 0.79 mmol) in trifluoroacetic acid (5 mL) was heated to 70 °C for 2 h then evaporated to dryness

under reduced pressure. The residue was dissolved in ethyl acetate (25 mL) and washed with saturated aqueous sodium bicarbonate solution (2× 25 mL). The organic layer was dried and evaporated to leave the product as a brown gum (0.19 g, 89%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.26 (dd, 1H, J = 8.0 Hz, J = 7.7 Hz), 7.19 (d, 1H, J = 8.0 Hz), 6.72 (d, 1H, J = 7.7 Hz), 3.62 (br s, 4H), 3.26 (m, 4H). LC/MS (Method A), tR = 0.32 min (purity = 84.5%), $[M+H]^+$ = 271.

2.1.9. 2-(3-Azido-2-nitrophenoxy)-1-(4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethanone (59)

A sample of 2-(3-azido-2-nitrophenoxy)acetic acid (**58**, 75 mg, 0.32 mmol) was dissolved in anhydrous dimethylformamide (2 mL) and treated with 4-(piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[d]imidazole (**37**, 94 mg, 0.35 mmol) and diisopropylethylamine (45 mg, 0.35 mmol, 63 μL). The mixture was purged with a nitrogen atmosphere and cooled in an ice bath. HATU (0.13 g, 0.35 mmol) was added and the mixture stirred for 2 h. The reaction mixture was treated with water (0.5 mL), and the product was purified directly without workup by reversed phase HPLC (Method E) to leave the product as a foamy solid (88 mg, 56%). ^{1}H NMR (300 MHz, CD_3OD) δ = 7.44 (dd, 1H, J = 8.5, J = 8.3), 7.25 (dd, 1H, J = 8.2 Hz, J = 7.7 Hz), 7.18 (d, 1H, J = 7.7 Hz), 6.99 (d, 1H, J = 8.5 Hz), 6.94 (d, 1H, J = 8.3 Hz), 6.72 (d, 1H, J = 7.6 Hz), 4.83 (s, 2H), 3.79 (m, 2H), 3.72 (m, 2H), 3.38 (m, 2H), 3.32 (m, 2H). LC/MS (Method B), tR = 1.33 min (purity = 99.9%), $[M+H]^+$ = 491.

2.1.10. 2-(2-Thioxo-2,3-dihydro-1H-benzo[*d*]imidazol-4-yloxy)-1-(4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethanone (60 and 2)

2-(3-Azido-2-nitrophenoxy)-1-(4-(2-(trifluoromethyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)ethanone (**59**, 80 mg, 0.16 mmol) was hydrogenated over 10% palladium on carbon (25 mg) in methanol (2 mL) for 5 h. The catalyst was filtered over diatomaceous earth, washed with methanol (2× 5 mL), and the combined filtrates were evaporated under reduced pressure to leave the phenylenediamine **60**, which was used without further purification or characterization. The residue was dissolved in anhydrous tetrahydrofuran (3 mL) and treated with 1,1′-thiocarbonyldiimidazole (43 mg, 0.24 mmol). The reaction mixture stirred for 18 h and then it was diluted with water (25 mL) and extracted with ethyl acetate (3× 25 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated. The crude product was purified by silica gel chromatography (Isco instrument, 12 g normal phase column, eluted with a gradient of 50–100% ethyl acetate in hexanes) to leave the pure product **2** as a gum (14 mg, 18%). ^{1}H NMR (300 MHz, CD_3COCD_3) δ = 7.25–7.10 (m, 3H), 7.03 (dd, 1H, J = 8.1 Hz, J = 8.0 Hz), 6.84 (d, 1H, J = 8.1 Hz), 6.81 (d, 1H, J = 8.3 Hz), 6.67 (br s, 1H), 5.01 (s, 1H), 3.60 (m, 4H), 2.77 (dd, 2H, J = 7.5 Hz, J = 8.3 Hz), 2.55 (dd, 2H, J = 6.0 Hz, J = 7.0 Hz). HPLC (Method D), tR (purity) = 8.67 min (79.6%). HR ESMS $[M+H]^+$ = 477.1313, calcd for $C_{21}H_{19}F_3N_6O_2S$: 477.1315.

2.1.11. 4-(2-(4-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethyl)-1H-benzo[*d*]imidazole-2(3H)-thione (47, 38, 39 and 5b)

A solution of 2,3-dinitrotoluene (**46**, 7.3 g, 40 mmol) in dimethylformamide (20 mL) was treated with dimethylformamide dimethylacetal (9.9 g, 83 mmol, 11 mL). The mixture was stirred at 150 °C for 2.5 h. The solvents were removed by vacuum distillation, and the crude product was crystallized from acetonitrile. The solid was filtered and air-dried to leave **47** as a dark purple solid. A 0.20 g sample of this solid and 4-(piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[d]imidazole (**37**, 0.10 g, 0.37 mmol) was dissolved in a mixture of tetrahydrofuran (2 mL), acetic acid (0.2 mL) and water (0.2 mL) and stirred for 90 min. Sodium cyanoborohydride (47 mg, 0.74 mmol) was added and stirring continued an additional 90 min. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with saturated aqueous sodium bicarbonate solution (15 mL), water (15 mL), and brine (15 mL). The organic layer was dried ($MgSO_4$), and evaporated under reduced pressure to leave the crude product which was purified by reversed phase HPLC (Method 2). The product fractions were combined, diluted with ethyl acetate (50 mL), washed with saturated aqueous sodium bicarbonate solution (50 mL), dried ($MgSO_4$) and evaporated to leave the product **38** as a yellow gum (75 mg, 44%). A 64 mg sample of **38** (0.14 mmol) was hydrogenated over 10% Pd/C (10 mg) in methanol (2 mL) at 1 atmosphere hydrogen pressure for 2 h. The catalyst was filtered, washed with methanol (2× 5 mL), and the combined filtrates were evaporated under reduced pressure to leave the phenylenediamine product **39** as a brown gum (50 mg, 88%). A 22 mg sample of **39** (54 μmol) was dissolved in tetrahydrofuran (0.50 mL) in an 8-mL scintillation vial and treated with 1,1′-thiocarbonyldiimidazole (19 mg, 0.11 mmol). The reaction mixture was purged with nitrogen, capped and heated to 70 °C for 3 h. Water (0.1 mL) was added and the product was purified by reversed phase HPLC (Method 2), and the product fractions were lyophilized. The product **5b** was obtained as a yellow powder (14 mg, 58%). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 13.78 (br s, 1H), 12.80 (br s, 1H), 12.47 (br s, 1H), 7.24 (dd, 1H, J = 8.0 Hz, J = 8.2 Hz), 7.09 (d, 1H, J = 7.4 Hz), 7.05 (dd, 1H, J = 7.4 Hz, J = 7.7 Hz), 7.01 (dd, 1H, J = 7.7 Hz, J = 1.1 Hz), 6.98 (dd, 1H, J = 7.7 Hz, J = 1.1 Hz), 6.64 (d, 1H, J = 6.9 Hz), 3.55 (br s, 4H), 2.99 (t, 2H, J = 7.4 Hz), 2.73 (br s, 4H), 2.63 (t, 3H, J = 7.4 Hz). Anal. Calcd for $C_{21}H_{22}F_3N_6S$: C, 56.49; H, 4.74; N, 18.82. Found: C, 56.35; H, 5.02; N, 18.70. ESMS $[M-H]^-$ = 445, $[M+H]^+$ = 447.

2.1.12. 2-(Trifluoromethyl)-4-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)ethyl)piperazin-1-yl)-1H-benzo[*d*]-imidazole (5a)

A sample of the phenylenediamine (**39**, 22 mg, 54 μmol) was dissolved in trifluoroacetic acid (1 mL) and heated to 70 °C for 3 h. The solvent was evaporated and the residue was purified by reversed phase HPLC (Method 2). The product fractions were lyophilized to leave **5a** as a free base (16 mg, 61%). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 13.80 (br s, 2H), 7.47 (br s, 1H), 7.30 (dd, 1H, J = 7.4 Hz, J = 7.7 Hz), 7.25 (dd, 1H, J = 6.0 Hz, J = 6.9 Hz), 7.24 (d, 1H, J = 8.0 Hz), 7.11 (br s, 1H), 6.65 (br s, 1H), 3.50 (br s, 4H), 3.20 (t, 2H, J = 7.4 Hz), 2.77 (m, 6H). Anal. Calcd for $C_{22}H_{20}F_6N_6$: C, 54.77; H, 4.18; N, 17.42. Found: C, 54.60; H, 4.30; N, 17.05. ESMS $[M-H]^-$ = 481, $[M+H]^+$ = 483.

2.1.13. 2-(3-Chloropropoxy)-6-nitroaniline (55)

A solution of 2-amino-3-nitrophenol (**54**, 2.0 g,12.98 mmol) in dimethylformamide (26 mL) was treated with potassium carbonate (1.97 g, 14.27 mmol) followed by 1-bromo-3-chloropropane (1.92 mL, 19.46 mmol), and the reaction mixture was heated to 90 °C in an oil bath and stirred overnight. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (300 mL), washed with water (3× 75 mL), 1 N sodium hydroxide (75 mL), brine (100 mL), dried ($MgSO_4$), and concentrated to dryness. The crude product was purified by flash chromatography on silica gel eluted with 20% ethyl acetate in hexanes to yield 1.38 g of 2-(3-chloropropoxy)-6-nitroaniline, **55**, as an orange solid (46%). ^{1}H NMR (DMSO) δ = 7.59 (d, 1H, J = 8.8 Hz), 7.11 (m, 3H), 6.59 (t, 1H, J = 8.4 Hz), 4.15 (t, 2H, J = 5.7 Hz), 3.92 (t, 2H, J = 6.5 Hz), 2.24 (t, 2H, J = 6.1 Hz). LC/MS (Method A), tR = 1.60 min, $[M+H]^+$ = 231/233 [Cl pattern].

2.1.14. 2-Nitro-6-(3-(4-(2-(trifluoromethyl)-1H-benzo[*d*]-imidazol-4-yl)piperazin-1-yl)propoxy)aniline (40)

A solution of 4-(piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (**37**, 0.10 g, 0.26 mmol) in DMSO (3 mL) was added triethylamine (0.08 mL, 0.57 mmol) followed by 2-(3-chloropropoxy)-6-nitroaniline (**55**, 0.09 g, 0.39 mmol), and the reaction was heated to 90 °C in an oil bath and stirred for 20 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (100 mL) washed with water (3× 50 mL) and brine (50 mL), and dried ($MgSO_4$). The solvents were concentrated to dryness and the crude product was purified by flash chromatography on silica gel with 5% methanol in methylene chloride as eluant to yield 50 mg of **40** as an orange-yellow solid. (41% yield). ^{1}H NMR ($CDCl_3$) δ = 7.72 (d, 1H, *J* = 8.9 Hz), 7.27 (m, 2H), 7.08 (br s, 1H), 6.91 (d, 1H, *J* = 7.6 Hz), 6.70 (br s, 1H), 6.60 (m, 3H), 4.11 (dd, 2H, *J* = 11.9 Hz, *J* = 6.0 Hz), 3.58 (br s, 4H), 2.71 (br s, 4H), 2.62 (t, 2H, *J* = 7.1 Hz), 2.08 (m, 2H). LC/MS (Method A), *t*R = 0.94 min $[M+H]^+$ = 465, $[M-H]^-$ = 463.

2.1.15. 3-(3-(4-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)propoxy)benzene-1,2-diamine (43)

2-Nitro-6-(3-(4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)propoxy)aniline (**40**, 50 mg, 0.108 mmol) was hydrogenated at 1 atmosphere H_2 pressure in methanol (2 mL) over 10% Pd/C (11 mg) for 18 h. The reaction mixture was filtered and washed with methanol (2× 5 mL) and the filtrate was concentrated to dryness in vacuo to leave the product **43** as an off-white solid (49 mg, 100%). ^{1}H NMR (DMSO) δ = 14.0 (br s, 1H), 7.31 (t, 1H, *J* = 7.8 Hz), 7.19 (d, 1H, *J* = 8.1 Hz), 6.74 (d, 1H, *J* = 7.8 Hz), 6.40 (m, 1H), 6.26 (m, 2H), 3.99 (m, 2H), 3.58 (br s, 4H), 2.71 (br s, 4H), 2.62 (m, 2H), 2.08 (m, 2H). LC/MS (Method A), *t*R = 0.48 min, $[M+H]^+$ = 435, $[M-H]^-$ = 433.

2.1.16. 4-(3-(4-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)propoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (3)

A solution of 3-(3-(4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)propoxy)benzene-1,2-diamine (**43**, 50 mg, 0.115 mmol) in THF (5 mL) was treated with thiocarbonyldiimidazole (31 mg, 0.17 mmol), and the mixture was stirred and heated to 70 °C for 18 h. The crude product was purified by direct injection reversed phase HPLC (Method E) to yield **3** as a light yellow solid (22 mg, 40% yield). ^{1}H NMR (DMSO) δ = 14.0 (s, 1H), 12.61 (d, 2H, *J* = 21 Hz), 7.32 (t, 1H, *J* = 7.9 Hz), 7.21 (m, 1H), 7.08 (t, 1H, *J* = 8.1 Hz), 6.78 (m, 3H), 4.45 (d, 2H, *J* = 10.7 Hz), 4.20 (t, 2H, *J* = 5.3 Hz), 3.77–3.54 (m, 4H), 3.37 (q, 2H, *J* = 10.4 Hz), 3.19 (m, 2H), 2.21 (m, 2H). HPLC (Method C), *t*R = 12.9 min (purity = 93.6%). HR ESMS $[M+H]^+$ = 477.1689, calcd for $C_{22}H_{23}F_3N_6OS$: 471.1686.

2.1.17. 3-(2-Amino-3-nitrophenyl)propan-1-ol (52)

A solution of 2-allyl-6-nitroaniline (**51**, 1.94 g, 10.9 mmol) in THF (10 mL) under a nitrogen atmosphere was cooled in ice, stirred and treated with a solution of borane in THF (1.0 M, 10.9 mL, 10.9 mmol). After 2.5 h the reaction mixture was treated with sodium perborate tetrahydrate (1.67 g, 10.9 mmol) and water (20 mL). The mixture was allowed to warm to room temperature and stirred for 18 h. The tetrahydrofuran was evaporated, the aqueous residue was treated with ethyl acetate (50 mL) and brine (30 mL). The aqueous layer was further extracted with ethyl acetate (50 mL) and the combined layers were dried (Na_2SO_4) and evaporated. The residue was purified by silica gel chromatography using a gradient of ethyl acetate in hexanes (40–60–75–100%) to leave **52** (1.3 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.04 (dd, 1H, *J* = 8.7 Hz, *J* = 1.3 Hz), 7.28 (dd, 1H, *J* = 8.7 Hz, *J* = 1.3 Hz), 6.64 (dd, 1H, *J* = 8.7 Hz, *J* = 8.7 Hz), 6.55 (br s, 1H), 3.74 (t, 2H, *J* = 5.8 Hz), 2.72 (t, 2H, *J* = 7.6 Hz), 1.90 (m, 2H). LC/MS (Method A), *t*R = 0.91 min (purity = 100%); ESMS $[M+H]^+$ = 197.

2.1.18. 2-(3-Chloropropyl)-6-nitroaniline (53)

A solution of 3-(2-amino-3-nitrophenyl)propan-1-ol (**52**, 0.58 g, 3.0 mmol) and triethylamine (0.38 g, 3.8 mmol, 0.54 mL) in methylene chloride (40 mL) was stirred and cooled in an ice bath. Thionyl chloride (0.45 g, 3.8 mmol, 0.27 mL) was added dropwise to the reaction mixture. After the addition was completed the mixture was allowed to warm to room temperature and stirred for 18 h. Ice (50 g) and 1 N HCl were added to the reaction mixture. After the ice melted the organic layer was separated and washed with saturated aqueous sodium bicarbonate solution (50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was filtered through a pad of silica gel eluted with 50% ethyl acetate in hexanes to leave the product as a yellow-orange powder (0.37 g, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.06 (dd, 1H, *J* = 8.7 Hz, *J* = 1.3 Hz), 7.30 (d, 1H, *J* = 8.6 Hz), 6.67 (dd, 1H, *J* = 8.7 Hz, *J* = 8.6 Hz), 6.35 (br s, 2H), 3.63 (t, 2H, *J* = 6.0 Hz), 2.78 (t, 2H, *J* = 7.3 Hz), 2.11 (m, 2H). LC/MS (Method A), *t*R = 1.64 min (purity = 90.0%), ESMS $[M+H]^+$ = 215, 217 (Cl pattern).

2.1.19. 2-Nitro-6-(3-(4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)propyl)aniline (41)

A solution of 2-(3-chloropropyl)-6-nitroaniline (**53**, 0.29 g, 1.3 mmol), 4-(piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[*d*]-imidazole (**37**, 0.30 g, 1.1 mmol), and diisopropylethylamine (0.14 g, 1.1 mmol, 0.20 mL) in anhydrous dimethylformamide (20 mL) was stirred at 80 °C for 18 h. The mixture was cooled to room temperature, diluted with ethyl acetate (50 mL) and washed with water (3× 50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was purified by silica gel chromatography eluted with a gradient of methanol in methylene chloride (2–5–10%) to leave the product as an orange powder (0.11 g, 22%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 9.70 (br s, 1H), 8.04 (dd, 1H, *J* = 8.7 Hz, *J* = 1.4 Hz), 7.42 (br s, 1H), 7.30 (dd, 1H, *J* = 9.4 Hz, *J* = 1.3 Hz), 7.09 (d, 1H, *J* = 8.1 Hz), 6.72 (d, 1H, *J* = 7.8 Hz), 6.62 (dd, 1H, *J* = 8.7 Hz, *J* = 8.1 Hz), 3.64 (br s, 4H), 2.77 (m, 4H), 2.72 (t, 2H, *J* = 6.5 Hz), 2.42 (t, 2H, *J* = 6.0 Hz), 1.90 (m, 2H). ESMS $[M+H]^+$ = 449.

2.1.20. 2-(Trifluoromethyl)-4-(3-(4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)propyl)-1H-benzo[*d*]-imidazole (44 and 4)

A solution of 2-nitro-6-(3-(4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)propyl)aniline (**41**, 0.34 g, 0.76 mmol) in *N*-methylpyrrolidinone (10 mL) was treated with tin(II) chloride dihydrate (0.86 g, 4.6 mmol) and heated to 65 °C for 3.5 h. Another portion of tin(II) chloride dihydrate (0.43 g) was added and heating continued for 2 h. The mixture was cooled to room temperature and stirred 18 h then diluted with methylene chloride (50 mL) and extracted with 1 N HCl (3× 50 mL). The combined acidic layers were neutralized with 25% sodium hydroxide solution and extracted with ethyl acetate (3× 50 mL). The combined organic layers were washed with water (5× 50 mL) and brine (50 mL) and dried (Na_2SO_4) and evaporated to leave **44** as a brown powder (0.20 g, 63%). A portion of this powder (45 mg, 0.11 mmol) was dissolved in trifluoroacetic acid (4 mL), capped tightly and heated to 70 °C for 2 h. The solvent was evaporated and the residue was purified by reversed phase HPLC (Method 2). The product fractions were lyophilized to leave the product **4** as a bistrifluoroacetate salt (28 mg, 42%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.01 (br s, 1H), 9.58 (br s, 1H), 7.53 (d, 1H, *J* = 6.1 Hz), 7.38 (br s, 1H), 7.30 (dd, 1H, *J* = 8.0 Hz, *J* = 8.0 Hz), 7.25 (br s, 1H), 7.19 (d, 1H, *J* = 8.0 Hz), 6.73 (d, 1H, *J* = 7.7 Hz), 4.40 (d, 2H,

J = 12.6 Hz), 3.69 (d, 2H, J = 11.5 Hz), 3.29 (br s, 4H), 3.12 (t, 2H, J = 12.6 Hz), 3.05 (br s, 4H). Mass. Spec. (ESI) m/z $[M+H]^+$ = 497. Anal. Calcd for $C_{27}H_{24}F_{12}N_6O_4$: C, 44.76; H, 3.34; N, 11.60. Found: C, 44.86; H, 3.71; N, 11.12.

2.1.21. 2-Amino-3-nitrobenzoic acid (48)

To a suspension of 3-nitrophthallic anhydride (5.0 g, 26 mmol) in benzene (anhydrous, 70 mL) was added trimethylsilyl azide, and the resulting suspension was heated in an 80 °C bath for 2.5 h. The solution was cooled to rt and concentrated under reduced pressure to one-fourth its original volume. The concentrated solution was heated in a 100 °C bath overnight. The yellow solution was cooled and concentrated to afford a yellow solid. To the crude material was added EtOH (20 mL), and the solution was concentrated to dryness to provide 8-nitro isatoic anhydride (5.4 g, 99%). ^{1}H NMR 300 MHz (300 MHz, DMSO-d_6): δ = 11.2 (br s, 1H), 8.51 (dd, 1H, J = 8.5, 1.5 Hz), 8.35 (dd, 1H, J = 7.7, 1.5 Hz), 7.42 (t, 1H, J = 8.0 Hz). A suspension of 8-nitro isatoic anhydride (3.0 g, 14.4 mmol) in concd HCl (100 mL) was heated in a 95 °C bath for 3 h. The solution was cooled to rt. The precipitate which formed was collected to provide the product **48** as a yellow powder (1.50 g, 57%). LC/MS (Method A), tR = 1.05 min (purity = 95%), MS (ESI-NEG) $[M-H]^-$ = 181.

2.1.22. (2-Amino-3-nitrophenyl)methanol (49)

To a suspension of 2-amino-3-nitro benzoic acid (**48**, 2.12 g, 10 mmol) in benzene (75 mL) was added thionyl chloride (1.8 mL, 25 mmol) dropwise. The suspension was heated to reflux overnight. The mixture was cooled to room temperature and concentrated under reduced pressure to afford a golden solid. The crude material was dissolved in THF (40 mL) and cooled in an ice bath. Sodium borohydride (0.83 g, 22 mmol) was added in portions, and the reaction was allowed to come to room temperature with stirring. The mixture was again cooled in ice, and H_2O (15 mL) was slowly added to the reaction. Once gas evolution had subsided, the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ (2× 50 mL) and brine (50 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The reside was adsorbed onto silica gel and purified by column chromatography, eluting with a gradient of 25% EtOAc/hexane to 50% EtOAc/hexane to afford the alcohol **49** (0.89 g, 46%) as an orange solid. ^{1}H NMR 300 MHz (300 MHz, DMSO-d_6): δ = 7.92 (dd, 1H, J = 8.7, 1.4 Hz), 7.49 (d, 1H, J = 6.8 Hz), 7.12 (br s, 2H), 6.66 (dd, 1H, J = 8.7, J = 7.1 Hz), 5.45 (t, 1H, J = 5.4 Hz), 4.52 (d, 2H, J = 5.4 Hz). MS (ESI-POS): $[M+H]^+$ = 169.

2.1.23. 2-Chloromethyl-6-nitroaniline (50)

To a solution of (2-amino-3-nitrophenyl)methanol (**49**, 5.83 g, 35 mmol) in CH_2Cl_2 (250 mL) cooled in an ice bath was added triethyl amine (6.3 mL, 45 mmol), followed by dropwise addition of thionyl chloride (3.16 mL, 43 mmol). The solution was stirred at room temperature overnight. The ice bath was replaced, and ice was added to quench the reaction. The reaction mixture was washed with 0.1 N HCl (30 mL), H_2O (2× 30 mL), and brine (30 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude material was adsorbed onto silica gel and purified by column chromatography, eluting with a gradient of 20% EtOAc/hexane to 30% EtOAc/hexane to afford the chloromethyl compound **50** (5.3 g, 82%) as a yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ = 7.89 (dd, 1H, J = 8 Hz, J = 2 Hz), 7.44 (d, 1H, J = 7 Hz), 7.12 (br s, 2H), 6.69–6.66 (m, 1H), 4.61 (s, 2H).

2.1.24. 2-Nitro-6-((4-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)methyl)aniline (42)

A solution of 2-chloromethyl-6-nitroaniline (**50**, 79 mg, 0.42 mmol), 4-(piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[d]-imidazole (**37**, 0.11 g, 0.42 mmol), and diisopropylethylamine (81 mg, 0.63 mmol, 0.11 mL) in anhydrous dimethylformamide (10 mL) was stirred at 80 °C for 18 h. The mixture was cooled to room temperature, diluted with water (50 mL) and ethyl acetate (50 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (50 mL). The combined organic layers were washed with water (4× 50 mL) and brine (50 mL), dried (Na_2SO_4) and evaporated under reduced pressure. The product was purified by flash chromatography on silica gel eluted with a gradient of 2–3% methanol in methylene chloride to leave **42** as a foamy, orange solid (0.15 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 11.22 (br s, 1H), 8.09 (dd, 1H, J = 8.7 Hz, J = 1.0 Hz), 7.69 (br s, 2H), 7.25 (dd, 1H, J = 8.0 Hz, J = 8.0 Hz), 7.18 (d, 1H, J = 6.8 Hz), 7.09 (d, 1H, J = 8.1 Hz), 6.70 (d, 1H, J = 7.8 Hz), 6.58 (dd, 1H, J = 8. Hz, J = 8.6 Hz), 3.56 (s, 2H), 3.52 (br s, 4H), 2.54 (br s, 4H). LC/MS (Method A), tR = 1.04 min. ESMS $[M+H]^+$ = 421.

2.1.25. 3-((4-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)methyl)benzene-1,2-diamine (45)

To a solution of 2-nitro-6-((4-(2-(trifluoromethyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)aniline (**42**, 0.15 g, 0.36 mmol) in N-methlpyrrolidinone (10 mL) was added tin(II) chloride dihydrate (0.41 g, 2.1 mmol) and the mixture was heated to 70 °C for 1.5 h. Another portion of tin(II) chloride dehydrate was added (0.41 g, 2.1 mmol), and heating continued another 1.5 h. The mixture was cooled to room temperature, diluted with 1 N HCl (50 mL) and methylene chloride (50 mL). The layers were separated, the organic layer was extracted with 1 N HCl (50 mL), and the combined aqueous layers were neutralized to pH 7 with 25% aqueous sodium hydroxide, extracted with ethyl acetate (3× 25 mL) and the combined organic layers were dried (Na_2SO_4) and evaporated to a yellow oil (70 mg, 50%). ^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.26 (dd, 1H, J = 7.9 Hz, J = 7.9 Hz), 7.17 (d, 1H, J = 8.0 Hz), 6.74 (d, 1H, J = 8.8 Hz), 6.70 (dd, 1H, J = 7.4 Hz, J = 3.0 Hz), 6.56 (d, 1H, J = 6.4 Hz), 6.55 (d, 1H, J = 3.0 Hz), 3.58 (s, 2H), 3.40 (br s, 4H), 2.67 (m, 4H). LC/MS (Method A), tR = 0.71 min (purity = 94.0%); ESMS $[M+H]^+$ = 391.

2.1.26. 4-((4-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)methyl)-1H-benzo[*d*]imidazole-2(3H)-thione (6b)

A solution of 3-((4-(2-(trifluoromethyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)benzene-1,2-diamine (**45**, 50 mg, 0.13 mmol) and 1,1′-thiocarbonyldiimidazole (30 mg, 0.22 mmol) in anhydrous THF (10 mL) under a nitrogen atmosphere was stirred and heated to 70 °C for 2 h. The mixture was cooled to room temperature, water (0.2 mL) was added, and the mixture was evaporated. The crude product was purified by reversed phase HPLC (Method 2) and the product fractions were lyophilized to leave **6b** as a bistrifluoroacetate salt (18 mg, 32%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.00 (br s, 1H), 12.88 (br s, 1H), 12.80 (br s, 1H), 9.80 (br s, 1H), 7.18–7.35 (m, 5H), 6.75 (d, 1H, J = 7.3 Hz), 4.56 (s, 2H), 4.30–4.45 (m, 2H), 3.32–3.40 (m, 4H), 3.08–3.20 (m, 2H). Mass. Spec. (ESI) m/z $[M+H]^+$ = 433. Anal. Calcd for $C_{24}H_{21}F_9N_6O_4S$: C, 43.64; H, 3.20; N, 12.72. Found: C, 43.75; H, 3.18; N, 13.00.

2.1.27. 2-(Trifluoromethyl)-4-(4-((2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)methyl)piperazin-1-yl)-1H-benzo[*d*]imidazole (6a)

A solution of 3-((4-(2-(trifluoromethyl)-1H-benzo[d]imidazol-4-yl)piperazin-1-yl)methyl)benzene-1,2-diamine (**45**, 80 mg, 0.20 mmol) in trifluoroacetic acid (4 mL) was capped tightly and heated to 70 °C for 2 h. The solvent was evaporated and the residue

was purified by reversed phase HPLC (Method 2), and the product fractions were lyophilized to leave the product **6a** as a powder (33 mg, 24% based on the bistrifluoroacetate salt). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.38 (br s, 1H), 13.99 (br s, 1H), 10.00 (br s, 1H), 7.79 (br s, 1H), 7.60 (d, 1H, J = 7.3 Hz), 7.53 (br s, 1H), 7.27 (dd, 1H, J = 7.9 Hz, J = 7.9 Hz), 7.17 (d, 1H, J = 6.1 Hz), 6.70 (br s, 1H), 4.81 (s, 2H), 4.38 (br s, 2H), 3.45 (br s, 2H), 3.15 (br s, 4H). Mass. Spec. (ESI) m/z $[M-H]^-$ = 467. Anal. Calcd for $C_{25}H_{20}F_{12}N_6O_4$: C, 43.11; H, 2.89; N, 12.07. Found: C, 43.28; H, 3.38; N, 12.27.

2.1.28. 4-(2,2-Diethoxyethoxy)-1,2-dinitrobenzene (62)

To a solution of 2,2-diethoxyethanol (0.25 g, 1.9 mmol) in THF (5 mL) under nitrogen atmosphere was added a 60% dispersion of sodium hydride in mineral oil (76 mg, 1.9 mmol). The mixture stirred for 20 min and was then added dropwise via syringe to an ice cooled solution of 4-fluoro-1,2-dinitrobenzene (**61**, 0.35 g, 1.9 mmol) in THF (5 mL) under a nitrogen atmosphere. The reaction mixture stirred an hour and was diluted with water (50 mL) and ethyl acetate (50 mL). The organic layer was separated, dried ($MgSO_4$) and evaporated. The crude product was purified by chromatography on silica gel eluted with 25% ethyl acetate in hexanes. The purified product **62** was isolated as an oil (0.22 g, 39%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.02 (d, 1H, J = 9.0 Hz), 7.29 (d, 1H, J = 2.5 Hz), 7.18 (dd, 1H, J = 9.0 Hz, J = 2.5 Hz), 4.85 (t, 1H, J = 5.0 Hz), 4.16 (d, 2H, J = 5.0 Hz), 3.79 (dq, 2H, J = 9.0 Hz, J = 7.1 Hz), 3.63 (dq, 2H, J = 9.0 Hz, J = 7.1 Hz), 1.27 (t, 6H, J = 7.1 Hz).

2.1.29. 4-(2,2-Diethoxyethoxy)benzene-1,2-diamine (63)

4-(2,2-Diethoxyethoxy)-1,2-dinitrobenzene (**62**, 0.20 g, 0.67 mmol) was hydrogenated over 10% Pd/C (40 mg) at one atmosphere hydrogen pressure in methanol (3 mL) for 18 h. The mixture was filtered, washed (methanol, 2× 2 mL) and the filtrate was evaporated under reduced pressure to provide the product **63** as a dark gum (0.15 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.61 (d, 1H, J = 8.3 Hz), 6.35 (d, 1H, J = 2.6 Hz), 6.27 (dd, 1H, J = 8.3 Hz, J = 2.6 Hz), 4.80 (t, 1H, J = 5.2 Hz), 3.92 (d, 2H, J = 5.2 Hz), 3.75 (dq, 2H, J = 9.0 Hz, J = 7.0 Hz), 3.62 (dq, 2H, J = 9.0 Hz, J = 7.0 Hz), 1.24 (t, 6H, J = 7.0 Hz). LC/MS (Method A), tR = 0.21 min (purity = 99%), $[M+H]^+$ = 241.

2.1.30. 5-(2,2-Diethoxyethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (64)

A solution of 4-(2,2-diethoxyethoxy)benzene-1,2-diamine (**63**, 0.13 g, 0.54 mmol) and 1,1′-dithiocarbonyldiimidazole (0.19 g, 1.1 mmol) in THF (2 mL) was stirred and heated to 70 °C for 2 h. After cooling to room temperature the mixture was diluted with ethyl acetate (20 mL) and washed with 1 N HCl (10 mL) and water (10 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to leave the product **64** as a yellow solid (0.15 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.47 (s, 1H), 12.40 (s, 1H), 7.02 (d, 1H, J = 8.6 Hz), 6.75 (dd, 1H, J = 9.7 Hz, J = 2.2 Hz), 6.70 (d, 1H, J = 2.2 Hz), 4.79 (t, 1H, J = 5.1 Hz), 3.93 (d, 2H, J = 5.1 Hz), 3.66 (dq, 2H, J = 9.4 Hz, J = 7.2 Hz), 3.57 (dq, 2H, J = 9.4 Hz, J = 7.2 Hz), 1.14 (t, 6H, J = 7.2 Hz). LC/MS (Method A), tR = 1.14 min (purity = 93.3%), $[M+H]^+$ = 283.

2.1.31. 2-(2-Thioxo-2,3-dihydro-1H-benzo[*d*]imidazol-5-yloxy)acetaldehyde (65)

A solution of 5-(2,2-diethoxyethoxy)-1H-benzo[d]imidazole-2(3H)-thione (**64**, 0.13 g, 0.46 mmol) in 1 N HCl (1 mL) and terahydrofuran (2 mL) was stirred at 70 °C for 2 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (20 mL), washed with water (10 mL), dried ($MgSO_4$) and evaporated under reduced pressure to leave the product as an amorphous solid. Used as is with no characterization.

2.1.32. 5-(2-(4-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (7)

A solution of 4-(piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[d]imidazole (**37**, 46 mg, 0.17 mmol) and 2-(2-thioxo-2,3-dihydro-1H-benzo[d]imidazol-5-yloxy)acetaldehyde (**65**, 29 mg, 0.14 mmol) in *N*-methylpyrrolidinone (1.3 mL) was stirred and treated with sodium triacetoxyborohydride (40 mg, 0.19 mmol) for an hour. The reaction mixture was diluted with water (0.3 mL) and purified by reversed phase HPLC (Method F) to leave the product **7** as an amorphous solid bistrifluoroacetate salt (20 mg, 21%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.00 (br s, 1H), 12.55 (br s, 1H), 12.44 (br s, 1H), 9.90 (br s, 1H), 7.31 (dd, 1H, J = 8.0 Hz, J = 7.8 Hz), 7.20 (d, 1H, J = 8.0 Hz), 7.09 (d, 1H, J = 8.4 Hz), 6.85 (d, 1H, obscured), 6.83 (s, 1H), 6.77 (d, 1H, J = 7.8 Hz), 4.40 (m, 2H), 3.65 (m, 10H). LC/MS (Method A), tR = 0.74 min (purity = 100%), $[M+H]^+$ = 463. Anal. Calcd for $C_{25}H_{23}F_9N_6O_5S$: C, 43.48; H, 3.36; N, 12.17. Found: C, 43.91; H, 3.81; N, 12.51.

2.1.33. *tert*-Butyl 4-(3,4-dinitrophenyl)piperazine-1-carboxylate (66)

A mixture of 4-fluoro-1,2-dinitrobenzene (**61**, 0.50 g, 2.7 mmol), *tert*-butyl piperazine-1-carboxylate (0.65 g, 3.5 mmol), and *N*-methylmorpholine on polystyrene (3.4 mmol/g, 1.6 g, 5.4 mmol) in *N*-methylpyrrolidinone (10 mL) was shaken for 2 h. Isocyanate on polystyrene resin (1.2 mmol/g, 1.2 g, 1.4 mmol) and additional solvent (10 mL) were added and the mixture was shaken at 60 °C for 2 h. After cooling to room temperature the mixture was filtered, the residue washed (ethyl acetate, 3× 20 mL) and the combined organic layers were washed with water (5× 100 mL), dried ($MgSO_4$) and evaporated to leave the product as a yellow solid (0.95 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.01 (d, 1H, J = 9.2 Hz), 6.92–6.85 (m, 2H), 3.63 (m, 4H), 3.48 (m, 4H), 1.50 (s, 9H). LC/MS (Method A), tR = 1.73 min (purity = 88%), $[M+H]^+$ = 353.

2.1.34. *tert*-Butyl 4-(3,4-diaminophenyl)piperazine-1-carboxylate (67)

tert-Butyl 4-(3,4-dinitrophenyl)piperazine-1-carboxylate (**66**, 0.92 g, 2.6 mmol) was hydrogenated over 10% Pd/C (0.10 g) at 1 atmosphere hydrogen pressure in methanol (20 mL) for 18 h. The catalyst was filtered, washed (methanol, 2× 10 mL), and the filtrate was evaporated to leave the product as a dark oil (0.74 g, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.65 (d, 1H, J = 8.3 Hz), 6.37 (d, 1H, J = 2.3 Hz), 6.32 (dd, 1H, J = 8.3 Hz, J = 2.3 Hz), 3.56 (m, 4H), 3.40 (br s, 4H), 2.96 (m, 4H), 1.49 (s, 9H). LC/MS (Method A), tR = 0.77 min (purity = 91%), $[M+H]^+$ = 293.

2.1.35. 5-(Piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (68)

A solution of *tert*-butyl 4-(3,4-diaminophenyl)piperazine-1-carboxylate (**67**, 0.70 g, 2.4 mmol) in TFA (15 mL) was stirred and heated to 70 °C for 2 h. The solvent was evaporated and the residue was dissolved in ethyl acetate (50 mL), washed with saturated aqueous sodium bicarbonate solution (50 mL), dried ($MgSO_4$) and evaporated to leave the product as a tan amorphous solid (0.43 g, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.50 (br s, 2H), 7.62 (d, 1H, J = 8.9 Hz), 7.17 (dd, 1H, J = 8.9 Hz, J = 1.6 Hz), 7.09 (d, 1H, J = 1.6 Hz), 3.30 (m, 4H), 3.21 (m, 4H). LC/MS (Method A), tR = 0.33 min (purity = 88%), $[M+H]^+$ = 271.

2.1.36. 1-Azido-3-(2,2-diethoxyethoxy)-2-nitrobenzene (69)

A solution of 2,2-diethoxyethanol (0.12 g, 0.90 mmol) in THF (1 mL) under nitrogen atmosphere was treated with sodium

hydride (36 mg, 0.90 mmol, 60% dispersion in mineral oil) and stirred 20 min. The mixture was added dropwise via syringe to a stirring, ice cooled solution of 1-azido-3-fluoro-2-nitrobenzene (**33**, 0.11 g, 0.60 mmol) in tetrahydrofuran (1 mL) under a nitrogen atmosphere. After 2 h the reaction mixture was diluted with water (15 mL) and ethyl acetate (15 mL), the layers were separated, and the aqueous layer was extracted with ethyl acetate (15 mL). The combined organic layers were dried ($MgSO_4$) and evaporated to leave the product as a yellow foamy solid (0.16 g, 90%) which was used for subsequent reactions without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.40 (dd, 1H, J = 8.2, J = 8.4 Hz), 6.47 (d, 1H, J = 8.2), 6.33 (d, 1H, J = 8.4 Hz), 4.75 (t, 1H, J = 4.8 Hz), 4.10 (d, 2H, J = 5.2 Hz), 3.75 (m, 2H), 3.59 (m, 2H), 1.22 (t, 6H, J = 6.4 Hz). HPLC (Method 1), tR = 1.74 min (purity = 84%).

2.1.37. 2-(3-Azido-2-nitrophenoxy)acetaldehyde (70)

A solution of 1-azido-3-(2,2-diethoxyethoxy)-2-nitrobenzene (**69**, 0.16 g, 0.54 mmol) was stirred in 1 N HCl (1 mL) and tetrahydrofuran (1 mL) at 70 °C for 3 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (20 mL), washed with water (2× 15 mL), dried ($MgSO_4$), and evaporated. The crude product was chromatographed on silica gel eluted with 50% ethyl acetate in hexanes to afford the product as a light yellow amorphous solid (74 mg, 62%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 9.81 (s, 1H), 7.45 (dd, 1H, J = 8.6 Hz, J = 8.1 Hz), 6.97 (d, 1H, J = 8.1 Hz), 6.68 (d, 1H, J = 8.6 Hz), 4.66 (s, 2H).

2.1.38. 5-(4-(2-(3-Azido-2-nitrophenoxy)ethyl)piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (71)

A solution of 2-(3-azido-2-nitrophenoxy)acetaldehyde (**70**, 60 mg, 0.27 mmol) and 5-(piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (**37**, 73 mg, 0.27 mmol) in DMF (2 mL) was stirred and treated with sodium triacetoxyborohydride (63 mg, 0.30 mmol). The reaction mixture was stirred for 2 h, diluted with ethyl acetate (20 mL), washed with water (3× 20 mL), dried ($MgSO_4$), and evaporated to leave the product as a thick gum (57 mg, 44%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 10.60 (br s, 1H), 7.71 (d, 1H, J = 8.9 Hz), 7.40 (dd, 1H, J = 8.3 Hz, J = 8.5 Hz), 7.08 (d, 1H, J = 8.3 Hz), 6.88 (s, 1H), 6.87 (d, 1H, J = 8.2 Hz), 9.80 (d, 1H, J = 8.5 Hz), 4.25 (t, 2H, J = 5.4 Hz), 3.20 (m, 4H), 2.88 (t, 2H, J = 5.4 Hz), 2.67 (m, 4H). LC/MS (Method A), tR = 1.05 min (purity = 91%), $[M-H]^-$ = 475.

2.1.39. 4-(2-(4-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-5-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (72 and 9)

5-(4-(2-(3-Azido-2-nitrophenoxy)ethyl)piperazin-1-yl)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (**71**, 50 mg, 0.11 mmol) was hydrogenated over 10% Pd/C (10 mg, 1 atmosphere hydrogen pressure) in methanol (1.5 mL) for 18 h. The catalyst was filtered, washed (methanol, 2× 2 mL), and the filtrate was evaporated under reduced pressure to leave phenylenediamine **72**. The residue was dissolved in anhydrous tetrahydrofuran (1 mL), treated with 1,1′-thiocarbonyldiimidazole and shaken at 70 °C under a nitrogen atmosphere for 1.5 h. The mixture was diluted with water (250 μL) and purified by reversed phase HPLC (Method F) to leave the product as a bistrifluoroacetate salt (27 mg, 36%). ^{1}H NMR (300 MHz, CD_3OD) δ = 7.63 (d, 1H, J = 8.9 Hz), 7.27 (dd, 1H, J = 9.0 Hz, J = 2.1 Hz), 7.22 (d, 1H, J = 2.1 Hz), 7.17 (dd, 1H, J = 8.1 Hz, J = 8.1 Hz), 6.92 (d, 1H, J = 8.1 Hz), 6.88 (d, 1H, J = 8.1 Hz), 4.60 (t, 2H, J = 4.1 Hz), 3.79 (t, 2H, J = 4.1 Hz), 3.70 (br m, 8H). ESMS $[M-H]^-$ = 461, $[M+H]^+$ = 463. Anal. Calcd for $C_{25}H_{23}F_9N_6O_5S$: C, 43.48; H, 3.36; N, 12.17. Found: C, 43.10; H, 3.60; N, 11.96.

2.1.40. *tert*-Butyl 4-(3-amino-2-nitrophenyl)piperazine-1-carboxylate (73)

A solution of *tert*-butyl 4-(3-azido-2-nitro-phenyl)-piperazine-1-carboxylate (**35**, 300 mg, 0.861 mmol) in methanol (15 mL) at 0 °C under nitrogen atmosphere was treated with tin(II) chloride (171 mg, 0.904 mmol) and the resulting yellow mixture was stirred for 1 h then allowed to warm to room temperature. After 2 h at 20 °C the dark orange mixture was concentrated in vacuo. The resulting dark red powder was partitioned between water (30 mL) and ethyl acetate (30 mL). Aqueous sodium hydroxide 15% (2 mL) was added and the aqueous layer was extracted with ethyl acetate (2× 30 mL). Brine (20 mL) was added and the aqueous layer was further extracted with ethyl acetate (2× 15 mL). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The product was purified by chromatography on silica gel eluted with 0.5% MeOH in CH_2Cl_2 to afford **73** as a red powder (226 mg, 82% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.10 (t, 1H, J = 7.88 Hz) 6.56 (dd, 1H, J = 7.88, J = 0.83 Hz), 6.39 (dd, 1H, J = 7.88 Hz, J = 0.83 Hz), 5.91 (br s, 2H), 3.79 (m, 4H), 3.34 (m, 4H), 1.41 (s, 9H). MS (ESI) m/z $[M+H]^+$ = 323. Anal. Calcd for $C_{15}H_{22}N_4O_4$: C, 55.89; H, 6.88; N, 17.38. Found: C, 55.47; H, 6.66; N, 16.99.

2.1.41. *tert*-Butyl 4-{2-nitro-3-[(trifluoroacetyl)amino]phenyl}-piperazine-1-carboxylate (74)

To a solution of 4-(3-nitro-2-nitro-phenyl)-piperazine-1-carboxylic acid *tert*-butyl ester (**73**, 200 mg, 0.620 mmol) in methylene chloride (10 mL) at 0 °C under a nitrogen atmosphere was added di-isopropylethylamine (137 μL, 0.744 mmol) followed by trifluoroacetic anhydride (92 μL, 0.651 mmol). The resulting red solution was stirred at 0 °C for 1 h. The reaction was terminated with the addition of saturated aqueous sodium bicarbonate solution (10 mL). The organic layer was separated and the aqueous layer was diluted with brine (10 mL) and extracted with methylene chloride (2× 10 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The product was purified by chromatography on silica gel eluted with 0.5% MeOH in CH_2Cl_2 to afford **74** as a red powder (220 mg, 99% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.57 (br s, 1H), 7.28 (dd, 1H, J = 7.5 Hz, J = 0.8 Hz), 7.10 (t, 1H, J = 7.9 Hz), 6.56 (dd, 1H, J = 7.6 Hz, J = 0.8 Hz), 3.37 (m, 4H), 2.91 (m, 4H), 1.41 (s, 9H). MS (ESI) m/z $[M+H]^+$ = 417. Anal. Calcd for $C_{17}H_{21}F_3N_4O_5$: C, 48.81; H, 5.06; N, 13.39. Found: C, 48.61; H, 4.82; N, 12.95.

2.1.42. *tert*-Butyl 4-{3-[methyl(trifluoroacetyl)amino]-2-nitrophenyl}piperazine-1-carboxylate (75)

To a solution of 4-[2-nitro-3-(2,2,2,-trifluoro-acetylamino)-phenyl]-piperazine-1-carboxylic acid *tert*-butyl ester (**74**, 220 mg, 0.526 mmol) in tetrahydrofuran (10 mL) at 0 °C under a nitrogen atmosphere was added triphenylphosphine (268 mg, 1.03 mmol), methanol (48 μL, 1.18 mmol), and diisopropylazodicarboxylate (207 μL, 1.03 mmol). The resulting yellow solution was allowed to warm to room temperature and stirred 15 h. Concentration in vacuo followed by chromatography on silica gel eluted with 0.5% MeOH in CH_2Cl_2 afforded **75** as an orange gum (201 mg, 89% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.63 (t, 1H, J = 7.4 Hz), 7.59 (dd, 1H, J = 7.4, J = 0.7 Hz), 7.43 (dd, 1H, J = 7.2, J = 0.7 Hz), 3.33 (m, 4H), 3.22 (s, 3H), 2.91 (m, 4H), 1.41 (s, 9H). ESMS m/z $[M+H]^+$ = 433. Anal. Calcd for $C_{18}H_{23}F_3N_4O_5$: C, 50.00; H, 5.36; N, 12.96. Found: C, 49.88; H, 5.40; N, 12.46.

2.1.43. *tert*-Butyl 4-[1-methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-yl]piperazine-1-carboxylate (76)

tert-Butyl 4-{3-[methyl(trifluoroacetyl)amino]-2-nitrophenyl}-piperazine-1-carboxylate (**75**, 2.92 g, 6.75 mmol) was hydrogenated over 5% Pd/C (300 mg) at 20 °C and one atmosphere hydrogen pressure in methanol (75 mL). The catalyst was filtered through Celite, and the filtrate was concentrated in vacuo. The

product was purified by chromatography on silica gel eluted with 40–70% ethyl acetate in hexanes to afford **76** as a white powder (1.78 g, 68% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.30 (t, 1H, J = 8.7 Hz), 7.23 (d, 1H, J = 8.7 Hz), 6.29 (d, 1H, J = 8.7 Hz), 3.93 (s, 3H), 3.52 (m, 4H), 3.46 (m, 4H), 1.48 (s, 9H). MS (ESI) m/z $[M+H]^+$ = 385. Anal. Calcd for $C_{18}H_{23}F_3N_4O_2$: C, 56.24; H, 6.03; N, 14.58. Found: C, 55.87; H, 5.74; N, 14.34.

2.1.44. 1-Methyl-4-piperazin-1-yl-2-(trifluoromethyl)-1*H*-benzimidazole (77)

A solution of *tert*-butyl 4-[1-methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-yl]piperazine-1-carboxylate (**76**, 1.75 g, 4.55 mmol) in methylene chloride (25 mL) and TFA (10 mL) was stirred 1 h. Methylene chloride (30 mL) was added, and the mixture was washed with 30% aq NaOH (100 mL). The aqueous layer was extracted with methylene chloride (30 mL), and the combined organic layers were dried ($MgSO_4$) and filtered through a silica pad eluted with 10% methanol in methylene chloride. The filtrate was concentrated in vacuo to a brown gum (920 mg, 71% yield). ^{1}H NMR (DMSO-d_6): δ = 7.28 (t, 1H, J = 6.9 Hz), 7.18 (d, 1H, J = 6.9 Hz), 6.65 (d, 1H, J = 6.9 Hz), 3.91 (br s, 1H), 3.43 (m, 4H), 3.36 (s, 3H), 2.91 (m, 4H). HPLC (Method A) tR = 0.23 min (purity = 95.6%), m/z $[M+H]^+$ = 285.

2.1.45. 3-(2-{4-[1-Methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-yl]piperazin-1-yl}ethoxy)benzene-1,2-diamine (78 and 79)

To a solution of 1-methyl-4-piperazin-1-yl-2-(trifluoromethyl)-1*H*-benzimidazole (**77**, 500 mg, 1.76 mmol) and 2-(3-azido-2-nitrophenoxy)-acetaldehyde (**70**, 469 mg, 2.11 mmol), in *N*-methyl-2-pyrrolidone (10 mL), at room temperature under nitrogen were added sodium triacetoxyborohydride (746 mg, 3.52 mmol) and acetic acid (10 μL). The resulting mixture was stirred for 90 min, quenched with water (1 mL) and partitioned between ethyl acetate (50 mL) and saturated aq sodium bicarbonate solution (50 mL). The organic layer was dried ($MgSO_4$)and concentrated in vacuo. The product was purified by chromatography on silica gel eluted with 0.5% methanol in methylene chloride to afford **78** as an orange gum (390 mg, 45% yield) which was used immediately without characterization. 4-{4-[2-(Azido-2-nitro-phenoxy)-ethyl]piperazin-1-yl}-1-methyl-2-trifluoromethyl-1H-benzimidazole (**78**, 390 mg, 0.795 mmol) was hydrogenated over 5% Pd/C in methanol (8 mL) at room temperature and one atmosphere of hydrogen pressure. The catalyst was filtered, washed (methanol), and the filtrate was evaporated in vacuo to leave **79** as a light brown powder (310 mg, 90% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 7.30 (t, 1H, J = 7.4 Hz), 7.21 (d, 1H, J = 7.4 Hz), 6.67 (d, 1H, J = 7.4 Hz), 6.35 (d, 1H, J = 7.7 Hz), 6.22 (td, 2H, J = 7.7 Hz, J = 1.4 Hz), 4.43 (br s, 2H), 4.11 (br s, 2H), 4.03 (t, 2H, J = 5.7 Hz), 3.92 (s, 3H), 3.54 (m, 4H), 2.74 (t, 2H, J = 5.7 Hz), 2.72 (m, 4H). HPLC (Method A), tR = 0.22 min, m/z $[M+H]^+$ = 435.

2.1.46. 4-(2-{4-[1-Methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-yl]piperazin-1-yl}ethoxy)-1,3-dihydro-2*H*-benzimidazole-2-thione (8b)

To a solution of 3-(2-{4-[1-methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-yl]piperazin-1-yl}ethoxy)benzene-1,2-diamine (**79**, 50 mg, 0.12 mmol) in tetrahydrofuran (5 mL) at room temperature under nitrogen was added 1,1′-thiocarbonyldiimidazole (61 mg, 0.345 mmol). The resulting mixture was capped and heated to 70 °C for 2.5 h. The solvent was evaporated, and the product was purified by reverse phase HPLC (Method F) to afford a yellow powder (38 mg, 56% yield) as the bistrifluoroacetate. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.68 (s, 1H), 12.64 (s, 1H), 9.60 (br s, 1H), 7.34 (m, 2H), 7.08 (t, 1H, J = 8.2 Hz), 6.82 (m, 3H), 4.50 (m, 2H), 4.44 (d, 2H, J = 12.37 Hz), 3.96 (s, 3H), 3.83 (d, 2H, J = 12.37 Hz), 3.71 (m, 2H), 3.48 (t, 2H, J = 11.54 Hz), 3.23 (t, 2H, J = 11.54 Hz). MS (ESI) m/z $[M+H]^+$ = 476. Anal. Calcd for $C_{26}H_{25}F_9N_6O_5S$: C, 44.32; H, 3.58; N, 11.93. Found: C, 44.60; H, 4.03; N, 11.80.

2.1.47. 1-Methyl-2-(trifluoromethyl)-4-[4-(2-{[2-(trifluoromethyl)-1*H*-benzimidazol-4-yl]oxy}ethyl)piperazin-1-yl]-1*H*-benzimidazole (8a)

A solution of 3-(2-{4-[1-methyl-2-(trifluoromethyl)-1*H*-benzimidazol-4-yl]piperazin-1-yl}ethoxy)benzene-1,2-diamine (**79**, 51 mg, 0.117 mmol) in TFA (2 mL) was capped and heated to 70°C for 2.5 h. The solvent was evaporated, and the product was purified by reverse phase HPLC (Method F) to afford **8a** as a brown powder (19.6 mg, 27% yield) as the bistrifluoroacetate. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 3.21 (t, 2H, J = 11.54 Hz), 3.49 (t, 2H, J = 11.54 Hz), 3.75 (m, 2H), 3.84 (d, 2H, J = 12.37 Hz), 3.95 (s, 3H), 4.44 (d, 2H, J = 12.37 Hz), 4.68 (m, 2H), 6.79 (d, 1H, J = 7.69 Hz), 6.99 (br s, 1H), 7.32 (m, 4H), 9.99 (br s, 1H), 14.05 (br s, 1H). MS (ESI) m/z $[M+H]^+$ = 512. Anal. Calcd for $C_{27}H_{24}F_{12}N_6O_5$: C, 43.79; H, 3.27; N, 11.35. Found: C, 43.88; H, 3.50; N, 11.51.

2.1.48. 1-(2-(3-Azido-2-nitrophenoxy)ethyl)piperazine (80)

A solution of 2-(piperazin-1-yl)ethanol (5.7 g, 43 mmol) in tetrahydrofuran (40 mL) under a nitrogen atmosphere was cooled in an ice bath and treated with sodium hydride (60% mineral oil dispersion, 1.7 g, 43 mmol) in portions over 5 min. After 1 h the mixture was cooled to −78 °C and a solution of 1-azido-3-fluoro-2-nitrobenzene (**33**, 6.0 g, 33 mmol) in tetrahydrofuran (40 mL) was added dropwise over 15 min. The mixture warmed to 20 °C over 2 h, 1 N HCl (50 mL) was added cautiously followed by ethyl acetate (200 mL) and water (200 mL). The aqueous layer was separated, washed with ethyl acetate (100 mL), neutralized with solid sodium carbonate and the product was extracted with chloroform (3× 100 mL). The combined chloroform layers were dried ($MgSO_4$) and evaporated under reduced pressure to leave the product as a gum (1.4 g, 15%). The original ethyl acetate layers were combined and extracted with 1 N HCl (2× 100 mL). The combined acidic layers were neutralized with solid sodium carbonate and extracted with chloroform (3× 100 mL). The combined chloroform layers were dried ($MgSO_4$) and evaporated to leave additional product (5.7 g, 59%) for a total yield of 7.1 g (74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.40 (dd, 1H, J = 8.4 Hz, J = 8.3 Hz), 6.85 (d, 1H, J = 8.3 Hz), 6.80 (d, 1H, J = 8.4 Hz), 4.20 (t, 2H, J = 5.7 Hz), 2.88 (m, 4H), 2.78 (t, 2H, J = 5.7 Hz), 2.51 (m, 4H).

2.1.49. *tert*-Butyl 4-(2-(3-azido-2-nitrophenoxy)ethyl)piperazine-1-carboxylate (81)

To a solution of 1-(2-(3-azido-2-nitrophenoxy)ethyl)piperazine (**80**, 7.1 g, 24 mmol) in methylene chloride (100 mL) was added di-*tert*-butyldicarbonate (7.4 g, 34 mmol), and the mixture stirred for 30 min. Aminomethylpolystyrene resin was added (3.2 mmol/g, 7.5 g, 24 mmol) and the mixture stirred an additional hour. The resin was filtered, washed with methylene chloride (3× 100 mL), and the combined filtrates were evaporated under reduced pressure to leave the product **81** as a brown gum (9.4 g, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.41 (dd, 1H, J = 8.4 Hz, J = 8.3 Hz), 6.87 (d, 1H, J = 8.3 Hz), 6.81 (d, 1H, J = 8.4 Hz), 4.20 (t, 2H, J = 5.6 Hz), 3.41 (m, 4H), 2.78 (t, 2H, J = 5.6 Hz), 2.47 (m, 4H), 1.47 (s, 9H). LC/MS (Method A), tR = 1.11 min (purity = 90.0%), $[M+H]^+$ −56 (*tert*-butyl) = 337.

2.1.50. *tert*-Butyl 4-(2-(2,3-diaminophenoxy)ethyl)piperazine-1-carboxylate (82)

A solution of *tert*-butyl 4-(2-(3-azido-2-nitrophenoxy)ethyl)piperazine-1-carboxylate (**81**, 4.46 g, 11.4 mmol) in methanol (120 mL) was hydrogenated at one atmosphere hydrogen pressure over 5% palladium on carbon (0.63 g) for 4 h. The cat-

alyst was filtered through diatomaceous earth, washed with methanol (2× 20 mL), and the combined filtrates were evaporated under reduced pressure to leave the product as a dark brown foamy solid (3.55 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 6.64 (dd, 1H, J = 8.1 Hz, J = 8.1 Hz), 6.41 (d, 1H, J = 8.1 Hz), 6.40 (d, 1H, J = 8.1 Hz), 4.12 (t, 2H, J = 5.8 Hz), 3.53 (br s, 2H), 3.49 (br s, 2H), 3.45 (m, 4H), 2.81 (t, 2H, J = 5.8 Hz), 2.52 (m, 4H), 1.46 (s, 9H). LC/MS (Method A), tR = 0.57 min (purity = 99%), $[M+H]^+$ = 337.

2.1.51. 4-(2-(4-(3-Azido-2-nitrophenyl)piperazin-1-yl)ethoxy)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (83 and 86)

A solution of *tert*-butyl 4-(2-(2,3-diaminophenoxy)ethyl)piperazine-1-carboxylate (**82**, 260 mg, 0.77 mmol) in TFA (3 mL) was stirred for 30 min at room temperature then 2 h at 75 °C. The TFA was evaporated and the residue was dissolved in water (10 mL), and lyophilized to leave product **83** as a dark brown gum. The residue was dissolved in dimethylsulfoxide (4 mL), treated with diisopropylethylamine (0.60 g, 4.6 mmol, 0.83 mL) and 1-azido-3-fluoro-2-nitrobenzene (**37**, 0.21 g, 1.2 mmol) and stirred at 60 °C for 20 h. The reaction mixture was cooled to 20 °C, diluted with ethyl acetate (50 mL) and washed with 1 M sodium carbonate (50 mL), water (2× 50 mL), and brine (50 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed on silica gel (Isco instrument, 40 g column; 50% ethyl acetate in hexanes to 100% ethyl acetate) to leave the product **86** as a yellow, foamy solid (232 mg, 63%, two steps). ^{1}H NMR (300 MHz, CD_3OD) δ = 7.49 (dd, 1H, J = 8.3 Hz, J = 8.2 Hz), 7.26 (d, 1H, J = 7.2 Hz), 7.25 (dd, 1H, J = 8.2 Hz, J = 7.2 Hz), 7.14 (d, 1H, J = 8.2 Hz), 7.14 (d, 1H, J = 8.2 Hz), 6.87 (d, 1H, J = 8.3 Hz), 4.34 (t, 2H, J = 5.5 Hz), 3.00 (m, 4H), 2.94 (t, 2H, J = 5.5 Hz), 2.70 (m, 4H). LC/MS (Method A), tR = 1.40 min (purity = 87.2%), $[M+H]^+$ = 476.

2.1.52. 3-(4-(2-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)benzene-1,2-diamine (88)

A solution of 4-(2-(4-(3-azido-2-nitrophenyl)piperazin-1-yl)ethoxy)-2-(trifluoromethyl)-1H-benzo[*d*]imidazole (**86**, 0.21 g, 0.44 mmol) in methanol (4 mL) was hydrogenated at 1 atmosphere hydrogen pressure over 5% palladium on carbon (40 mg) for 6 h. The mixture was filtered, the catalyst was washed with methanol (2× 5 mL), and the combined filtrates were evaporated under reduced pressure to leave the product **88** as a tan foamy solid (94 mg, 51%). ^{1}H NMR (300 MHz, CD_3OD) δ = 7.20–7.30 (m, 2H), 6.88 (d, 1H, J = 7.0 Hz), 6.46–6.57 (m, 3H), 4.38 (t, 2H, J = 5.5 Hz), 2.98 (t, 2H, J = 5.5 Hz), 2.88 (m, 4H), 2.82 (m, 4H). LC/MS (Method A), tR = 1.07 min (purity = 95.3%), $[M+H]^+$ = 421.

2.1.53. 4-(4-(2-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)-1H-benzo[*d*]imidazol-2(3H)-one (10a)

A solution of 3-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)benzene-1,2-diamine (**88**, 40 mg, 0.095 mmol) in tetrahydrofuran (1 mL) was treated with 1,1′-carbonyldiimidazole (23 mg, 0.14 mmol) and stirred for 18 h. The mixture was diluted with water (0.2 mL) and purified by reversed phase HPLC (Method 2) to leave the bistrifluoroacetate product **10a** as a gum (39 mg, 61%). ^{1}H NMR (300 MHz, CD_3OD) δ = 7.42 (dd, 1H, J = 8.1 Hz, J = 8.2 Hz), 7.31 (d, 1H, J = 8.1 Hz), 7.17 (dd, 1H, J = 8.0 Hz, J = 8.0 Hz), 6.99 (d, 1H, J = 8.0 Hz), 6.97 (d, 1H, J = 8.1 Hz), 6.92 (d, 1H, J = 8.2 Hz), 4.65 (t, 2H, J = 4.9 Hz), 3.87 (m, 4H), 3.57 (m, 4H), 3.27 (t, 2H, J = 4.9 Hz). ^{13}C NMR (300 MHz, CD_3OD) δ = 169.30, 150.40, 141.00 (q), 136.99, 136.27, 134.89, 132.99, 127.57, 127.17, 124.83, 118.50, 112.99, 107.86, 107.09, 106.05, 62.29, 56.61, 53.20, 53.19. HPLC (Method C), t_R = 7.01 min (purity = 99.9%). HRMS $[M+H]^+$ = 447.1744, calcd for $C_{21}H_{21}F_3N_6O_2$: 447.1751.

2.1.54. 4-(4-(2-(2-(Trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)-1H-benzo[*d*]imidazole-2(3H)-thione (11)

A solution of 3-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)benzene-1,2-diamine (**88**, 40 mg, 0.095 mmol) in tetrahydrofuran (1 mL) was treated with 1,1′-thiocarbonyldiimidazole (25 mg, 0.14 mmol) and stirred for 18 h. The mixture was diluted with water (0.2 mL) and purified by reversed phase HPLC (Method 2) to leave the bistrifluoroacetate product **11** as a gum (43 mg, 66%). ^{1}H NMR (300 MHz, CD_3OD) δ = 7.42 (dd, 1H, J = 8.1 Hz, J = 8.1 Hz), 7.31 (d, 1H, J = 8.2 Hz), 7.04 (dd, 1H, J = 8.0 Hz, J = 8.0 Hz), 6.97 (d, 1H, J = 8.0 Hz), 6.86 (d, 1H, J = 8.0 Hz), 6.82 (d, 1H, J = 8.2 Hz), 4.64 (t, 2H, J = 4.9 Hz), 3.86 (m, 4H), 3.53 (m, 4H), 3.23 (t, 2H, J = 4.9 Hz). ^{13}C NMR (300 MHz, CD_3OD) δ = 157.00, 150.50, 141.41, 140.88, 137.05, 135.79, 133.05, 131.85, 127.65, 124.01, 123.29, 122.00, 118.43, 112.01, 107.85, 106.93, 106.10, 62.37, 56.70, 53.43, 53.40. HPLC (Method C), tR = 6.68 min (purity = 99.9%). HRMS $[M+H]^+$ = 463.1522, calcd for $C_{21}H_{21}F_3N_6OS$: 463.1522.

2.1.55. *tert*-Butyl 4-(2-(2-thioxo-2,3-dihydro-1H-benzo[*d*]-imidazol-4-yloxy)ethyl)piperazine-1-carboxylate (84)

A solution of *tert*-butyl 4-(2-(2,3-diaminophenoxy)ethyl)piperazine-1-carboxylate (**82**, 8.0 g, 24 mmol) was dissolved in anhydrous tetrahydrofuran (100 mL), purged with a nitrogen atmosphere, treated with 1,1′-thiocarbonyldiimidazole (7.6 g, 43 mmol) and stirred 18 h. Water (15 mL) was added and the reaction mixture stirred an additional 24 h. The solvent was evaporated, the residue was dissolved in ethyl acetate (300 mL), washed with water (3× 100 mL) and brine (100 mL). The organic layer was dried and evaporated under reduced pressure to leave the crude product **84**, which was purified by flash chromatography on silica gel eluted with a gradient of 50% ethyl acetate in hexanes to 100% ethyl acetate. The desired product was obtained as a colorless gum (4.3 g, 47%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.70 (br s, 1H), 12.48 (br s, 1H), 7.03 (dd, 1H, J = 8.1 Hz, J = 8.1 Hz), 6.77 (d, 1H, J = 8.1 Hz), 6.74 (d, 1H, J = 8.1 Hz), 4.20 (t, 2H, J = 5.6 Hz), 3.31 (m, 4H), 2.56 (t, 2H, J = 5.6 Hz), 2.46 (m, 4H), 1.38 (s, 9H). LC/MS (Method A), tR = 0.64 min (purity = 95.3%), $[M+H]^+$ = 378.

2.1.56. 4-(2-(4-(3-Azido-2-nitrophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (85 and 87)

A mixture of *tert*-butyl 4-(2-(2-thioxo-2,3-dihydro-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazine-1-carboxylate (**84**, 4.3 g, 11 mmol) and dimethylsilane on polystyrene resin (1.7 mmol/g, 13 g, 22 mmol) in methylene chloride (80 mL) was treated with trifluoroacetic acid (20 mL) and stirred for an hour. The reaction mixture was filtered, the polymer residue was washed with methylene chloride (2× 50 mL), and the combined filtrates were evaporated under reduced pressure. The crude product was dissolved in water (100 mL), saturated with sodium carbonate, and extracted with *n*-butanol (3× 100 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to leave the product **85** as a sticky, light tan gum (2.1 g, 69%). A portion of this intermediate (**85**, 1.8 g, 6.5 mmol) was dissolved in DMSO (18 mL), treated with 1-azido-3-fluoro-2-nitrobenzene (**37**, 1.8 g, 9.7 mmol) and stirred at 60 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with ethyl acetate (300 mL), washed with 1 M sodium carbonate (100 mL), and the aqueous layer was further extracted with ethyl acetate (50 mL). The combined ethyl acetate layers were washed with water (3× 100 mL) and brine (100 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel eluted with a gradient of 75% ethyl acetate in hexanes to 100% ethyl acetate. The product **87** was obtained as a yellow foamy solid (1.9 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$)

δ = 12.70 (br s, 1H), 10.42 (br s, 1H), 7.48 (dd, 1H, *J* = 8.1 Hz, *J* = 8.1 Hz), 7.37 (dd, 1H, *J* = 8.3 Hz, *J* = 1.1 Hz), 7.06 (dd, 1H, *J* = 8.0 Hz, *J* = 8.1 Hz), 7.02 (dd, 1H, *J* = 8.3 Hz, *J* = 1.1 Hz), 6.92 (d, 1H, J = 7.9 Hz), 6.80 (d, 1H, J = 7.9 Hz), 4.22 (t, 2H, J = 5.4 Hz), 3.35 (m, 4H), 2.81 (m, 6H). LC/MS (Method A), *t*R = 1.03 min (purity = 92.3%), $[M+H]^+$ = 441.

2.1.57. 4-(2-(4-(2,3-Diaminophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (89)

A solution of 4-(2-(4-(3-azido-2-nitrophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (**87**, 1.8 g, 4.1 mmol) in *N*-methylpyrrolidinone (40 mL) was treated with tin(II) chloride dihydrate (9.2 g, 41 mmol) and stirred 5 min at 20 °C then 1.5 h at 100 °C. The reaction mixture was cooled to 20 °C, diluted with 1 N HCl (30 mL) and filtered. The filtrate was neutralized with solid sodium carbonate, diluted with ethyl acetate (200 mL), stirred for 15 min, and filtered. The filtrate layers were separated, and the organic layer was washed with water (5× 100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$) and evaporated under reduced pressure to leave the product as a light yellow amorphous solid (0.68 g, 43%). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 12.40 (br s, 1H), 10.65 (br s, 1H), 7.05 (dd, 1H, *J* = 8.1 Hz, *J* = 8.1 Hz), 6.85 (d, 1H, *J* = 8.0 Hz), 6.81 (dd, 1H, *J* = 7.9, Hz, *J* = 1.1 Hz), 6.73 (d, 1H, J = 7.9 Hz), 6.70 (dd, 1H, *J* = 7.9 Hz, *J* = 7.9 Hz), 6.55 (dd, 1H, *J* = 7.9 Hz, *J* = 1.1 Hz), 4.38 (t, 2H, *J* = 4.8 Hz), 3.60 (br s, 4H), 3.15 (t, 2H, *J* = 4.8 Hz), 2.89 (m, 4H). LC/MS (Method A), *t*R = 0.22 min (purity = 92.5%), $[M+H]^+$ = 385.

2.1.58. 4-(4-(2-(2-Thioxo-2,3-dihydro-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)-1H-benzo[*d*]imidazol-2(3H)-one (10b)

A solution of 4-(2-(4-(2,3-diaminophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (**89**, 57 mg, 0.16 mmol) and carbonyldiimidazole (22 mg, 0.12 mmol) in tetrahydrofuran (1 mL) was stirred for 18 h. The reaction mixture was diluted with water (0.2 mL) and purified by reversed phase HPLC (Method 2). The product **10b** was obtained as a bistrifluoroacetate salt (2.4 mg, 3.7%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.62 (br s, 1H), 12.59 (br s, 1H), 10.80 (br s, 1H), 10.68 (br s, 1H), 9.55 (s, 1H), 7.12 (dd, 1H, *J* = 8.1 Hz), 6.92 (dd, 1H, *J* = 8.0 Hz, *J* = 8.0 Hz), 6.85 (d, 1H, *J* = 7.3 Hz), 6.82 (d, 1H, *J* = 7.4 Hz), 6.79 (d, 1H, *J* = 7.3 Hz), 6.76 (d, 1H, *J* = 8.0), 4.50 (br t, 2H, *J* = 5.7 Hz), 3.72 (br s, 4H), 3.48 (partially obscured bs, 4H), 3.03 (br t, 2H, *J* = 5.7 Hz). HPLC (Method C), *t*R = 7.34 min (purity = 97.8%). ESMS $[M-H]^-$ = 409. HRMS $[M+H]^+$ = 411.1605, calcd for $C_{20}H_{23}N_6O_2S$: 411.1611.

2.1.59. 5-(4-(2-(2-Thioxo-2,3-dihydro-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)quinoxaline-2,3(1H,4H)-dione (12)

A solution of 4-(2-(4-(2,3-diaminophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (**89**, 30 mg, 0.078 mmol) and oxalyldiimidazole (22 mg, 0.12 mmol) in tetrahydrofuran (1 mL) was stirred for 18 h. The reaction mixture was diluted with water (0.2 mL) and purified by reversed phase HPLC (Method 2). The product was obtained as a bistrifluoroacetate salt (13 mg, 30%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.63 (br s, 2H), 12.01 (br s, 1H), 10.96 (br s, 1H), 9.94 (br s, 1H), 7.12 (d, 1H, *J* = 8.0 Hz), 7.09 (m, 2H), 7.00 (d, 1H, *J* = 8.9 Hz), 6.86 (d, 1H, *J* = 7.8 Hz), 6.84 (d, 1H, *J* = 7.8 Hz), 4.53 (br t, 2H, *J* = 4.7 Hz), 3.74 (br t, 2H, *J* = 4.7 Hz), 3.68 (m, 4H), 3.12 (m, 4H). ^{13}C NMR (300 MHz, DMSO-d_6) δ = 167.71, 158.00 (q), 155.85, 155.05, 141.86, 138.11, 133.53, 126.29, 123.39,123.21, 121.93, 120.84, 115.59, 112.20, 105.71, 103.44, 62.72, 54.99, 51.72, 48.71. HPLC (Method C), *t*R = 4.50 min (purity = 96.2%). HRMS $[M+H]^+$ = 438.1471, calcd for $C_{21}H_{23}N_6O_3S$: 438.1474.

2.1.60. 4-(2-(4-(Quinoxalin-5-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (13)

A solution of 4-(2-(4-(2,3-diaminophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (**89**, 10 mg, 26 μmol) in methanol (1 mL) was treated with 40% aqueous glyoxal (8 μL, 52 μmol) and stirred for 2 h. Water (0.4 mL) was added and the reaction mixture was purified by reversed phase HPLC (Method 2) to leave the monotrifluoracetate product **13** as an amorphous solid (5.2 mg, 38%). ^{1}H NMR (300 MHz, CD_3OD) δ = 8.86 (d, 1H, *J* = 2.0 Hz), 8.84 (d, 1H, *J* = 2.0 Hz), 7.75 (d, 1H, *J* = 4.0 Hz), 7.74 (d, 1H, *J* = 4.0 Hz), 7.34 (dd, 1H, *J* = 4.0 Hz, *J* = 4.0 Hz), 7.13 (dd, 1H, *J* = 8.0 Hz, *J* = 8.2 Hz), 6.88 (d, 1H, *J* = 8.0 Hz), 6.85 (d, 1H, *J* = 8.2 Hz), 4.58 (t, 2H, *J* = 4.7 Hz), 4.20 (m, 2H), 3.86 (m, 2H), 3.81 (t, 2H, *J* = 4.7 Hz), 3.68 (m, 2H), 3.31 (m, 2H). HPLC (Method C), *t*R = 5.21 min (purity = 98.5%). HR ESMS $[M+H]^+$ = 407.1653, calcd for $C_{21}H_{23}N_6OS$: 407.1649.

2.1.61. 4-(2-(4-(1H-Benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (14)

A solution of 4-(2-(4-(2,3-diaminophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (**89**, 41 mg, 0.12 mmol) in 90% formic acid (1 mL) was heated to 90 °C for 2 h. The formic acid was evaporated, and the product was purified by reversed phase HPLC (Method F). The pure product **14** was obtained as a bistrifluoroacetate salt (4.0 mg, 5.4%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.62 (br s, 2H), 9.80 (br s, 1H), 9.15 (br s, 1H), 7.42 (s, 1H), 7.43 (d, 1H, *J* = 6.6 Hz), 7.12 (dd, 1H, *J* = 6.7 Hz, *J* = 8.1 Hz), 7.00 (d, 1H, *J* = 5.7 Hz), 6.88 (d, 1H, *J* = 8.1 Hz), 6.85 (d, 1H, *J* = 7.8 Hz), 4.68 (br t, 2H, *J* = 5.6 Hz), 4.55 (br s, 4H), 3.74 (br s, 4H), 3.21 (br t, 2H, *J* = 5.7 Hz). HPLC (Method C), *t*R = 6.63 min (purity = 91.0%). HR ESMS $[M+H]^+$ = 395.1650, calcd for $C_{20}H_{23}N_6OS$: 395.1656.

2.1.62. 4-(2-(4-(2-Methyl-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (15)

Prepared from **89** and acetic acid as above for compound **14** on 50 mg scale. Purification by HPLC (Method F). Yield = 58%. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.66 (br s, 1H), 12.61 (br s, 1H), 9.65 (br s, 1H), 7.34 (dd, 1H, *J* = 8.0 Hz, *J* = 7.9 Hz), 7.22 (d, 1H, *J* = 8.1 Hz), 7.10 (dd, 1H, *J* = 8.1, Hz, *J* = 8.0 Hz), 6.88 (d, 1H, *J* = 8.1 Hz), 6.81 (d, 1H, *J* = 8.0 Hz), 6.80 (d, 1H, *J* = 7.9 Hz), 4.50 (br t, 2H), 3.84 (br t, 2H), 3.68 (br s, 4H), 3.40 (obscured m, 4H), 2.81 (s, 3H). HPLC (Method C), *t*R = 9.8 min (purity = 91.0%). HR ESMS $[M+H]^+$ = 408.1728, calcd for $C_{21}H_{24}N_6OS$: 408.1732.

2.1.63. 4-(2-(4-(2-(Perfluoroethyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (16)

Prepared as above for compound **14** from **89** (15 mg, 39 μmol) and perfluoropropionic acid (0.30 mL). Purified by reversed phase HPLC (Method F) to leave the product **16** bistrifluoroacetate salt as a foamy solid (10 mg, 35%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 14.10 (br s, 1H), 12.68 (br s, 1H), 12.65 (br s, 1H), 9.65 (br s, 1H), 7.33 (dd, 1H, *J* = 8.0 Hz, *J* = 7.9 Hz), 7.21 (d, 1H, *J* = 8.1 Hz), 7.10 (dd, 1H, *J* = 8.1, Hz, *J* = 8.0 Hz), 6.85 (d, 1H, *J* = 8.1 Hz), 6.83 (d, 1H, *J* = 8.0 Hz), 6.80 (d, 1H, *J* = 7.9 Hz), 4.52 (broad t, 2H), 3.85 (br t, 2H), 3.70 (br s, 4H), 3.90 (obscured m, 4H). HPLC (Method 3), *t*R = 10.2 min (purity = 82.6%). ESMS $[M+H]^+$ = 513. HR ESMS $[M+H]^+$ = 513.1506, calcd for $C_{22}H_{22}F_5N_6OS$: 513.1498.

2.1.64. 4-(2-(4-(2-Phenyl-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (17)

A solution of 4-(2-(4-(2,3-diaminophenyl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (**89**, 40 mg, 0.10 mmol) and benzaldehyde (22 mg, 0.20 mmol) in isopropyl alcohol was kept open to the atmosphere, stirred rapidly, and heated to 70 °C

for 3 h. The mixture was cooled to room temperature, diluted with DMSO (0.5 mL) and water (0.5 mL), and purified by reversed phase HPLC (Method 2). The product **17** was obtained as a bistrifluoroacetate salt (35 mg, 50%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.67 (br s, 1H), 12.64 (br s, 1H), 10.08 (br s, 1H), 8.21 (d, 1H, J = 8.1 Hz), 8.19 (d, 1H, J = 8.1 Hz), 7.59 (m, 3H), 7.25 (m, 2H), 7.12 (dd, 1H, J = 8.1 Hz, J = 8.1 Hz), 6.87 (d, 1H, J = 8.1 Hz), 6.85 (d, 1H, J = 8.1 Hz), 6.79 (d, 1H, J = 8.1 Hz), 4.56 (t, 3H, J = 4.7 Hz), 4.40 (m, 2H), 3.87 (m, 2H), 3.74 (t, 2H, J = 4.7 Hz), 3.57 (m, 2H), 3.28 (m, 2H). ^{13}C NMR (300 MHz, DMSO-d_6) δ = 167.71, 158.40 (q), 148.87, 141.90, 141.89, 141.88, 140.24, 135.96, 133.51, 130.32, 128.93, 128.62, 126.82, 124.00, 123.19, 108.41, 105.79, 103.42, 62.90, 54.91, 51.70, 46.47. HPLC (Method C), tR = 7.28 min (purity = 92.2%). HR ESMS [M+H]$^+$ = 470.1974, calcd for $C_{26}H_{27}N_6OS$: 471.1969.

2.1.65. 4-(2-(4-(2-*m*-Tolyl-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (18)

To an 8-mL screw cap vial were added *N*-methyl pyrrolidinone (4 mL/vial) and 279 μL of a 0.2 M solution of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uronium hexafluorophosphate (HATU) in NMP. To this was added 17.8 mg (0.046 mmol) of 4-{2-[4-(2,3-diaminophenyl)-piperazin-1-yl]-ethoxy}-1,3-dihydro-benzimidazole-2-thione (**89**) followed by *m*-toluic acid (7.5 mg, 0.056 mmol) and the vial was capped tightly. After shaking overnight on an orbital shaker the vial was treated with 0.5 mL of glacial acetic acid, recapped and shaken at 110 °C for 2 h. It was then allowed to cool to room temperature and was shaken overnight. Sulfonic acid resin (Argonaut, 132 mg, 1.4 mmol/g) was then added and the vial was shaken for 6 h followed by filtration using polypropylene filter tubes (15 mL). The resin was washed with methanol (3× 3 mL) followed by dichloromethane (2× 3 mL). The filter tube containing the washed resin was treated with 1.5 mL of 9:1 methanol/triethylamine. After loosely shaking for 3 min the reaction was filtered into a test tube (13 × 100 mm) and the solvent removed by vacuum. The crude product was then purified using automated RP-HPLC (Method E) and the product fractions were evaporated in a 8 mL scintillation vial to yield 3 mg (13% yield) of 4-(2-(4-(2-*m*-tolyl-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (**18**). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.68 (s, 1H), 12.66 (s, 1H), 9.72 (br s, 1H), 8.13 (s, 1H), 7.95 (d, 1H, J = 7.8 Hz), 7.70 (d, 1H, J = 7.8 Hz), 7.35 (t, 1H, J = 7.8 Hz), 7.23 (m, 2H), 7.15 (t, 1H, J = 8.3 Hz), 6.89 (m, 2H), 6.73 (m, 1H), 4.59 (br s, 4H), 3.89 (m, 2H), 3.78 (m, 2H), 3.60 (m, 2H), 3.31 (t, 2H, J = 15 Hz), 2.35 (s, 3H). HRMS [M+H]$^+$ = 485.2112, calcd for $C_{27}H_{29}N_6OS$: 485.2116. HPLC (Method D): tR = 9.8 min (purity = 96.4%).

By the same method and scale were prepared:

2.1.66. 4-(2-(4-(2-(4-Ethylphenyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (20b)

Prepared as above for compound **18** from **89** and 4-ethylbenzoic acid (15% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ = 12.68 (s, 1H), 12.66 (s, 1H), 9.71 (br s, 1H), 8.11 (d, 2H, J = 8.3 Hz), 7.44 (d, 2H, J = 8.3 Hz), 7.23 (m, 2H), 7.15 (t, 1H, J = 8.3 Hz), 6.89 (m, 2H), 6.73 (m, 1H), 4.59 (br s, 4H), 3.89 (m, 2H), 3.78 (m, 2H), 3.60 (m, 2H), 3.31 (t, 2H, J = 15 Hz), 2.72 (m, 2H), 1.26 (t, 3H, J = 9 Hz). HRMS [M+H]$^+$ = 499.2272, calcd for $C_{28}H_{31}N_6OS$: 499.2275. A-HPLC (Method D), tR = 8.6 min (purity = 90.2%).

2.1.67. 4-(2-(4-(2-(2,4-Dimethylphenyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)ethoxy)-1H-benzo[*d*]imidazole-2(3H)-thione (23)

Prepared as above for compound **18** from **89** and 2,4-dimethylbenzoic acid (10% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.68 (br s, 1H), 12.66 (br s, 1H), 9.71 (br s, 1H), 7.95 (d, 1H, J = 8.0 Hz), 7.35 (d, 1H, J = 8.0 Hz), 7.30 (s, 1H), 7.23 (m, 2H), 7.15 (t, 1H, J = 8.3 Hz), 6.89 (m, 2H), 6.73 (m, 1H), 4.59 (br s, 4H), 3.89 (m, 2H), 3.78 (m, 2H), 3.60 (m, 2H), 3.31 (t, 2H, J = 15 Hz), 2.38 (s, 3H), 2.29 (s, 3H). HRMS [M+H]$^+$ = 499.2270, calcd for $C_{28}H_{31}N_6OS$: 499.2273. HPLC (Method D), tR = 8.6 min (purity = 99.1%).

2.1.68. 2-(4-*tert*-Butylphenyl)-4-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)-1H-benzo[*d*]imidazole (22)

To an 8-mL screw cap vial were added *N*-methyl pyrrolidinone (3 mL/vial) and 1 mL of a 0.14 M solution of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyl-uroniumhexafluoro phosphate (HATU) in NMP. To this was added 50.0 mg (0.119 mmol) of 3-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)benzene-1,2-di-amine (**88**) followed by 4-*tert*-butylbenzoic acid (25 mg, 0.143 mmol), and the vial was capped tightly. After shaking overnight on an orbital shaker the vial was treated with 0.5 mL of glacial acetic acid, recapped and shaken at 110 °C for 2 h. It was then allowed to cool to room temperature and shaken overnight. Sulfonic acid resin (Argonaut, 340 mg, 1.4 mmol/g) was then added and the vial was shaken for 6 h followed by filtration using a polypropylene filter tube (15 mL). The resin was washed with methanol (2× 3 mL) followed by dichloromethane (2× 3 mL). The resin was shaken with 1.0 mL of 9:1 methanol/triethylamine for 3 min, and the mixture was filtered into a test tube (13 × 100 mm) and the solvent removed by vacuum. The crude product was then purified using automated RP-HPLC (Method F) and the fractions evaporated in a 8-mL scintillation vial to yield 23 mg (34% yield) of 2-(4-*tert*-butyl-phenyl)-4-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl) piperazin-1-yl)-1H-benzo[*d*]-imidazole (**22**). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.2 (br s, 1H), 8.09 (d, 2H, J = 8.4 Hz), 7.60 (d, 2H, J = 8.4 Hz), 7.35 (m, 2H), 7.20 (m, 2H), 7.02 (d, 1H, J = 8.1 Hz), 6.72 (d, 1H, J = 8.1 Hz), 4.71 (br s, 2H), 4.42 (br s, 2H), 3.86 (br s, 2H), 3.79 (br s, 2H), 3.57 (br s, 2H), 3.23 (br s, 2H), 1.35 (s, 9H). ^{13}C NMR (300 MHz, DMSO-d_6) δ = 158.39, 153.02, 148.87, 140.17, 139.20, 135.97, 126.49, 126.08, 125.66, 125.25, 123.67, 122.02, 120.71, 118.12, 117.12, 114.21, 110.3, 106.39, 105.76, 63.45, 54.81, 51.71, 46.37, 34.56, 30.85. HRMS [M+H]$^+$ = 563.2739, calcd for $C_{31}H_{34}F_3N_6O$: 563.2741. HPLC (Method D), tR = 10.5 min (purity = 100%).

2.1.69. 2-(4-Isopropylphenyl)-4-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)-1H-benzo[*d*]imidazole (21)

Prepared as above for compound **22 88** and 4-*i*-propylbenzoic acid (38% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.2 (br s, 1H), 8.11 (d, 2H, J = 8.3 Hz), 7.48 (d, 2H, J = 8.3 Hz), 7.39 (m, 2H), 7.22 (m, 2H), 7.04 (d,1H, J = 7.9 Hz), 6.74 (d, 1H, J = 7.9 Hz), 4.74 (br s, 2H), 4.48 (br s,2H), 3.91 (br s, 2H), 3.82 (br s, 2H), 3.60 (br s, 2H), 3.25 (br s, 2H), 3.04 (m, 1H), 1.30 (d, 6H, J = 10.6 Hz). HRMS [M+H]$^+$ = 549.2593, calcd for $C_{30}H_{32}F_3N_6O$: 549.2582. HPLC (Method D), tR = 8.1 min (purity = 100%).

2.1.70. 2-(4-Ethylphenyl)-4-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-4-yloxy)ethyl)piperazin-1-yl)-1H-benzo[*d*]imidazole (20a)

Prepared as above for compound **18** from **88** and 4-ethylbenzoic acid (10% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.2 (br s, 1H), 8.11 (d, 2H, J = 8.3 Hz), 7.45 (d, 2H, J = 8.3 Hz), 7.39 (m, 2H), 7.23 (m, 2H), 7.04 (d, 1H, J = 7.8 Hz), 6.75 (d, 1H, J = 7.8 Hz), 4.75 (m, 2H), 4.48 (br s, 2H), 3.91 (br s, 2H), 3.82 (m, 2H), 3.60 (br s, 2H), 3.25 (br s, 2H), 2.73 (q, 2H, J = 9.0 Hz), 1.28 (t, 3H, J = 9.0 Hz). HRMS [M+H]$^+$ = 535.2430, calcd for $C_{29}H_{30}F_3N_6O$: 535.2426. HPLC (Method D), tR = 8.1 min (purity = 100%).

2.1.71. 2-*p*-Tolyl-4-(4-(2-(2-(trifluoromethyl)-1H-benzo[*d*]imidazol-7-yloxy)-ethyl)piperazin-1-yl)-1H-benzo[*d*]Imidazole (19)

Prepared as above for compound **18** from **88** and *p*-toluic acid (36% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 10.2 (br s, 1H), 8.04 (d, 2H, J = 8.1 Hz), 7.42 (d, 2H, J = 8.1 Hz), 7.39 (m, 2H), 7.23 (m, 2H), 7.04 (d, 1H, J = 7.8 Hz), 6.75 (d, 1H, J = 7.8 Hz), 4.75 (m, 2H), 4.48 (br s, 2H), 3.91 (br s, 2H), 3.82 (m, 2H), 3.60 (br s, 2H), 3.25 (br s, 2H), 2.34 (s, 3H). HRMS $[M+H]^+$ = 521.2268, calcd for $C_{28}H_{28}F_3N_6O$: 521.2271. HPLC (Method D), tR = 9.4 min (purity = 91%).

2.1.72. *tert*-Butyl 4-(2-(4-*tert*-butylphenyl)-1H-benzo[*d*]imidazol-4-yl)piperazine-1-carboxylate (90)

A mixture of *tert*-butyl 4-(2,3-diaminophenyl)piperazine-1-carboxylate (**36**, 1.8 g, 5.9 mmol), 4-*tert*-butylbenzaldehyde (1.1 g, 7.1 mmol, 1.2 mL), and 10% palladium on carbon (0.60 g) in isopropanol (40 mL) was stirred rapidly and heated to 80 °C for 2 h open to air. After cooling to room temperature the catalyst was filtered with the aid of diatomaceous earth, washed with isopropanol, and the combined filtrates were evaporated under reduced pressure. The crude product was chromatographed on silica gel eluted with 75% hexanes in ethyl acetate to leave the product as a foamy solid (2.5 g, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.72 (br s, 1H), 8.06 (d, 2H, J = 8.3 Hz), 7.56 (d, 2H, J = 8.3 Hz), 7.05 (m, 2H), 6.53 (d, 1H, J = 5.1 Hz), 3.56 (brs, 4H), 3.51 (brs, 4H), 1.44 (s, 9H), 1.33 (s, 9H). LC/MS (Method I), tR = 2.83 min (purity = 100%), $[M+H]^+$ = 435.

2.1.73. 2-(4-*tert*-Butylphenyl)-4-(piperazin-1-yl)-1H-benzo[*d*]imidazole (91)

A solution of *tert*-butyl 4-(2-(4-*tert*-butylphenyl)-1H-benzo[*d*]imidazol-4-yl)piperazine-1-carboxylate (**90**, 2.4 g, 5.3 mmol) in trifluoroacetic acid (20 mL) and dichloromethane (20 mL) was stirred for 2 h. The solvents were evaporated, and the crude product was purified by reversed phase HPLC (Method E). The product containing fractions were combined, neutralized with 1 M sodium carbonate solution, and extracted with ethyl acetate (2× 50 mL). The extracts were combined, dried ($MgSO_4$), and evaporated under reduced pressure to provide the product as a white, foamy solid (1.0 g, 54%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.68 (br s, 1H), 8.05 (d, 2H, J = 8.1 Hz), 7.56 (d, 2H, J = 8.1 Hz), 7.05 (m, 2H), 6.49 (d, 1H, J = 7.1 Hz), 3.48 (brs, 4H), 2.97 (brs, 4H), 1.33 (s, 9H). LC/MS (Method I), tR = 1.40 min (purity = 95.0%), $[M+H]^+$ = 335.

2.1.74. 2-{4-[2-(4-*tert*-Butyl-phenyl)-1*H*-benzoimidazol-4-yl]piperazin-1-ylmethyl}-6-nitro-phenylamine (95)

To a solution of 2-(4-*tert*-butyl-phenyl)-4-piperazin-1-yl-1H-benzoimidazole (**91**, 1.30 g, 3.89 mmol) and 2-chloromethyl-6-nitro-phenylamine (**50**, 660 mg, 3.53 mmol) in anhydrous NMP (40 mL) was added diisopropylethylamine (1.0 mL, 4.24 mmol) and the solution stirred at room temperature under nitrogen for 18 h. The solution was diluted with ethyl acetate (150 mL) and washed with water (10× 50 mL). The organic extracts were combined and washed with brine (2× 100 mL), dried (Na_2SO_4), filtered, and the solvent removed to give a tan solid with some amber colored oil (1.84 g). This material was adsorbed onto silica gel and purified by column chromatography eluted with a solution of 20% ethyl acetate in hexane to afford the nitro amino product (610 mg, 36% yield) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.25 (d, 1H, J = 7.5 Hz), 8.00 (d, 2H, J = 8.2 Hz), 7.50 (d, 1H, J = 6.9 Hz), 7.35 (d, 2H, J = 8.4 Hz), 7.00 (m, 1H), 6.95 (m, 1H), 6.75 (m, 1H), 6.65 (d, 1H, J = 6.7 Hz), 4.15 (s, 2H), 3.33 (br s, 4H), 3.15 (br s, 4H), 1.25 (s, 9H). HPLC (Method D) tR = 12.5 min (purity = 100%). MS, $[M+H]^-$ = 483, $[M+H]^+$ = 485.

2.1.75. 2-({4-[2-(4-*tert*-Butylphenyl)-1*H*-benzimidazol-4-yl]piperazin-1-yl}methyl)-6-nitroaniline (96)

2-{4-[2-(4-*tert*-Butylphenyl)-1*H*-benzoimidazol-4-yl]piperazin-1-ylmethyl}-6-nitrophenylamine (290 mg, 0.60 mmol) and tin(II) chloride (681 mg, 3.59 mmol) were stirred in anhydrous NMP (40 mL) at 70 °C under nitrogen for 18 h. The reaction was quenched with 1 N HCl (100 mL) and extracted with methylene chloride (2× 50 mL). The organic extracts were combined and extracted with 1 N HCl (2× 50 mL). The aqueous extracts were combined and neutralized with 2 N NaOH solution. This aqueous phase was extracted with ethyl acetate (4× 100 mL). The organic extracts were combined and washed with distilled water (10× 50 mL). The ethyl acetate extracts were combined, dried (Na_2SO_4), and the solvent removed to give a brown oil. This material was dissolved in aqueous DMSO and purified by RP-HPLC. The product fractions were collected and the acetonitrile removed in vacuo. The aqueous residue lyophilized to give a white solid (55 mg, 20% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.08 (d, 2H, J = 8.2 Hz), 7.57 (d, 2H, J = 8.5 Hz), 7.15 (m, 2H), 6.90 (m, 2H), 6.65 (m, 2H), 4.35 (s, 2H), 3.50 (br s, 8H), 1.35 (s, 9H). HPLC (Method D), tR = 4.9 min (purity = 99.0%), $[M+H]^-$ = 453, $[M+H]^+$ = 455.

2.1.76. 4-{4-[2-(4-*tert*-Butyl-phenyl)-1*H*-benzoimidazol-4-yl]-piperazin-1-ylmethyl}-1,3-dihydro-benzoimidazole-2-thione (24)

2-Amino-3-({4-[2-(4-*tert*-butylphenyl)-1*H*-benzimidazol-4-yl]-piperazin-1-yl}methyl)phenylamine (**96**, 140 mg, 0.31 mmol) and 1,1′-thiocarbonyldiimidazole (67 mg, 0.34 mmol) were stirred in anhydrous THF (10 mL) at 60 °C for 2 h. The solvent was removed, in vacuo, to give a reddish brown oil (220 mg). This material was adsorbed onto silica and purified by column chromatography, eluting with a 1:1 solution of hexane in ethyl acetate to give an off-white solid (30 mg, 20% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.05 (d, 2H, J = 8.4 Hz), 7.55 (d, 2H, J = 8.5 Hz), 7.05 (m, 5H), 6.48 (m, 1H), 3.75 (s, 2H), 3.55 (br s, 4H), 2.65 (br s, 4H), 1.30 (s, 9H). HPLC (Method D) tR = 9.0 min (purity = 90.7%). HRMS $[M+H]^+$ = 497.2484, calcd for $C_{29}H_{33}N_6S$: 497.2482.

2.1.77. 4-{4-[2-(4-*tert*-Butyl-phenyl)-1*H*-benzoimidazol-4-yl]-piperazin-1-ylmethyl}-1,3-dihydro-benzoimidazol-2-one (27)

2-Amino-3-({4-[2-(4-*tert*-butylphenyl)-1*H*-benzimidazol-4-yl]-piperazin-1-yl}methyl)phenylamine (**96**, 150 mg, 0.33 mmol) and 1,1′-carbonyldiimidazole (59 mg, 0.36 mmol) were stirred in anhydrous THF at 60 °C for 2 h. The solvent was removed in vacuo, dissolved in aqueous DMSO and purified by RP-HPLC (Method F). The product fractions were combined and lyophilized to give a white solid (45 mg, 28% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 11.10 (s, 1H), 10.90 (s, 1H), 8.08 (d, 2H, J = 8.5 Hz), 7.60 (d, 2H, J = 8.5 Hz), 7.10 (m, 3H), 7.05 (m, 2H), 6.65 (m, 1H), 4.45 (s, 2H), 3.55 (br s, 8H), 1.33 (s, 9H). HPLC tR = 8.9 min (purity = 88.0%). HRMS $[M+H]^+$ = 481.2708 calcd for $C_{29}H_{33}N_6O$: 481.2710.

2.1.78. 5-({4-[2-(4-*tert*-Butylphenyl)-1*H*-benzimidazol-4-yl]piperazin-1-yl}methyl)-1,4-dihydroquinoxaline-2,3-dione (28)

2-Amino-3-({4-[2-(4-*tert*-butylphenyl)-1*H*-benzimidazol-4-yl]-piperazin-1-yl}methyl)phenylamine (**96**, 100 mg, 0.22 mmol) and oxalyldiimidazole (63 mg, 0.33 mmol) were stirred at room temperature in anhydrous tetrahydrofuran (10 mL) for 2 h. The reaction was quenched with water (5 mL) and stirred for 1 h. The solvent was removed in vacuo and the aqueous residue dissolved in dimethylsulfoxide and purified by RP-HPLC (Method F). The product fractions were combined and lyophilized to afford the product as a white powder (7 mg, 6%). ^{1}H NMR (300 MHz, DMSO-d_6) δ = 8.07 (d, 2H, J = 8.4 Hz), 7.58 (d, 2H, 8.4 Hz), 7.27 (m, 3H), 7.14 (m, 2H), 6.75 (m, 1H), 4.64 (m, 4H), 3.16 (s, 2H),

1.34 (s, 9H). HPLC (Method D) tR = 9.1 min (purity = 92.3%). MS $[M-H]^-$ = 507, $[M+H]^+$ = 509.

2.1.79. 4-(4-Benzylpiperazin-1-yl)-2-(4-*tert*-butylphenyl)-1*H*-benzimidazole (25)

2-(4-*tert*-Butylphenyl)-4-piperazin-1-yl-1*H*-benzimidazole (**91**, 40 mg, 0.12 mmol), benzaldehyde (15.8 μL, 0.16 mmol), and sodium triacetoxyborohydride (51 mg, 0.24 mmol) were stirred in dichloroethane (2 mL) at room temperature for 18 h. The product was concentrated, in vacuo, and dissolved in dimethylsulfoxide/water and purified by RP-HPLC (method). The product fractions were combined and the solvent removed to afford the product as a clear oil as the di-TFA salt (51 mg, 100% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.36 (s, 9H), 3.02–3.21 (m, 2H), 3.40 (d, J = 2.44 Hz, 2H), 3.47–3.59 (m, 2H), 4.49 (s, 4H), 6.69 (d, J = 7.32 Hz, 1H), 7.20 (s, 1H), 7.18 (d, J = 7.32 Hz, 1H), 7.58–7.66 (m, 5H), 8.10 (d, J = 7.50 Hz, 2H), 9.97 (s, 1H). HPLC (Method D), tR = 9.7 min (purity = 100%). HRMS, $[M+H]^+$ = 425.2702, $[M+H]^+$ calcd for $C_{28}H_{33}N_4$: 425.2700.

2.1.80. 2-(4-*tert*-Butylphenyl)-4-[4-(2-naphthylmethyl)piperazin-1-yl]-1*H*-benzimidazole (26)

2-(4-*tert*-Butylphenyl)-4-piperazin-1-yl-1*H*-benzimidazole (**91**, 40 mg, 0.12 mmol), 2-napthaldehyde (24 mg, 0.16 mmol), and sodium triacetoxyborohydride (51 mg, 0.24 mmol) were stirred in dichloroethane (2 mL) at room temperature for 18 h. The product was concentrated, in vacuo, and dissolved in dimethylsulfoxide/water and purified by RP-HPLC (Method F). The product fractions were combined, and the solvent was removed to afford the product as a clear oil as the di-TFA salt (48 mg, 84% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 1.36 (s, 9H), 3.01–3.25 (m, 4H), 3.61 (br s, 4H), 4.43 (br s, 1H), 4.99 (br s, 2H), 6.68 (d, J = 7.32 Hz, 1H), 7.19 (d, J = 4.39 Hz, 2H), 7.49 - 7.77 (m, 6H), 7.88 (d, J = 6.84 Hz, 1H), 8.09 (d, J = 8.30 Hz, 2H), 8.49 (d, J = 8.79 Hz, 2H), 9.73–9.97 (m, 1H). HPLC (Method D), tR = 11.0 min (purity = 92.0%). HRMS, $[M+H]^+$ = 475.2860, calcd for $C_{32}H_{35}N_4$ $[M+H]^+$: 475.2856.

2.1.81. 2-(4-*tert*-Butylphenyl)-4-(4-(3-fluoro-4-nitrobenzyl)piperazin-1-yl)-1H-benzoimidazole (92)

In a round bottom flask were combined the 3-fluoro-4-nitrobenzaldehyde (0.52 g, 3.07 mmol) and NMP (20 mL). To the stirring solution were added the 2-(4-*tert*-butyl-phenyl)-4-(piperazin-1-yl)-1H-benzo[*d*]imidazole (**91**, 0.86 g, 2.56 mmol) and sodium triacetoxyborohydride (1.36 g, 6.4 mmol), and the resulting mixture stirred overnight. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with saturated sodium bicarbonate solution, water, and brine. The organic layer was dried ($MgSO_4$) and concentrated. The crude product was purified by column chromatography with 40% ethyl acetate in hexane as eluant to leave 850 mg (68% yield) of 2-(4-*tert*-butylphenyl)-4-(4-(3-fluoro-4-nitrobenzyl)piperazin-1-yl)-1H-benzimidazole as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.69 (s, 1H), 8.16 (t, 1H, J = 8.2 Hz), 8.04 (d, 2H, J = 8.5 Hz), 7.59 (m, 1H), 7.55 (d, 2H, J = 8.5 Hz), 7.47 (d, 1H, J = 8.6 Hz), 7.03 (m, 2H), 6.50 (dd, 1H, J = 7.0 Hz, 1.8 Hz), 3.71 (s, 2H), 3.60 (br s, 4H), 2.66 (br s, 4H), 1.33 (s, 9H). LC/MS (Method A), tR = 1.24 min (purity = 100%), $[M+H]^+$ = 488, $[M-H]^-$ = 486.

2.1.82. 4-[4-(3-Azido-4-nitro-benzyl)-piperazin-1-yl]-2-(4-*tert*-butyl-phenyl)-1H-benzo-imidazole (93)

In a round bottom flask were combined 2-(4-*tert*-butylphenyl)-4-[4-(3-fluoro-4-nitrobenzyl)piperazin-1-yl]-1H-benzoimidazole (0.197 g, 0.40 mmol), dimethyl sulfoxide (3 mL), and sodium azide (0.029 g, 0.44 mmol), and the mixture stirred overnight. The mixture was diluted with ethyl acetate (100 mL), washed with water (2× 50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$) and concentrated to leave 190 mg (92% yield) of 4-[4-(3-azido-4-nitrobenzyl)-piperazin-1-yl]-2-(4-*tert*-butylphenyl)-1H-benzoimidazole as a light yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.69 (s, 1H), 8.04 (d, 2H, J = 8.7 Hz), 8.01 (d, 1H, J = 8.3 Hz), 7.57 (m, 1H), 7.54 (d, 2H, J = 8.6 Hz), 7.39 (dd, 1H, J = 8.5, 1.2 Hz), 7.03 (m, 2H), 6.50 (dd, 1H, J = 5.5, 1.4 Hz), 3.70 (s, 2H), 3.60 (br s, 4H), 2.68 (br s, 4H), 1.33 (s, 9H). LC/MS (Method A), tR = 1.41 min (purity = 89.8%), $[M+H]^+$ = 511, $[M-H]^-$ = 509.

2.1.83. 4-{4-[2-(4-*tert*-Butyl-phenyl)-1H-benzoimidazol-4-yl]-piperazin-1-ylmethyl}-benzene-1,2-diamine (94)

In a round bottom flask under nitrogen were combined 4-[4-(3-azido-4-nitro-benzyl)-piperazin-1-yl]-2-(4-*tert*-butyl-phenyl)-1H-benzoimidazole (**93**, 0.19 g, 0.37 mmol), methanol (20 mL), and 5% platinum on carbon (0.145 g). A hydrogen balloon was attached, the flask evacuated, and a hydrogen atmosphere established. After stirring for 4 h, the balloon was removed, the flask purged with nitrogen, and the reaction mixture filtered thru Celite with a large excess of methanol. The solution was concentrated to dryness on a rotary evaporator to yield 170 mg (100% yield) of 4-{4-[2-(4-*tert*-butyl-phenyl)-1H-benzoimidazol-4-yl]-piperazin-1-ylmethyl}-benzene-1,2-diamine as a brown oil. LC/MS (Method A), tR = 0.90 min (purity = 100%), $[M+H]^+$ = 455, $[M-H]^-$ = 453.

2.1.84. 6-{4-[2-(4-*tert*-Butyl-phenyl)-1H-benzoimidazol-4-yl]-piperazin-1-ylmethyl}-1,4-dihydro-quinoxaline-2,3-dione (29)

In a round bottom flask under nitrogen were combined 4-{4-[2-(4-*tert*-butyl-phenyl)-1H-benzoimidazol-4-yl]-piperazin-1-yl-methyl}-benzene-1,2-diamine (**94**, 0.17 g, 0.37 mmol), THF (20 mL), and 1,1′-oxalyldiimidazole (0.284 g, 1.5 mmol) and the solution stirred overnight. The solution was diluted with ethyl acetate (100 mL) and washed with water (100 mL). A precipitate forms and was collected by filtration. The filtrate was concentrated to dryness on a rotary evaporator and combined with the precipitate. Purification by RP-HPLC (Method E) yielded 46 mg (24% yield) of 6-{4-[2-(4-*tert*-butyl-phenyl)-1H-benzoimidazol-4-yl]-piperazin-1-ylmethyl}-1,4-dihydro-quinoxaline-2,3-dione as an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ = 12.19 (s, 1H), 12.09 (s, 1H), 9.82 (br s, 1H), 8.07 (d, 2H, J = 8.6 Hz), 7.58 (d, 2H, J = 8.6 Hz), 7.27 (m, 2H), 7.22 (d, 1H, J = 8.6 Hz), 7.13 (m, 2H), 6.64 (m, 1H), 4.48 (br s, 2H), 4.44 (s, 2H), 3.50 (br s, 2H), 3.38 (br s, 2H), 3.09 (m, 2H), 1.34 (s, 9H). HPLC (Method D), tR = 5.84 min (purity = 79%). HRMS $[M+H]^+$ = 509.2659, calcd for $C_{30}H_{33}N_6O_2$: 509.2659.

2.1.85. 5-((4-(2-(4-*tert*-Butylphenyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)methyl)pyrimidine-2,4(1H,3H)-dione (30)

2-(4-*tert*-Butylphenyl)-4-piperazin-1-yl-1*H*-benzimidazole (**91**, 75 mg, 0.22 mmol), uracil-5-carboxaldehyde (41 mg, 0.29 mmol), and sodium triacetoxyborohydride (95 mg, 0.45 mmol) were stirred in NMP (2 mL) at room temperature for 18 h. The mixture was treated with ethyl acetate (50 mL), washed with water (7× 30 mL) and brine (40 mL), dried ($MgSO_4$), and evaporated. The crude product was purified by RP-HPLC (Method F). The product fractions were combined, treated with saturated sodium bicarbonate solution (50 mL), and extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with brine (50 mL), dried ($MgSO_4$), and evaporated to leave the product as a white solid (15 mg, 15%). ^{1}H NMR (400 MHz, MeOH-d_4) δ = 7.98 (d, 2H, J = 8.5 Hz), 7.51 (d, 2H, J = 8.5 Hz), 7.43 (s, 1H), 7.10 (m, 2H), 6.67 (m, 1H), 3.40 (m, 4H), 3.35 (s, 2H), 2.77 (m,. 4H), 1.33 (s, 9H). ^{13}C NMR (300 MHz, DMSO-d_6) δ = 164.33, 152.03, 151.23, 148.08, 142.79, 140.32, 136.08, 135.26, 127.56, 125.96, 125.57, 123.09, 107.88, 106.19, 103.29, 52.70, 52.36, 49.08, 34.48, 30.95. HPLC (Method D), tR = 8.5 min (purity = 99.9%). HRMS, $[M+Na]^+$ = 481.2327, calcd for $C_{26}H_{30}N_6O_2$: 481.2323.

2.1.86. 5-((4-(2-(4-*tert*-Butylphenyl)-1H-benzo[*d*]imidazol-4-yl)piperazin-1-yl)methyl)-1-ethylpyrimidine-2,4(1H,3H)-dione (31)

2-(4-*tert*-Butylphenyl)-4-piperazin-1-yl-1*H*-benzimidazole (**91**, 75 mg, 0.22 mmol), 1-ethyluracil-5-carboxaldehyde (49 mg, 0.29 mmol) and sodium triacetoxyborohydride (95 mg, 0.45 mmol) were stirred in NMP (2 mL) at room temperature for 18 h. The mixture was treated with ethyl acetate (50 mL), washed with water (7× 30 mL) and brine (40 mL), dried ($MgSO_4$), and evaporated. The crude product was purified by RP-HPLC (Method F). The product fractions were combined, treated with saturated sodium bicarbonate solution (50 mL), and extracted with ethyl acetate (3× 30 mL). The combined extracts were washed with brine (50 mL), dried ($MgSO_4$), and evaporated to leave the product as a white solid (50 mg, 46%). ^{1}H NMR (400 MHz, MeOH-d_4) δ = 7.97 (d, 2H, J = 8.6 Hz), 7.59 (s, 1H), 7.51 (d, 2H, J = 8.6 Hz), 7.10 (m, 2H), 6.65 (m, 1H), 3.77 (q, 2H, J = 7.2 Hz), 3.38 (m, 4H), 3.34 (s, 2H), 2.75 (m,. 4H), 1.31 (s, 9H), 1.25 (t, 3H, J = 7.2 Hz). ^{13}C NMR (300 MHz, DMSO-d_6) δ = 163.81, 152.03, 150.72, 158.08, 143.80, 142.79, 139.09, 135.27, 127.57, 125.97, 125.55, 123.08, 108.66, 106.18, 103.30, 52.82, 52.37, 49.03, 42.64, 34.47, 30.94, 14.16. HPLC (Method D), tR = 8.8 min (purity = 90.5%). HRMS, $[M+Na]^+$ = 481.2638, calcd for $C_{28}H_{34}N_6O_2$: 481.2323.

2.2. Radio-ligand binding assay

Radio-ligand binding assays were done according to the procedure described by Millar et al. (*Methods Neurosci.* **1995**, *25*, 145–162). Briefly, the recombinant cells expressing human or rat GnRH receptors were harvested in binding buffer, (25 mM Tris–Cl, pH 7.4, 0.1% sodium azide, 0.1% BSA), homogenized with a polytron and centrifuged at 12,000g for 15 min. The membrane pellets were washed, resuspended in binding buffer and the protein concentration was determined (Pierce, Rockford, IL). Typically, 50 μg of the membrane protein was incubated with approximately 50,000 cpm of ^{125}I-(D-trp^6)-LHRH for 2 h at 4 °C in a total volume of 200 μL. Competition studies were performed by the addition of increasing concentrations of unlabeled competitor. The samples were filtered through glass-fiber filters (Skatron Corp. Sterling, VA) presoaked in wash buffer (50 mM Tris, pH 7.4, 10 mM $MgCl_2$, 0.5 mM EDTA) containing 1% BSA. The filters were washed twice with the wash buffer, and the retained radioactivity was counted on a gamma counter (Packard Instruments). Assays were always performed in triplicates. Non-specific binding was assessed in the presence of 1 μM unlabeled (D-trp^6)-LHRH.

2.3. Inositol phosphate (IP) accumulation assay

The quantitation of inositol phosphates was carried out according to the procedure described by Chengalvala et al. (*J. Biochem. Biophys. Methods* **1999**, *38*, 163–170). Briefly, recombinant cells expressing either human or rat GnRH receptors pre-labeled with myo-(1,2)-H^3-Inositol were incubated with GnRH agonist, D-trp^6-LHRH, in the presence or absence GnRH antagonist compound in culture medium containing Lithium chloride for 1 h. At the end of this period, the cells were lysed and labeled inositol phosphates were measured after separating from inositol by rapid filtration method. IP assays were performed in triplicates and repeated in three independent experiments.

2.4. LH release assay

Anterior pituitaries were collected from CO_2 asphyxiated adult rats. Tissue was washed with sterile Hepes-buffered saline (HBSS) containing 5% glucose, and minced using a sterile razor blade into pieces approximately 1 mm in size. HBSS was removed by aspiration and replaced with an HBSS enzyme solution consisting of 3.5 mg/mL collagenase type 1 (Worthington Biochemicals), 1.2 mg/mL hyaluronidase type III (Calbiochem), and 3% BSA (Sigma). Tissue was incubated at 37 for 1 h and then dissociated by gentle trituration through a fire polished Pasteur pipet. Cells were strained through a cell strainer (Costar) to remove clumps and pelleted by centrifugation. Dissociated cells were resuspended in DMEM media (low glucose—Invitrogen) supplemented with 25 mM Hepes, 1× glutamax (Invitrogen), 1× antibiotic/antimycotic (Invitrogen), and 10% fetal bovine serum (growth media) and plated into flat bottom 96-well tissue culture plates at 30,000 cells/well. Cultures were incubated at 37 °C with 5% CO_2 for 72 h.

Treatments prepared in challenge media, which is growth media where the FBS is replaced with 0.1% BSA. Treatment consisted of a dose response of an antagonist of choice in the presence of an ED_{50} concentration of the GnRH analogue Dtrp6-LHRH. Cultures were washed 2× with challenge media to remove serum and then incubated with 100 μL/well of treatment for 3 h. Media was removed and saved for LH RIA analysis. Toxicity of compound was assessed directly on the cells using the cell titer-glo assay (Promega).

2.5. Efficacy studies in rats

Male Sprague–Dawley rats ten days post-castration were instrumented with indwelling catheters in jugular vein to facilitate frequent blood sampling, Basal blood samples were drawn from all the animals and either vehicle (2% Tween 80/0.04 N HCl) or small molecule GnRH antagonist was administered orally. Blood samples were drawn at regular intervals and plasma LH levels measured using a commercial ELISA kit (Endocrine Technologies, CA).

2.6. Statistical analysis

Data are analyzed using Excel/SAS analysis system (SAS Institute Inc., Cary, NC).

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