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Selection, synthesis, and structure–activity relationship of tetrahydropyrido[4,3-*d*]pyrimidine-2,4-diones as human GnRH receptor antagonists

Marion C. Lanier,[a,*] Miklos Feher,[b] Neil J. Ashweek,[a] Colin J. Loweth,[a] Jaimie K. Rueter,[a] Deborah H. Slee,[a] John P. Williams,[a] Yun-Fei Zhu,[a] Susan K. Sullivan[a] and Michael S. Brown[a]

[a]*Departments of Medicinal Chemistry and Pharmacology, Neurocrine Biosciences, 12790 El Camino Real, San Diego, CA 92130, USA*
[b]*The Campbell Family Institute for Breast Cancer Research, University Health Network, Toronto Medical Discovery Tower, 101 College Street, Suite 5-361, Toronto, Ont., Canada M5G 1L7*

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Abstract—The present article describes a selection of a new class of small molecule antagonists for the h-GnRH receptor, their preparation, and evaluation in vitro. Three computational methods were combined into a consensus score, to rank order virtual templates. The top 5% of templates were further evaluated in silico and assessed for novelty and synthetic accessibility. The tetrahydropyrido[4,3-*d*]pyrimidine-2,4-dione core was selected for synthesis and evaluated in vitro. Using an array approach for analog design and synthesis, we were able to drive the binding below 10 nM for the h-GnRH receptor after two rounds of optimization.

1. Introduction

Gonadotropin-releasing hormone (GnRH), also known as luteinizing hormone-releasing hormone (LHRH), is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2), which is produced and secreted by the hypothalamus in a pulsatile manner.[1] Through interaction with specific GnRH receptors in the pituitary, both follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are released from this site. These hormones, in turn, regulate the production of steroids and gametes.[2]

A number of disease states can be controlled via the regulation of this pituitary–gonadal hormone axis, in particular endometriosis, uterine fibroids, and prostate cancer. Interestingly, suppression of both FSH and LH production can be achieved via the agonism or antagonism of the GnRH receptor as continuous activation with an agonist ultimately leads to down regulation of the receptor. Peptide agonists such as Leuprolide®[3] are commercially available. However, treatment with a GnRH agonist initially leads to overproduction of both FSH and LH with a concomitant 'flare effect', which tends to initially exacerbate symptoms in patients. In contrast, GnRH antagonists act immediately at the receptor, quickly suppressing the release of FSH and LH. A number of peptide antagonists such as Cetrotide®[1b] are currently on the market. Due to the very low oral bioavailability of these peptides, administration is normally via injection or depot formulation. In response to the need for a more convenient route of administration, intensive efforts have been initiated toward the development of orally bioavailable small-molecule GnRH antagonists. TAK-013[4] was the most advanced small molecule antagonist as it proceeded to phase II clinical trials (has since been discontinued), followed by NBI-42902[5] and NBI-56418 from Neurocrine Biosciences Inc. NBI-56418 has entered Phase II clinical trials.

Several distinct classes of small molecule GnRH antagonists have been reported in the literature in the past

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* Corresponding author. Tel.: +1 858 617 7685; fax: +1 858 617 7619; e-mail addresses: mlanier@neurocrine.con; mfeher@uhnres.utoronto.ca

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few years.[6] Takeda has described benzodiazepines, spiroamines, thienopyridinones, and thienopyrimidinones. 2-Aryltryptamines and 3-arylquinolones apparently based compounds that were described by Merck. Pfizer/Agouron patents described a series of 2-furancarboxamides. Neurocrine's effort has been focused mainly around 5-aryluracils, and pyrrolo or imidazopyrimidinones. Although there are exceptions, most of these molecules have similar requirements: a basic protonatable nitrogen, one or two aromatic groups, and an aliphatic lipophilic group. Our goal was to find a new class of small molecule antagonists with a distinct SAR and to use in-house computational tools to help select the best template. Using the consensus scoring method[7] which utilized both in-house and published data; we evaluated roughly one hundred different templates via computational analysis as potential GnRH antagonists. These templates were proposed by medicinal chemists based on their knowledge of the GnRH literature and had to fill two requirements: have three points of diversity and be synthetically amenable to combinatorial chemistry. These templates ranged from conservative to more speculative ones. Those with the best scores were prepared at the bench and tested against the human GnRH receptor. In this paper, we describe one of them, the tetrahydropyrido[4,3-*d*]pyrimidine-2,4-dione, its in silico evaluation, synthesis, and biological binding with regard to the human GnRH receptor.

2. Computational methods

Ligand-based consensus scoring of virtual libraries was applied to select and rank order proposed templates. The details of this process, such as the description of the individual methods applied, their combination into a consensus scoring method, and the evaluation of their performance, are given elsewhere.[7] Briefly, during the template selection, the following methods were combined into a consensus score: MACCS,[8] TGT[9], and MP61[10] fingerprints, encapsulating structural, pharmacophore, and gross property information of the molecule, respectively, as implemented in the MOE modeling suite,[9] BCUT descriptors,[11,12] as implemented within the diverse solutions (DVS) software[13], and 3D pharmacophores using the Catalyst[14] and CombiCode[15] software. The individual scores were combined using sum ranks (ranking compounds on each property and adding these ranks) as well as logistic regression with a logistic curve fitted to the data and coefficients being determined from a second training set. These methods were trained on a set containing 100 actives and 1000 inactives (the fingerprint methods only used information from the active sets) and validated using 200 actives and 1500 inactives. The logistic regression coefficients were determined on a separate training set, containing 100 actives and 900 inactives. The training and validation sets were chosen using diverse subset selection from a collection of molecules synthesized for GnRH binding within Neurocrine or described in the literature, and which contained a diverse set of structural motifs.[7]

The consensus scoring method was used to select templates from 101 core structures proposed by medicinal chemists. Figure 1 includes a few examples of proposed templates.

For each template, virtual libraries were generated and the products were scored. In order to maximize the probability of finding actives, the top scoring templates, as opposed to the top scoring individual compounds, were identified. The top 5% of products based on consensus score were examined and five cores with the most examples in this set were chosen for further evaluation. In the second stage of the process, libraries around these five templates using readily available side chains were generated. The scoring process proceeded as before. Based on the computational results, as well as intellectual property and synthetic considerations, the tetrahydro[4,3-*d*]pyrimidine-2,4-dione template VI was selected as the first library for synthesis.

In order to establish similarities and differences in binding in comparison to uracil-containing GnRH antagonists, flexible alignments[16] were performed, as implemented in the MOE suite of programs.[9] In summary, different conformations and alignment poses of the molecules were randomly generated and evaluated using a scoring function that includes Gaussian distance dependence for pharmacophore and volume-like terms, as well as the relative energy of the given conformation. It was shown that such alignments reproduce well the

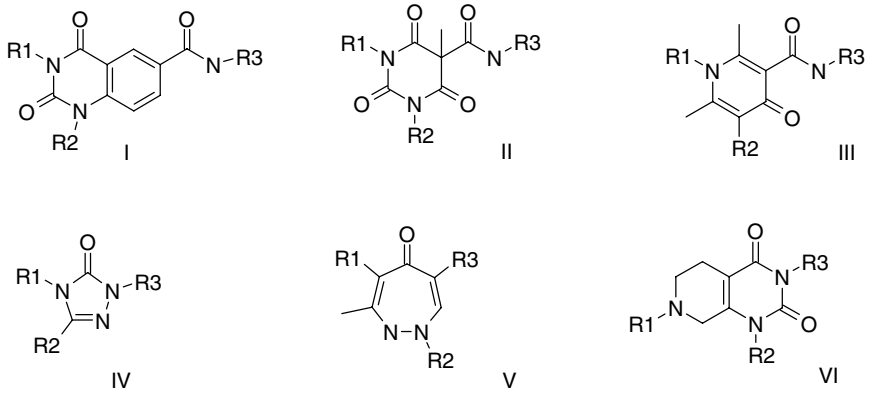


Figure 1. Examples of proposed templates.

relative orientation and conformations of molecules in co-crystallized ligands.[16]

The alignments proceeded in the following manner. First a stochastic conformational search was performed on the template molecule, NBI-42902,[17] using the Merck Force field and continuum solvation, as implemented in MOE. The conformations of this molecule were then clustered and since the lowest energy cluster was well separated from any other (>3 kcal/mol), only the lowest energy conformer was used as a template to which other GnRH compounds were aligned. This lowest energy conformer had very similar geometry to a crystallized close analog of NBI-42902 (unpublished results). Although based on the validation[16] the top scoring alignment solution is expected to reproduce the experimental binding mode best, for 10 randomly selected 5,6,7,8-tetrahydro[4,3-*d*]pyrimidine-2,4-diones the top-scoring 50 solutions were visually clustered employing metric scaling.[18] Using this method it was shown that all top scoring alignments represented similar binding modes regarding the relative position of the major pharmacophoric groups, common among the majority of GnRH antagonists. The alignment results are exemplified by 6-benzyl-1-(2,6-difluoro-benzyl)-3-{2-[methyl-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5,6,7,8-tetrahydro-1H-pyrido[4,3-*d*]pyrimidine-2,4-dione (**34**), shown in Figure 2.

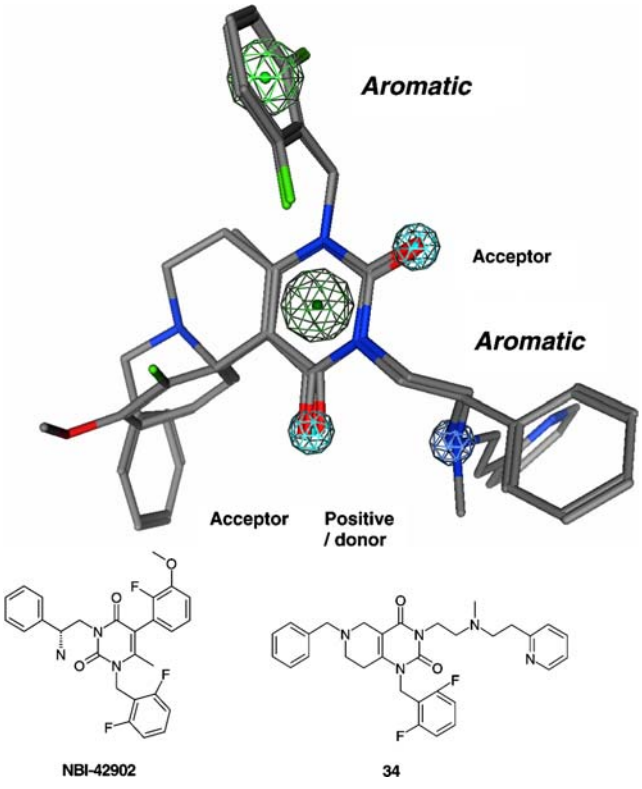


Figure 2. Flexible alignment of a tetrahydro[4,3-*d*]pyrimidine-2,4-dione (compound **34**) with a uracil-containing GnRH antagonist (NBI-42902). The five major pharmacophoric groups, at least four of which are found in the majority of GnRH antagonists, are also displayed.

3. Chemistry

There are two regioisomers for the selected template, the 5,6,7,8-tetrahydropyrido[3,4-*d*]pyrimidine-2,4-dione VI and the 5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-2,4-dione VII (Fig. 3). Both were prepared using similar routes and evaluated.

For regioisomer VI, when R3 was a benzyl or a phenyl, the commercially available 1-benzyl-4-ethoxycarbonyl-3-piperidone hydrochloride **1** was treated with ammonium acetate for an hour at room temperature to give the corresponding enamine **2**. Reaction of **2** with various isocyanates followed by a ring-closure under basic conditions gave the 3-substituted-7-benzyl-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione **3**. **3** was then alkylated at the N-1 position with various halides under basic conditions to give **4** which underwent hydrogenation to deprotect the amine at the 7 position. Treatment of **5** with various halides, acid chlorides, and aldehydes gave the final compounds **6** (Scheme 1). Similar synthetic schemes were used to prepare regioisomer VII, starting from the commercially available 1-benzyl-3-carbethoxy-4-piperidone hydrochloride (not shown).

When R3 was a substituted ethyl amine, the 4-oxo-piperidine-3-carboxylic acid ethyl ester **7** was Boc-protected on nitrogen. **8** underwent the same sequence of steps described in Scheme 1 using allyl isocyanate to give **11**. Boc-protection instead of benzyl was necessary to keep the integrity of the amine at the 7 position during the allyl oxidation step. The allyl group of **11** was oxidized by treatment with OsO_4/2,6-lutidine followed by $NaIO_4$ to form the intermediate aldehyde **12**. Reductive amination of **12** using standard conditions gave **13**. Boc-deprotection followed by alkylation, acylation or reductive amination gave the final compounds **15** (Scheme 2). To allow for more diversity at the 3 position, the *p*-methoxybenzyl (PMB) isocyanate was used as a protecting group. Chemistry proceeded as previously to give compound **17**. After switching protecting groups at the 6 position, the PMB group was removed in the presence of aluminum chloride in DCM and R3 was installed via a Mitsunobu displacement. Finally, the trifluoroacetate was removed from **19** and R1 installed at the 6 position by alkylation, acylation or reductive amination. The order of steps was modified depending on the desired point of diversity (Scheme 3).

4. Results and discussion

Initially, 8 libraries were prepared, 4 with each regioisomer VI and VII (Table 1). Within each library, R3 and R2 were kept constant and R1 was varied. Because

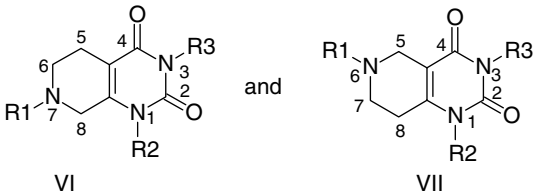
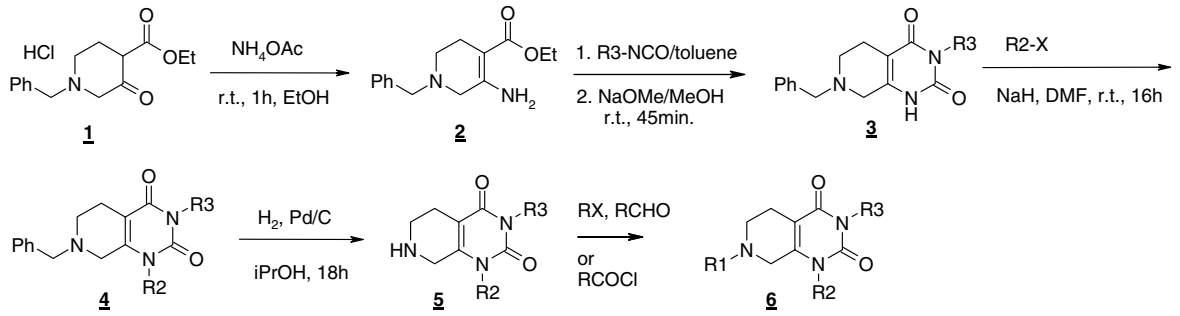
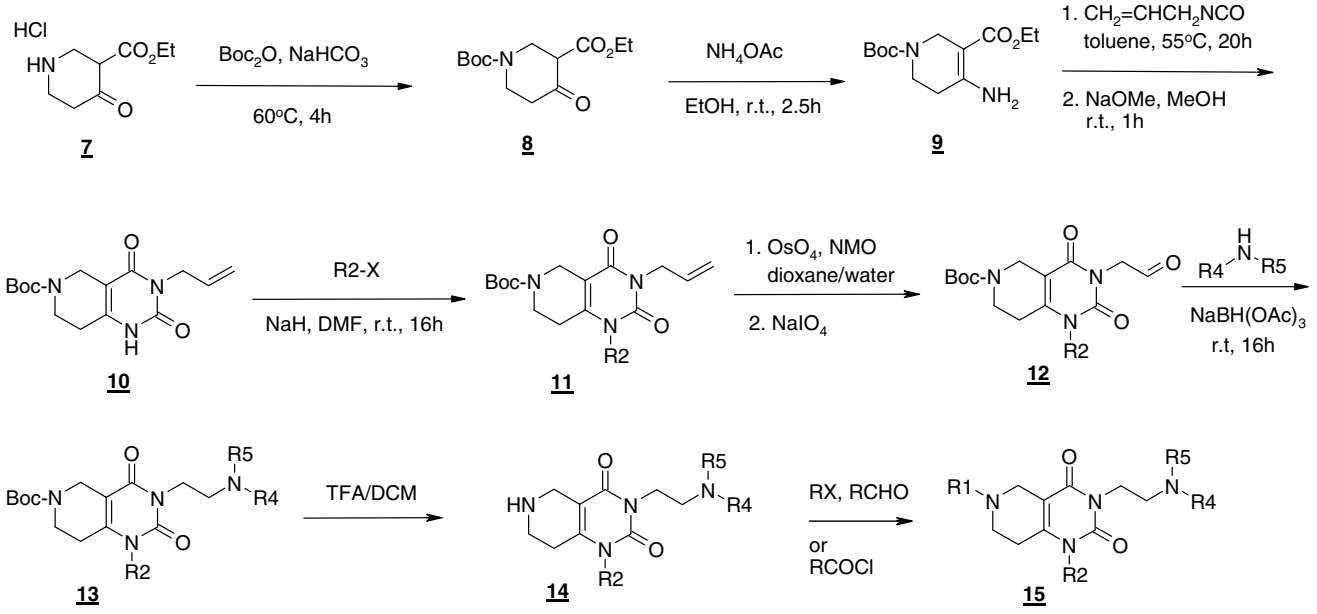


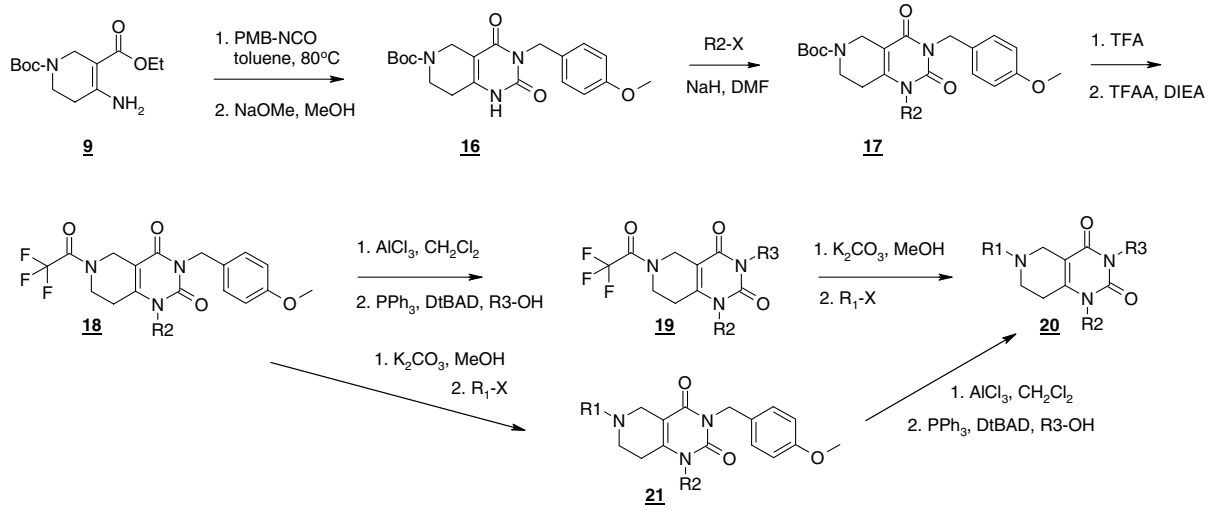
Figure 3. Templates VI and VII.



Scheme 1. Synthesis of template VI when R3 is a phenyl or benzyl.



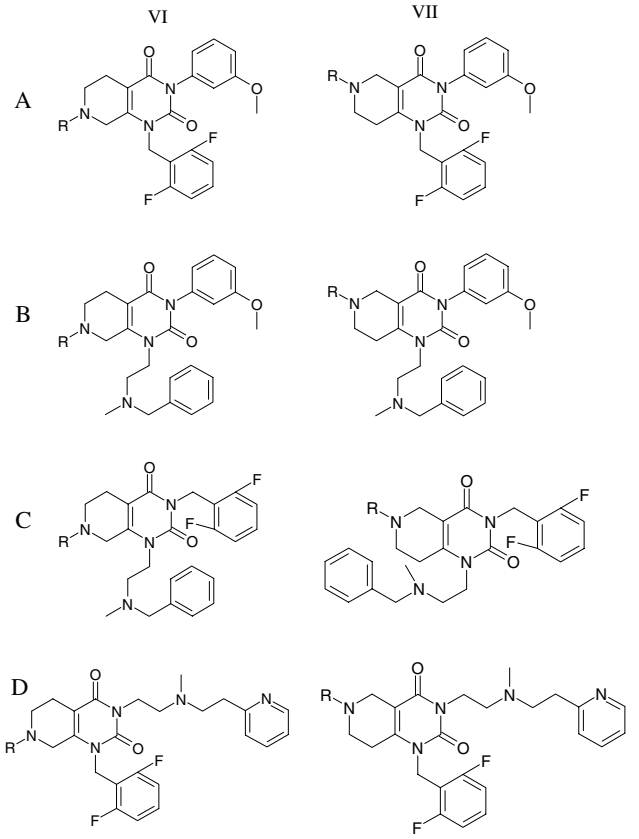
Scheme 2. Synthesis of template VII when R3 is an ethylamine.



Scheme 3. Synthesis of template VII via a PMB protecting group.

of the good overlap between the alignment models and the 5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-2,4-dione core, R2 and R3 at position 1 and 3, respectively, were selected from groups that had shown binding in previous uracil GnRH series.[6] R1 was a diversity set of 36 reagents leading to aliphatic and aromatic substituted alkyl and acyl groups (Fig. 4). The diversity set was generated by computational methods (using Tanimoto similarity on MACCS fingerprints) to avoid bias toward former known SAR.

Table 1. Eight libraries prepared in the first round



The compounds synthesized were evaluated for their ability to inhibit [^{125}I-His^{5}, D-Tyr^{6}]GnRH agonist binding to the cloned human GnRH-receptor as previously described.[19] Out of the eight libraries prepared and tested, VIA and VIID produced active compounds. SAR of libraries VIA and VIID were different suggesting a possible difference in binding modes. Both templates VIA and VIID have three features aligning perfectly with NBI-42902: the two acceptors and the aromatic interactions. Template VIID has a fourth interaction (positive/donor) compared to VIA which should be reflected in the binding data. Indeed in the first set prepared, library VIA showed at best binding in the micromolar range but library VIID had compound **37** with a binding K_i of 35 nM. Some examples are shown in Table 2.

4.1. Library VIA

The unsubstituted core (**24**) showed modest binding at 970 nM. None of the small or polar alkyl and acyl side chains (not shown) showed binding at the h-GnRH receptor above 30% at a concentration of 10^{-6} M. When R1 contained an aromatic group, the binding ranged from 500 to 2200 nM or worse. These data suggested that the core amine was essential to maintain the interaction with the receptor and a lipophilic and/or aromatic interaction was tolerated (Table 2). To optimize the series, further exploration was done around R1. Since the benzylic substitution gave the most active compounds in the first round, a library of 30 compounds around template VIA was prepared with various benzylic, heteroaromatic, and lipophilic groups. The substituted benzyls or benzyl replacements did not help to gain additional interactions with the receptor when compared to the unsubstituted benzyl **23**. The overall binding of the molecule was not significantly affected by the position and/or the nature of the benzyl substituents.

4.2. Library VIID

Analogs in the VIID library showed a similar trend to library VIA. Small or polar alkyl groups and acyl groups at the 6 position failed to generate tight binders at the human GnRH receptor but aromatic groups improved the binding substantially. Adding a *para*-substituent such as a methyl group to the benzyl moiety (compound **37**) boosted the binding more than 20-fold (35 nM). Following the same approach used for Library VIA, a more focused library was prepared around the R1 region of VIID, using systematic screening of substituted benzyls, heterocycles, and non-aromatic lipophilic groups. A total of 45 compounds were prepared. Some key examples are shown in Table 3.

Regarding the benzylic substitution, *para* and/or *ortho* groups (compounds **37**, **40**–**47**, **49**) improved the binding. The same substitution at the *meta* position had no effect on the binding compared to the unsubstituted benzyl (**48**, 850 nM). The 4-chloro benzyl compound (**42**) had a K_i of 17 nM compared to 990 and 850 nM, respectively, for the corresponding unsubstituted phenyl compound (**34**) and the 3-chloro compound (**48**). The 4-methyl substitution was twofold better than the 2-methyl (**37**: 35 nM vs **49**: 85 nM, respectively). The 2,4-disubstitution pattern was found optimal with the 2-methyl-4-chloro (**51**) and 2,4-dimethyl (**52**) both having a binding affinity of 5 nM against the h-GnRH receptor. The 4-methyl (**37**) and 4-ethyl (**40**) compounds had similar binding with 35 and 20 nM, respectively. When the steric bulk was increased more, the binding started to decrease as illustrated with 4-*tert*-butyl compound (**41**, 300 nM). Increased polarity at the *para* position also had a negative impact on the binding as shown with the 4-methylsulfone (**46**, 805 nM) and the 4-methoxy (**43**, 100 nM), although almost any substituent at the *para* position showed improved affinity over the parent benzyl compound. Strongly electron-deficient groups such as 4-trifluoromethyl (**45**, 30 nM) or electron-donating groups such as 4-methoxy (**43**, 100 nM) did not have a critical impact on binding to the receptor. The best substitutions were weakly activating and deactivating groups (methyl and chloro) strongly suggesting that a favored ring orientation or shape (2 and/or 4 substitution pattern) rather than a π–π interaction was critical to a good receptor pocket fit. Heterocycles and bicyclic systems tested were in most cases not as tight binding as the best substituted benzyls. The 3-methyl benzothiophene **53** (15 nM) was the exception and was comparable to the 2,4-dimethyl benzyl **52** (5 nM) and 4-chloro-2-methyl benzyl **51** (5 nM). These results were much more encouraging than for library VIA. We

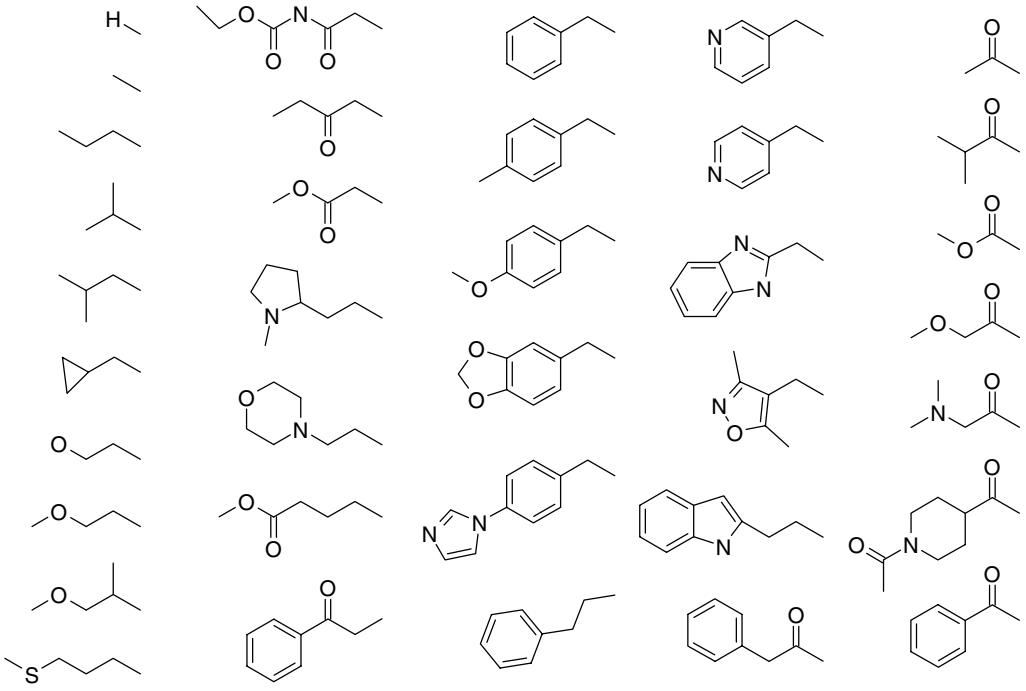
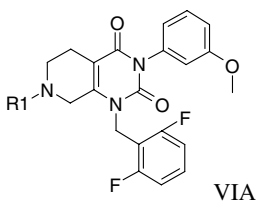
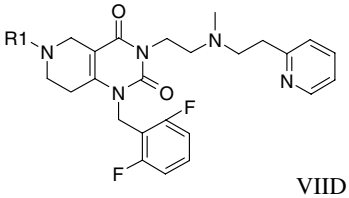


Figure 4. R1 diversity set generated by computational methods.

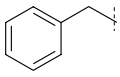
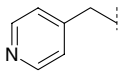
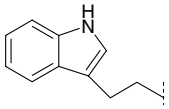
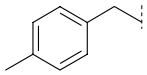
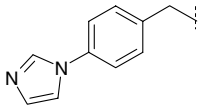
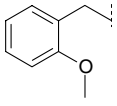
Table 2. Modification of the R1 group



VIA



VIID

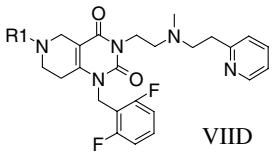
R1	Compound	K_i (nM)	Compound	K_i (nM)
	**23**	1290	**34**	990
	**24**	970	**33**	>10,000
	**25**	1580	**35**	>10,000
	**26**	2200	**36**	515
	**27**	>10,000	**37**	35
	**28**	540	**38**	>10,000
	**29**	980	**39**	NA

decided to focus our efforts on library VIID and explore the substitutions at the 1 and 3 positions (R2 and R3).

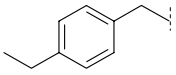
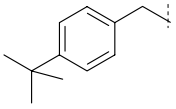
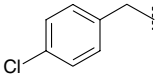
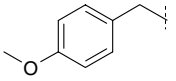
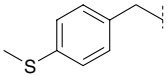
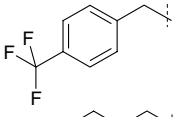
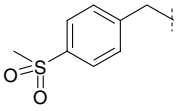
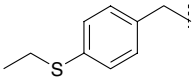
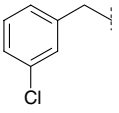
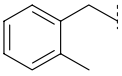
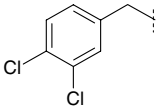
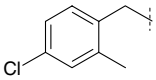
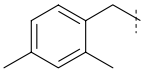
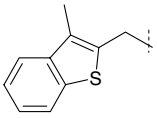
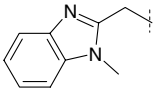
Based on flexible alignments (Fig. 1), it was assumed that the R2 region of VIID should contain an aromatic or hydrophobic group. Over 50 compounds were prepared using the methodology described in Scheme 2 to test this hypothesis. Representatives of this exploration are disclosed in Table 4. When R2 was hydrogen (**55**) or methyl (**56**), no binding was detected. Only the benzyl (**58**), preferably *ortho* substituted (**53**, **59**, **60**), and the cyclohexyl (**57**) showed competitive binding against the human GnRH receptor, highlighting an important lipophilic interaction. 2,6-Disubstitutions gave the best binding with a 20-fold increase in binding compared to the unsubstituted phenyl (**58**, 305 nM compared to **60**, 10 nM). From these results, it appeared that in this region of the molecule, the SAR of the VIID series mostly paralleled that of the uracil antagonist series.[20] Not only was lipophilicity important but the shape, orientation, and electron-deficiency were key factors for the interaction with the binding pocket of the receptor. This supported the alignment suggested by our computational studies where the uracil moieties overlap and the piperidine ring was flanking the pyridinedione core. It was worth noting that cyclohexyl was well tolerated and equipotent to benzyl in the present series. In the uracil series however, there was a fourfold binding difference in favor of the benzyl.[20]

Our last focus was to modify the R3 group on the right hand side of the templates (Table 5). Based on the alignment, the positive/donor interaction was in the correct region but the ethyl pyridine did not align well with the reference model and was not in a specific interaction with the receptor. A library was generated with two points of diversity: an amine linker and a substituent on that amine.

Table 3. Modification of the R1 group of template VIID



VIID

Compound	R1	K_i (nM)
40		20
41		300
42		17
43		100
44		10
45		30
46		805
47		15
48		850
49		85
50		255
51		5
52		5
53		15
54		>10,000

For the linker, substituted and unsubstituted ethyl amines, methyl 2-, 3-, and 4-piperidines (*R* and *S*), 2-, 3-, and 4-piperidines, 2-methyl morpholine (*RS*), methyl 2- and 3-pyrrolidines (*R* and *S*), 2- and 3-pyrrolidines (*S*, *RS*), 2-methyl azetidine (*RS*) and 1-tetrahydroisoquinolines (*R*) were used. The amine substitutions were H, methyl, isobutyl, cyclopentyl, benzyl, 2-methyl pyridine, and 2-ethyl pyridine. Amine functionality replacements such as amide, ester, cyanoguanidine, or amidine moieties were also considered. Over 100 compounds (not shown) were prepared and tested. Replacing the amine with any other functionality did not prove to be a successful approach. The ethyl was still the best linker. The 2-methyl piperidine (*RS*) and 2-methyl pyrrolidine (*R*) were the next best with a loss of at least twofold binding. The optimal distance between the core and the basic center was the two-carbon linker. For example, extending to three carbons as in **71c**, the binding dropped 20-fold compared to the two-carbon spaced analog **71b**. The stereochemistry was also crucial. The S isomer of **71d** was 1370 nM compared to 20 nM for the R isomer. This observation was found to be general and independent of the ring size. The *R*-phenylglycine compound was also made as it was one of the best side chains in the uracil series.[21] In the present series, its binding was a modest 45 nM (**66e**). Regarding the amine substitutions, a lipophilic interaction helped substantially and in most cases benzyl and pyridines were best. Compound **72d** was tested at 10 nM against the human GnRH receptor, only twofold less than **52**.

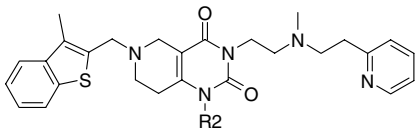
In summary, we successfully identified and synthesized a novel series of h-GnRH antagonists, the 5,6,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-2,4-dione, using a computationally driven approach. We have demonstrated that using a consensus scoring method, we could successfully identify a new template which included key features present in most GnRH series, but had only partial correlation with the SAR of other known small molecule GnRH antagonists. Template VIA had only three key features out of the four usually required. The binding for this series plateaued around one micromolar. When an additional feature was present like in series VIID, the binding was improved substantially. Using an array approach, we were able to explore all three points of diversity of the template and rapidly produce low nanomolar binding compounds (below 10 nM).

5. Experimental

5.1. General methods and materials

Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. Concentration refers to evaporation under vacuum using a Büchi rotary evaporator. Reaction products were purified, when necessary, by chromatography on silica gel (40–63 μm) with the solvent system indicated. Nuclear magnetic resonance data were recorded on a Varian Mercury 300 MHz Spectrometer using TMS as the internal standard. Compounds that were prepared in a library format were purified by HPLC using mass collection and their purities

Table 4. Modification of the R2 group



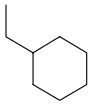
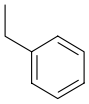
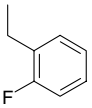
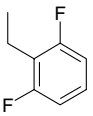
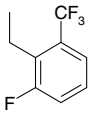
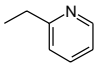
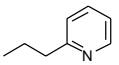
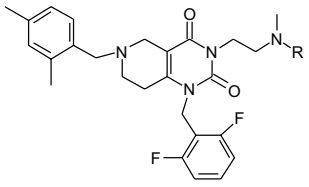
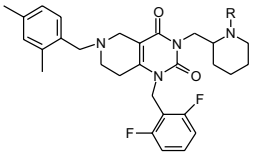
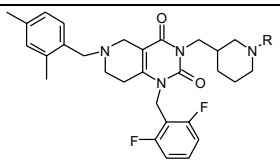
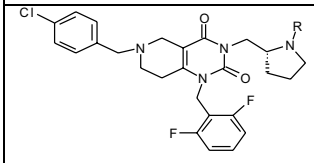
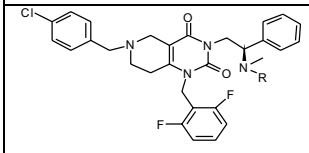
Compound	**55**	**56**	**57**	**58**	**59**	**53**	**60**
	H	Me					
K_i (nM)	>10,000	>10,000	380	305	80	15	10

Table 5. Modification of the R3 group

	R	H	Me					
		**N/A**	**67a***	**68a**	**N/A**	**N/A**	**71a**	**52**
	Ki		1400	1800			180	5
		**66b**	**67b**	**68b**	**69b**	**70b**	**71b**	**72b****
	Ki	>10000	1710	2590	520	945	25	(R) 30 (S) 2410
		**66c**	**67c**	**68c**	**69c**	**70c**	**71c**	**72c**
	Ki	2450	>10000	>10000	2580	160	490	600
		**66d**	**N/A**	**N/A**	**69d**	**N/A**	**71d**	**72d**
	Ki	>10000			1370		(R) 20 (S) 1370	10
		**66e**	**67e**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**
	Ki	45 nM	70 nM					

*4-Ethyl benzyl instead of 2,4-dimethyl benzyl.
**4-Chlorobenzyl instead of 2,4-dimethyl benzyl.

checked by LC–MS. All the compounds having a UV purity above 85% at two different wavelengths (220 and 254 nM) were tested in a biological assay. Analytical HPLC-MS Method 1 was run on an Agilent 1100 series platform equipped with an auto-sampler, a UV detector (220 and 254 nM), an MS detector (APCI); HPLC column: YMC ODS AQ, S-5, 5 μ, 2.0 × 50 mm cartridge; HPLC gradient: 1.0 mL/min, from 10% acetonitrile in water to 90% acetonitrile in water in 2.5 min, maintaining 90% for 1 min. Both acetonitrile and water have 0.025% TFA. Analytical HPLC-MS Method 2 was run on an Agilent 1100 equipped with an auto-sampler, an UV detector

(220 and 254 nM), a MS detector (APCI); HPLC column: Phenomenex Synergi-Max RP, 2.0 × 50 mm column; HPLC gradient: 1.0 mL/min, from 5% acetonitrile in water to 95% acetonitrile in water in 13.5 min, maintaining 95% for 2 min. Both acetonitrile and water have 0.025% TFA. Analytical HPLC-MS Method 3 was run on an Agilent 1100 series platform equipped with an auto-sampler, a UV detector (220 and 254 nM), an MS detector (APCI); HPLC column: Phenomenex Synergi-Max RP, 2.0 × 50 mm column; HPLC gradient: 1.0 mL/min, from 5% acetonitrile in water to 95% acetonitrile in water in 13.5 min, maintaining 95% for 2 min. Both acetonitrile and water have 0.025% TFA. Analytical HPLC-MS Method 4 was run on an Agilent 1100 series platform equipped with an auto-sampler, a UV detector (220 and 254 nM), an MS detector (APCI), and Berger FCM 1200 CO_2 pump module; HPLC column: Berger Pyridine, PYR 60A, 6 μ, 4.6 × 150 mm column; HPLC gradient: 4.0 mL/min, 120 bar; from 10% methanol in supercritical CO_2 to 60% methanol in supercritical CO_2 in 1.67 min, maintaining 60% for 1 min. Methanol has 1.5% water. Backpressure regulated at 140 bar. Analytical HPLC-MS Method 5 was run on a Dionex platform equipped with an autosampler, a UV detector (220 and 254 nM), an MS detector (APCI); HPLC column: Phenomenex CX18 4.6 × 150 mm; HPLC gradient: 95% 0.04% NH_4OH/H_2O to 90% 0.04% NH_4OH/ACN over 9.86 min, 12.30 min run. The binding assay was performed following the method described in reference 19. Each compound was tested at least twice.

5.2. Synthesis of library VIA via Scheme 1

5.2.1. 5-Amino-1-benzyl-1,2,3,6-tetrahydro-pyridine-4-carboxylic acid ethyl ester (2). To a solution of 1-benzyl-3-oxo-piperidine-4-carboxylic acid ethyl ester hydrochloride **1** (20 g, 67.2 mmol) in ethanol (200 mL) was added ammonium acetate (51.8 g, 672 mmol). The mixture was stirred for 1 h at room temperature, after which the TLC (5% methanol in dichloromethane) showed complete consumption of starting material. The solvent was removed in vacuo, and the resulting residue was partitioned between ethyl acetate and 1 N NaOH. The aqueous layer was further extracted with dichloromethane and the combined organic layers were washed with brine and dried over anhydrous magnesium sulfate, filtered and evaporated to give a beige solid, which was recrystallized from diethyl ether and hexanes to give the product as an off-white solid (11.9 g, 68%). A second crop of product (3.41 g, 19.5%) was obtained from the mother liquor. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.20–7.40, m, 5H; 4.01, q, 2H, (J = 6.9 Hz); 3.51, s, 2H; 3.32, s, 2H; 2.93, s, 2H; 2.46–2.51, bm, 2H; 2.21, t, 2H, (J = 5.1 Hz); 1.16, t, 3H, (J = 6.9 Hz). LCMS-1: t_R = 0.32 (100%); MS: m/z 261 $[M+H]^+$, expected 261 $[M+H]^+$.

5.2.2. 7-Benzyl-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione (22). To 3-amino-1-benzyl-1,2,5,6-tetrahydro-pyridine-4-carboxylic acid ethyl ester **2** (7.0 g, 26 mmol) in toluene (60 mL) was added 3-methoxyphenyl isocyanate (4.6 g, 32 mmol). The resulting mixture was stirred for 12 h at room temperature after which it was concentrated in vacuo and triturated with methanol (80 mL) to yield 1-benzyl-5-[3-(3-methoxy-phenyl)-ureido]-1,2,3,6-tetrahydro-pyridine-4-carboxylic acid ethyl ester as a white solid (8.0 g, 75%). To this intermediate were added methanol (100 mL) and 30% sodium methoxide in methanol (10 mL, 60 mmol). The resulting mixture was stirred at room temperature overnight, after which it was concentrated in vacuo, resuspended in water, and precipitated via pH adjustment to 7 with 1 N HCl. The resulting white solid was filtered, washed with water, and dried over anhydrous magnesium sulfate to yield the title compound **22** (6.2 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40–7.30, m, 6H; 6.96, dd, 1H, (J = 8.4 Hz and 0.9 Hz); 3.79, s, 3H; 3.70, br s, 2H; 3.24, br s, 2H; 2.76, br s, 2H; 2.52, br s, 2H. LCMS-2: t_R = 0.49 (100%); MS: m/z 363.9 $[M+H]^+$, expected 364 $[M+H]^+$.

5.2.3. 7-Benzyl-1-(2,6-difluoro-benzyl)-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione (23). To 7-benzyl-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione **22** (3.2 g, 8.8 mmol) in dimethylformamide (20 mL) was added sodium hydride (0.38 g, 9.7 mmol), followed by 2,6-difluorobenzyl bromide (2.0 g, 9.7 mmol). The resulting solution was stirred at room temperature for 2 h, after which it was poured into a solution of saturated aqueous sodium bicarbonate. The resulting solids were filtered and triturated with hexanes to yield compound **23** (3.7 g, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.36, t, 1H, (J = 7.8 Hz); 7.32–7.19, m, 6H; 6.93, dd, 1H, (J = 8.4 and 2.4 Hz); 6.84, t, 2H, (J = 8.4 Hz); 6.79, d, 1H, (J = 7.8 Hz); 6.73, t, 1H, (J = 1.5 Hz); 5.10, s, 2H; 3.79, s, 3H; 3.70, br s, 2H; 3.41, br s, 2H; 2.74–2.70, m, 2H; 2.60–2.57, m, 2H. LCMS-3: t_R = 4.88 (93%); MS: m/z 489.9 $[M+H]^+$, expected 490 $[M+H]^+$.

5.2.4. 1-(2,6-Difluoro-benzyl)-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione (24). To 7-benzyl-1-(2,6-difluoro-benzyl)-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione **23** (3.0 g, 6.1 mmol) in isopropanol (60 mL) was added 10% palladium hydroxide on carbon (1.0 g). The resulting mixture was placed under an atmosphere of hydrogen (1 atm) and stirred for 12 h at room temperature. It was then filtered through Celite and concentrated in vacuo to yield **24** as a white solid (2.0 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.37, t, 1H, (J = 8.4 Hz); 7.29–7.26, m, 1H; 6.96–6.86, m, 3H; 6.79, d, 1H, (J = 7.5 Hz); 6.73, br s, 1H; 5.15, br s, 2H; 3.86, br s, 2H; 3.79, s, 3H; 3.04, br s, 2H; 2.51, br s, 2H. LCMS-4: t_R = 1.82 (89%); MS: m/z 399.8 $[M+H]^+$, expected 400 $[M+H]^+$.

5.3. General procedure for the alkylation of 24

To 1-(2,6-difluoro-benzyl)-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione **24** (50 mg, 0.13 mmol) in dimethylformamide (1 mL) were added the appropriate alkyl halide (0.20 mmol), diisopropylethylamine (50 mg, 0.39 mmol), and tetrabutylammonium iodide (5.0 mg, 0.014 mmol). The resulting mixture was stirred at 50 °C for 12 h. Compounds were purified directly by preparative HPLC yielding the TFA salts of the

title compounds. Compounds **25**–**27** were prepared using this procedure.

5.3.1. 1-(2,6-Difluoro-benzyl)-7-[pyridin-4-yl-methyl]-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*d*]pyrimidine-2,4-dione (25). LCMS-3: t_R = 4.12 (90%): *m*/*z* 491 $[M+H]^+$, expected 491 $[M+H]^+$.

5.3.2. 1-(2,6-Difluoro-benzyl)-7-[2-(1*H*-indol-3-yl)-ethyl]-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*d*]pyrimidine-2,4-dione (26). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.10, br s, 1H; 7.58, d, 1H, (*J* = 7.8 Hz); 7.40–7.34, m, 2H; 7.28–7.11, m, 3H; 7.05, br s, 1H; 6.94, dd, 1H, (*J* = 9.0 and 2.7 Hz); 6.89, t, 2H, (*J* = 8.4 Hz); 6.79, d, 1H, (*J* = 6.9 Hz); 6.74, br s, 1H; 5.11, s, 2H; 3.79, s, 3H; 3.68, br s, 2H; 3.08–2.96, m, 4H; 2.89, br s, 2H; 2.64, br s, 2H. LCMS-3 t_R = 5.36 (94%): *m*/*z* 543 $[M+H]^+$, expected 543 $[M+H]^+$.

5.3.3. 1-(2,6-Difluoro-benzyl)-7-[4-methylbenzyl]-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*d*]pyrimidine-2,4-dione (27). LCMS-3: t_R = 5.20 (93%); MS: *m*/*z* 504 $[M+H]^+$, expected 504 $[M+H]^+$.

5.4. General procedure for the reductive amination of 24

To 1-(2,6-difluoro-benzyl)-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1H-pyrido[3,4-*d*]pyrimidine-2,4-dione **24** (50 mg, 0.13 mmol) in dichloroethane (1 mL) were added the appropriate aldehyde (0.15 mmol) and sodium triacetoxyborohydride (37 mg, 0.17 mmol). The resulting mixture was stirred at room temperature overnight, after which it was concentrated in vacuo and redissolved in methanol (1 mL). Preparative HPLC yielded the TFA salts of the title compounds. Compounds **28** and **29** were prepared using this procedure.

5.4.1. 1-(2,6-Difluoro-benzyl)-7-[4-imidazol-1-yl-benzyl]-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*d*]pyrimidine-2,4-dione (28). LCMS-3: t_R = 3.50 (98%); MS: *m*/*z* 556 $[M+H]^+$, expected 556 $[M+H]^+$.

5.4.2. 1-(2,6-Difluoro-benzyl)-7-(2-methoxy-benzyl)-3-(3-methoxy-phenyl)-5,6,7,8-tetrahydro-1*H*-pyrido[3,4-*d*]pyrimidine-2,4-dione (29). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.37, t, 1H, (*J* = 7.8 Hz); 7.38–7.24, m, 3H; 6.98–6.90, m, 3H; 6.88, t, 2H, (*J* = 8.4 Hz); 6.78, d, 1H, (*J* = 7.5 Hz); 6.73, t, 1H (*J* = 2.1 Hz); 5.12, s, 2H; 4.03, s, 2H; 3.84, s, 3H; 3.81, br s, 2H; 3.79, s, 3H; 2.99, br s, 2H; 2.69, bt, 2H (*J* = 10.5 Hz). LCMS-3: t_R = 4.68 (100%); MS: *m*/*z* 520 $[M+H]^+$, expected 520 $[M+H]^+$.

5.5. Synthesis of library VIID via Scheme 2

5.5.1. 1-*tert*-Butoxycarbonyl-3-carbethoxy-4-piperidone (8). Di-*tert*-butyl dicarbonate (39.3 g, 0.18 mol) was added in one portion to 3-carbethoxy-4-piperidone hydrochloride **7** (31.83 g, 0.15 mol), sodium bicarbonate (13.9 g, 0.16 mol), sodium chloride (26.3 g, 0.45 mol) in water/chloroform (100 mL/200 mL). The mixture was heated to 60 °C for 4 h. After cooling to room temperature the aqueous layer was separated and extracted with dichloroethane (3× 150 mL). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate, filtered, and evaporated to give a colorless oil which crystallized upon standing (40.1 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.23, q, 2H, (*J* = 6.9 Hz); 4.07, br s, 2H; 3.57, t, 2H, (*J* = 5.9 Hz); 2.37, t, 2H, (*J* = 5.9 Hz); 1.48, s, 9H; 1.31, t, 3H, (*J* = 6.9 Hz).

5.5.2. Ethyl-4-amino-1-*tert*-butoxycarbonyl-1,2,5,6-tetrahydropyridine-3-carboxylate (9). To a solution of 1-*tert*-butoxycarbonyl-3-carbethoxy-4-piperidone **8** (12.9 g, 47.9 mmol) in ethanol was added ammonium acetate (36.9 g, 479 mmol). The mixture was stirred for 2.5 h at room temperature, after which the TLC (50% ethyl acetate in hexanes) showed complete consumption of starting material. The solvent was removed in vacuo, and the resulting residue was partitioned between dichloroethane (300 mL) and 1 N sodium hydroxide (100 mL). The aqueous layer was further extracted with dichloroethane (3× 100 mL) and the combined organic layers were washed with brine and dried over anhydrous magnesium sulfate, filtered, and evaporated to give ethyl-4-amino-1-*tert*-butoxycarbonyl-1,2,5,6-tetrahydropyridine-3-carboxylate **9**, which was used without purification in the following reactions (11.4 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.15, q, 2H, (*J* = 7.2 Hz); 4.07, br s, 2H; 3.81, bm, 1H; 3.69, br s, 2H; 3.52, t, 2H, (*J* = 6.0 Hz); 2.28, t, 2H, (*J* = 6.0 Hz): 1.48, s, 9H; 1.27, t, 3H, (*J* = 7.2 Hz). LCMS-1: t_R = 2.62 (100%); MS: *m*/*z* 271 $[M+H]^+$, expected 271 $[M+H]^+$.

5.5.3. 3-Allyl-2,4-dioxo-1,3,4,5,7,8-hexahydro-2*H*-pyrido[4,3-*d*]pyrimidine-6-carboxylic acid *tert*-butyl ester (10). A solution of ethyl-4-amino-1-*tert*-butoxycarbonyl-1,2,5,6-tetrahydropyridine-3-carboxylate **9** (8.7 g, 32.4 mmol), allyl isocyanate (3.6 mL, 33.7 mmol), and diisopropylethylamine (0.9 mL, 5.6 mmol) in toluene (50 mL) was warmed to 55 °C. An additional volume of allyl isocyanate (3.6 mL, 33.7 mmol) was added and stirring at 55 °C was continued for 18 h. The reaction was monitored by TLC (20% ethyl acetate in hexanes) for completion. After cooling to room temperature, solvents were evaporated, the residue was diluted with methanol (50 mL), and a 30% weight solution of sodium methoxide in methanol (17.8 mL, 97.2 mmol) was added carefully. The solution was stirred at room temperature for 1 h. Upon completion, the solvent was removed in vacuo. The resulting residue was dissolved in water (200 mL), acidified to pH 4 with 1 N aqueous HCl. Product **10** precipitated as a pale yellow solid (8.0 g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 5.80–5.95, m, 1H; 5.10–5.30, dt, 2H, (*J* = 14.7 and 9 Hz); 4.53, d, 1H, (*J* = 5.7 Hz); 4.22, br s, 1H; 3.82, t, 2H, (*J* = 5.7 Hz); 3.67, t, 2H, (*J* = 5.7 Hz); 1.48, s, 9H.

5.5.4. 3-Allyl-1-(2,6-difluoro-benzyl)-2,4-dioxo-1,3,4,5,7,8-hexahydro-2*H*-pyrido[4,3-*d*]pyrimidine-6-carboxylic acid *tert*-butyl ester (30). A solution of **10** (5.5 g, 17.9 mmol) in anhydrous dimethylformamide (50 mL) was treated with sodium hydride (60% suspension in oil, 0.64 g, 16.0 mmol). After stirring at room temperature for 10 min, 2,6-difluorobenzyl bromide (3.3 g, 16.0 mmol) was added and stirring continued overnight at room temperature. The solvent was removed and the residue was dissolved in ethyl

acetate (75 mL) and washed with water (50 mL). The two layers were separated. The aqueous layer was extracted with ethyl acetate (3× 50 mL). The organic layers were combined, washed with brine (50 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated to give a yellow oil. After trituration in hexanes, the expected product **30** was obtained (5.8 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25–7.30, m, 1H; 6.80–6.95, m, 2H; 5.80–5.95, m, 1H; 5.09–5.29, m, 2H; 5.20, s, 2H; 4.57, d, 2H, (J = 5.7 Hz); 4.24, s, 2H; 3.80, t, 2H, (J = 5.7 Hz); 3.63, t, 1H, (J = 5.7 Hz); 2.60, t, 2H, (J = 5.7 Hz); 1.47, s, 9H.

5.5.5. 1-(2,6-Difluoro-benzyl)-2,4-dioxo-3-(2-oxo-ethyl)-1,3,4,5,7,8-hexahydro-2*H*-pyrido[4,3-*d*]pyrimidine-6-carboxylic acid *tert*-butyl ester (31). To a solution of **30** (5.2 g, 12.0 mmol) in dioxane/water (90 mL/30 mL) were added 2,6-lutidine (2.8 mL, 24.0 mmol), osmium tetroxide (2.4 mL of a 2.5% solution in isopropanol, 0.24 mmol), and sodium periodate (10.3 g, 48.0 mmol). The mixture was followed by TLC (50% ethyl acetate in hexanes). When the reaction was complete, the mixture was treated with water (150 mL), then extracted with dichloromethane (3× 50 mL). The organic layers were combined, washed with water (50 mL), then brine (50 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated. The crude aldehyde **31** was used immediately without further purification.

5.5.6. 1-(2,6-Difluoro-benzyl)-3-{2-[methyl-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-2,4-dioxo-1,3,4,5,7,8-hexahydro-2*H*-pyrido[4,3-*d*]pyrimidine-6-carboxylic acid *tert*-butyl ester (32). The crude aldehyde **31** (5.55 g, 12.8 mmol) was dissolved in 100 mL of dichloroethane with 2-(2-methylaminoethyl)pyridine (1.91 g, 14.0 mmol). Sodium triacetoxyborohydride (4.06 g, 19.1 mmol) was added and the mixture was stirred overnight at room temperature. The solvent was removed and the residue was extracted with ethyl acetate, washed with water (50 mL) and then brine (50 mL), dried over anhydrous magnesium sulfate, filtered, and evaporated. The mixture was purified by silica gel chromatography (5% methanol in dichloromethane) to give product **32** (5.17 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.52 d, 1H, (J = 4.8 Hz); 7.60, dt, 1H, (J = 7.8 Hz and 1.8 Hz); 7.20–7.29, m, 2H; 7.10–7.15, m, 1H; 6.85–6.96, m, 2H; 5.20, s, 2H; 4.23, s, 2H; 4.15, t, 2H, (J = 6.9 Hz); 3.63, t, 2H, (J = 5.1 Hz); 2.78–3.05, m, 4H; 2.80, t, 2H, (J = 6.9 Hz); 2.55–2.63, m, 2H; 2.46, s, 3H; 1.47, s, 9H. LCMS-5: t_R = 5.44 (91%); MS: m/z 556.3 $[M+H]^+$, expected 556 $[M+H]^+$.

5.5.7. 1-(2,6-Difluoro-benzyl)-3-{2-[methyl-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5,6,7,8-tetrahydro-1*H*-pyrido[4,3-*d*]pyrimidine-2,4-dione (33). Compound **32** (4.49 g, 8.09 mmol) was dissolved in dichloromethane (20 mL) and trifluoroacetic acid (15.5 mL, 202.3 mmol) was added. After 2-h stirring at room temperature the solvent was removed and a portion of the residue was extracted with ethyl acetate and a small amount of 1 N aqueous sodium hydroxide. The aqueous layer was further extracted with ethyl acetate and the combined organic layers were dried over anhydrous magnesium sulfate, filtered, and evaporated to give the free base as a slightly tacky foaming solid **33** (2.2 g, 59.8%) which was used without further purification. A sample was purified for biological testing. LCMS-3: t_R = 1.72 (100%); MS: m/z 455.8 $[M+H]^+$, expected 456 $[M+H]^+$.

5.6. General procedure for the alkylation of 33

To **33** (45 mg, 0.1 mmol) in dimethylformamide (1 mL) were added the appropriate alkyl or benzyl halide (0.15 mmol) and diisopropylethylamine (52 mg, 0.4 mmol). The resulting mixture was shaken at 45 °C overnight, after which the mixture was purified by preparative HPLC, affording the TFA salts of the title compounds. Compounds **34–36** were prepared using this method.

5.7. General procedure for the reductive amination of 33

To **33** (32 mg, 0.07 mmol) in dichloroethane (1 mL) were added the appropriate aldehyde (0.11 mmol) and sodium triacetoxyborohydride (22 mg, 0.11 mmol). The resulting mixture was stirred at room temperature overnight, after which it was concentrated in vacuo and redissolved in methanol (1 mL). Preparative HPLC purification yielded the TFA salts of the title compounds. Compounds **37–54** were prepared using this method.

Compound	LCMS method	t_R (% purity at 220 nM)	m/z $[M+H]^+$	Expected $[M+H]^+$
34	3	3.06 (100)	546.1	546
35	5	3.03 (91)	547.2	547
36	5	4.11 (97)	599.2	599
37	3	3.65 (99)	560	560
38	5	2.70 (100)	612.2	612
40	3	3.97 (100)	574.1	574
41	3	4.70 (100)	602.1	602
42	3	3.80 (99)	580	580
43	3	3.37 (92)	576.0	576
44	3	3.89 (100)	592.0	592
45	3	4.26 (100)	614	614
46	3	3.05 (100)	624.0	624
47	3	4.09 (100)	606.1	606
48	3	4.00 (99)	580.0	580
49	3	3.65 (98)	560	560
50	3	4.47 (100)	614	614
51	3	4.27 (100)	594.2	594
52	3	4.14 (100)	574.2	574
53	3	4.74 (100)	616.0	616
54	3	3.50 (94)	600.1	600

Compound **51**: ^{1}H NMR of TFA salt (300 MHz, DMSO-d_6) δ: 8.46 d, 1H, (J = 4.8 Hz); 7.80, t, 1H, (J = 6.6 Hz); 7.23–7.46, m, 6H; 7.08, t, 2H, (J = 8.1 Hz); 5.13, s, 2H; 4.16, s, 2H; 3.54, t, 2H, (J = 7.2 Hz); 3.34, t, 2H, (J = 7.2 Hz); 3.14, t, 2H, (J = 7.2 Hz); 2.90, s, 4H; 2.48, s, 8H; 2.35, s, 2H.

Compound **52**: ^{1}H NMR of TFA salt (300 MHz, DMSO-d_6) δ: 8.47 d, 1H, (J = 4.8 Hz); 7.80, t, 1H, (J = 7.5 Hz); 7.26–7.46, m, 4H; 7.05–7.16, m, 4H; 5.13, s, 2H; 4.17, s, 2H; 3.55, s, 2H; 3.34, s, 2H; 3.14, s, 2H; 2.90, s, 4H; 2.48, s, 6H; 2.33, s, 2H; 2.72, s, 2H.

Compound **53**: ^{1}H NMR of TFA salt (300 MHz, DMSO-d_6) δ: 8.45 d, 1H, (J = 4.5 Hz); 7.93, d, 1H, (J = 7.2 Hz); 7.74–7.84, m, 2H; 7.32–7.46, m, 4H; 7.29, t, 1H, (J = 6.0 Hz); 7.07, t, 2H, (J = 7.5 Hz); 5.14, s, 2H; 4.16, s, 2H; 3.53, t, 2H, (J = 6.9 Hz); 3.34, s, 2H; 3.14, t, (J = 6.9 Hz), 2H; 2.89, s, 4H; 2.48, s, 8H; 2.41, s, 2H.

5.7.1. 6-(3-Methyl-benzo[*b*]thiophen-2-ylmethyl)-3-{2-[methyl-(2-pyridin-2-yl-ethyl)-amino]-ethyl}-5,6,7,8-tetrahydro-1*H*-pyrido[4,3-*d*]pyrimidine-2,4-dione (55). To a solution of **10** (400 mg, 1.3 mmol) in dioxane/water (10 mL/2 mL) were added 2,6-lutidine (279 mg, 2.6 mmol), OsO_4 (0.26 mL of a 2.5% solution in isopropylalcohol), and $NaIO_4$ (1.1 g, 5.2 mmol). The mixture was stirred at room temperature and followed by TLC. After 4 h, the reaction was complete. Solvents were evaporated, a mixture of ethyl acetate/water 20 mL/20 mL was added, and the aldehyde extracted with ethyl acetate (2× 20 mL). The organic layers were combined, washed with water, then brine, dried over $MgSO_4$, filtered, and evaporated. The crude aldehyde (293 mg) was used immediately without further purification. It was dissolved in 5 mL of 1,2-dichloroethane and 2-methylamino ethyl pyridine (0.14 mL, 1 mmol) was added followed by sodium triacetoxyborohydride (290 mg, 1.6 mmol). The mixture was stirred overnight at room temperature. The solvent was removed and the residue was partitioned between dichloromethane and water, the organics were washed with brine, dried over $MgSO_4$, filtered, and evaporated. The integrity of the intermediate was checked by LC/MS and then used without further purification. The deprotection step and reduction amination steps were performed as described earlier for the library via Scheme 2 to give compound **55**.

Compounds **56**–**60**: were made using synthetic Scheme 2. In the alkylation step of **10**, the 2,6-difluorobenzyl bromide was replaced by methyl iodide, bromomethylcyclohxane, benzyl bromide, 2-fluorobenzyl bromide, and 2-fluoro-6-(trifluoromethyl)benzyl bromide, respectively.

Compound	LCMS method	t_R (% purity at 220 nM)	m/z $[M+H]^+$	Expected $[M+H]^+$
55	3	3.13 (100)	489.8	490
56	3	3.22 (100)	504	504
57	5	5.43 (100)	586.3	586
58	3	4.77 (100)	579.8	580
59	3	5.46 (100)	598.1	598
60	3	4.68 (98)	666.2	666

5.8. Library made via Scheme 3

5.8.1. 3-(4-Methoxy-benzyl)-2,4-dioxo-1,3,4,5,7,8-hexahydro-2H-pyrido[4,3-*d*]pyrimidine-6-carboxylic acid *tert*-butyl ester (16). To 4-amino-5,6-dihydro-2H-pyridine-1,3-dicarboxylic acid 1-*tert*-butyl ester 3-ethyl ester **8** (2.0 g, 7.3 mmol) in dry toluene (15 mL) was added 4-methoxybenzyl isocyanate (1.3 g, 8.1 mmol). The resulting mixture was stirred for 12 h at 80 °C, after which it was concentrated in vacuo and resuspended in methanol (30 mL). To this solution was added 30% sodium methoxide in methanol (2.0 mL, 11 mmol). The resulting mixture was stirred at room temperature for 30 min, after which it was concentrated in vacuo and partitioned between dichloromethane (50 mL) and 0.5 M citric acid (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The resulting residue was triturated with hexanes/dichloromethane 1:1 (30 mL) and dried to yield the title compound as an off-white solid (1.1 g, 39%). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.22, d, 2H, (J = 9.0 Hz); 6.83, d, 2H, (J = 9.0 Hz); 4.85, s, 2H; 3.98, s, 2H; 3.69, s, 3H; 3.52–3.46, m, 2H; 2.42–2.38, m, 2H; 1.39, s, 9H. LCMS-1: t_R = 2.56 (100%); MS: m/z 301.2 $[M+H]^+$, expected 301 $[M+H]^+$.

5.8.2. 1-(2,6-Difluoro-benzyl)-3-(4-methoxy-benzyl)-2,4-dioxo-1,3,4,5,7,8-hexahydro-2H-pyrido[4,3-*d*]pyrimidine-6-carboxylic acid *tert*-butyl ester (61). To **16** (4.3 g, 11.0 mmol) in dimethylformamide (30 mL) was added NaH (0.44 g, 11.0 mmol), followed by 2,6-difluorobenzyl bromide (2.3 g, 11.0 mmol). The resulting solution was stirred at room temperature for 2 h, after which it was partitioned between diethyl ether (50 mL) and 1 N NaOH (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield the title compound as a yellow oil (5.3 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43, d, 2H, (J = 9.0 Hz); 7.29, dd, 1H, (J = 8 and 9 Hz); 6.89, t, 2H (J = 8.4 Hz); 6.82, d, 2H (J = 9.0 Hz); 5.21, s, 2H; 5.08, s, 2H; 4.21, s, 2H; 3.77, s, 3H; 3.59, t, 2H, (J = 5.7 Hz); 2.54, br t, 2H (J = 5.4 Hz); 1.45, s, 9H. LCMS-6: t_R = 2.17 (100%); MS: m/z 414.0 $[M+H]^+$, expected 413 $[M+H]^+$.

5.8.3. 1-(2,6-Difluoro-benzyl)-3-(4-methoxy-benzyl)-6-(2,2,2-trifluoro-acetyl)-5,6,7,8-tetrahydro-1H-pyrido[4,3-*d*]pyrimidine-2,4-dione (62). To **61** (2.7 g, 5.2 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (7.5 g, 65 mmol). The resulting mixture was stirred for 1 hour at room temperature and then concentrated in vacuo. The residue was partitioned between dichloromethane (50 mL) and 1 M NaOH (50 mL), after which the organic layer was dried over $MgSO_4$ and concentrated in vacuo to give the free amine as a yellow oil (1.6 g, 74%). It was used without further purification in the next step. To this intermediate (0.80 g, 1.9 mmol) in dichloromethane (10 mL) was added diisopropylethylamine (0.50 g, 3.9 mmol) followed by trifluoroacetic anhydride (0.60 g, 2.9 mmol). The resulting mixture was stirred at room temperature for 48 h. The resulting mixture was partitioned between dichloromethane (50 mL) and 0.5 M citric acid (50 mL), after which the organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield the title compound as a yellow oil (0.96 g, 99%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43, d, 1H (J = 9.0 Hz); 7.42, d, 1H, (J = 9.0 Hz); 7.34–7.26, m, 1H; 6.91, t, 2H, (J = 8.4 Hz); 6.82, d, 1H (J = 8.7 Hz); 6.81, d, 1H (J = 8.7 Hz); 5.20, s, 1H; 5.18, s, 1H; 5.07, s, 2H; 4.47, s, 1H; 4.44, s, 1H; 3.87–3.79, m, 2H; 3.77, s, 3H; 2.72-2.65, m, 2H. LCMS-1: t_R = 2.72 (100%); MS: m/z 510.0 $[M+H]^+$, expected 510 $[M+H]^+$.

5.8.4. 1-(2,6-Difluoro-benzyl)-6-(2,2,2-trifluoro-acetyl)-5,6,7,8-tetrahydro-1H-pyrido[4,3-*d*]pyrimidine-2,4-dione (63). To **62** (0.96 g, 1.9 mmol) in dichloromethane (20 mL) was added anhydrous aluminum chloride

(0.51 g, 3.8 mmol). The resulting mixture was stirred at room temperature for 5 min, after which it was partitioned between dichloromethane (50 mL) and 0.5 M citric acid (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to yield the title compound as a beige solid (0.70 g, 95%). LCMS-1: $t_R = 2.52$ (100%); MS: *m/z* 388.0 $[M+H]^+$, expected 388 $[M+H]^+$.

5.8.5. 3-[1-(2,6-Difluoro-benzyl)-2,4-dioxo-6-(2,2,2-trifluoro-acetyl)-1,4,5,6,7,8-hexahydro-2*H*-pyrido[4,3-*d*]pyrimidin-3-ylmethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (64). To **63** (0.65 g, 1.7 mmol) in dry tetrahydrofuran (20 mL) were added triphenylphosphine (0.66 g, 2.5 mmol) and 3-hydroxymethyl-*N*-Boc-piperidine (0.54 g, 2.5 mmol) followed by di-*tert*-butylazodicarboxylate (0.58 g, 2.5 mmol) at 0 °C. The resulting mixture was warmed to room temperature and stirred for 16 h, after which it was absorbed onto silica gel and purified by silica gel chromatography eluting with 40% ethyl acetate in hexanes to yield the title compound with several impurities present (0.97 g) and was used in the next step without further purification.

5.8.6. 3-[1-(2,6-Difluoro-benzyl)-2,4-dioxo-1,4,5,6,7,8-hexahydro-2*H*-pyrido[4,3-*d*]pyrimidin-3-ylmethyl]-piperidine-1-carboxylic acid *tert*-butyl ester (65). Intermediate **64** (0.97 g, assume 1.7 mmol) was dissolved in methanol (25 mL) and K_2CO_3 (0.325 mesh, 0.47 g, 3.4 mmol) was added and stirred at room temperature for 3 h. Solids were filtered off washing with methanol (25 mL) and the solvent was removed in vacuo. Diethyl ether (75 mL) and 1 M NaOH (10 mL) were added and the aqueous phase was extracted with diethyl ether (75 mL). The combined organic extracts were washed with brine, dried over magnesium sulfate, and concentrated. The intermediate was used without further purification.

5.8.7. 1-(2,6-Difluoro-benzyl)-6-(2,4-dimethyl-benzyl)-3-piperidin-3-ylmethyl-5,6,7,8-tetrahydro-1*H*-pyrido[4,3-*d*]pyrimidine-2,4-dione (66c). The crude compound **65** (assume 1.7 mmol) was dissolved in 1,2-dichloroethane (15 mL) and 2,4-dimethylbenzaldehyde (0.27 g, 0.28 mL, 2.0 mmol) was added followed by $NaBH(OAc)_3$ (0.57 g, 2.7 mmol) and stirred at room temperature for 16 h. Polymer supported tosylhydrazine (2.0 g, ~8 mmol) was added and stirred at room temperature for 6 h to scavenge any remaining aldehyde. The polymer supported reagent was filtered off washing with dichloromethane (100 mL) and then TFA (4 mL) was added and the reaction mixture was stirred at room temperature for 16 h. The solvent and excess TFA were removed in vacuo and the mixture was basified with 2 M NaOH and the aqueous extracted with dichloromethane (3× 100 mL). The combined organic extracts were dried over magnesium sulfate and concentrated to give the crude amine which was used without further purification. An aliquot was purified by HPLC for biological testing.

5.8.8. 3-(1-Benzyl-piperidin-3-ylmethyl)-1-(2,6-difluoro-benzyl)-6-(2,4-dimethyl-benzyl)-5,6,7,8-tetrahydro-1*H*-pyrido[4,3-*d*]pyrimidine-2,4-dione (70c). To a solution of **66c** (50 mg, ~0.1 mmol) in 1,2-dichloroethane (1 mL) was added benzaldehyde (14 mg, 0.15 mmol) followed by $NaBH(OAc)_3$ (50 mg, 0.25 mmol) and stirred at room temperature for 3 h. The solvent was removed in vacuo and redissolved in methanol. Purification was by HPLC to give the TFA salt of the title compound **70c**.

The same series of steps was used to synthesize compounds **66a–e**, **67a–e**, **68a–e**, **69a–e**, **70a–e**, and **71a–e** from Table 5.

5.8.9. 1-(2,6-Difluoro-benzyl)-6-(2,4-dimethyl-benzyl)-3-[1-(2-pyridin-2-yl-ethyl)-piperidin-3-ylmethyl]-5,6,7,8-tetrahydro-1*H*-pyrido[4,3-*d*]pyrimidine-2,4-dione (72c). To a solution of the crude amine **67c** (50 mg, ~0.1 mmol) in 1,2-dichloroethane (1 mL) was added 2-vinyl pyridine (38 mg, 0.4 mmol) followed by acetic acid (24 mg, 0.4 mmol) and the reaction mixture was heated at 80 °C for 3 h. The solvent was removed in vacuo and redissolved in methanol. Purification was by HPLC to give the TFA salt of the title compound. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.60, d, 1H, ($J = 5.1$ Hz); 8.01, t, 1H, ($J = 7.2$ Hz); 7.62, d, 1H, ($J = 7.8$ Hz); 7.50, dd, 1H, ($J = 7.5$ and 5.4 Hz); 7.28–7.18, m, 2H; 7.06–7.03, m, 2H; 6.86, t, 2H, ($J = 8.2$ Hz); 5.13, d, 1H, ($J = 16.5$ Hz); 5.09, d, 1H, ($J = 16.8$ Hz); 4.40, septet, 1H, ($J = 6.4$ Hz); 4.32, s, 2H; 4.98–3.82, m, 4H; 3.66–3.36, m, 8H; 3.10–2.96, m, 2H; 2.80–2.54, m, 2H; 2.41–2.30, m, 1H; 2.36, s, 3H; 2.31, s, 3H; 2.01–1.88, m, 3H.

The same series of steps was used to synthesize compounds **72b** and **72d** from Table 5.

Compound	LCMS method	t_R (% purity at 220 nM)	*m/z* $[M+H]^+$	Expected $[M+H]^+$
66b	3	3.96 (100)	509.1	509
66c	5	4.25 (100)	509.2	509
66d	3	3.56 (100)	501.1	501
66e	4	2.08 (94)	551.0	551
67a	3	3.96 (98)	483.0	483
67b	3	3.99 (100)	523.2	523
67c	3	3.92 (100)	523	523
67e	3	4.33 (100)	565.0	565
68a	5	4.89 (100)	525.2	525
68b	3	4.34 (100)	565.2	565
68c	5	4.71 (97)	565.3	565
69b	3	4.24 (100)	577.2	577
69c	5	4.81 (94)	577.3	577
69d	3	4.38 (98)	569.2	569
70b	3	4.49 (89)	599.2	599
70c	5	5.01 (87)	599.4	599
71a	3	4.06 (96)	560.2	560
71b	3	4.20 (100)	600.2	600
71c	5	4.50 (98)	600.4	600
(*R*)**71d**	3	4.20 (100)	592.1	592
(*S*)**71d**	3	4.25 (98)	592.1	592
(*R*)**72b**	3	3.95 (95)	620.2	620
(*S*)**72b**	3	4.07 (100)	620.2	620
72c	5	4.11 (100)	614.4	614
72d	3	4.01 (100)	606.2	606

Compound **71b**: ^1H NMR (300 MHz, $CDCl_3$) δ: 8.55, d, 1H, ($J = 3.9$ Hz); 7.76, t, 1H, ($J = 7.2$ Hz); 7.67, d, 1H, ($J = 7.8$ Hz); 7.33, dd, 1H, ($J = 7.5$ and 4.8 Hz); 7.32–

7.21, m, 2H; 7.07–7.03, m, 2H; 6.86, t, 2H, (J = 5.4 Hz); 5.12, br s, 2H; 4.60, dd, 1H, (J = 15.0 and 8.1 Hz); 4.44–4.26, m, 4H; 4.03–3.84, m, 4H; 3.58–3.34, m, 2H; 3.24–2.96, m, 4H; 2.37, s, 3H; 2.31, s, 3H; 2.16–1.56, m, 3H.

Compound **69d**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.39, s, 4H; 7.31, dd, 1H, (J = 8.4 and 6.6 Hz); 6.89, t, 2H, (J = 8.4 Hz); 5.16, br s, 2H; 4.45–4.32, m, 2H; 4.26, br s, 2H; 3.92–3.64, m, 4H; 3.51–3.32, m, 2H; 3.07–2.93, m, 4H; 2.25–1.59, m, 12H.

Compound **66e**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.46–7.24, m, 10H; 6.86, t, 2H, (J = 8.4 Hz); 5.14, bd, 1H, (J = 16.2 Hz); 4.94, bd, 1H, (J = 16.2 Hz); 4.60, t, 1H, (J = 10.8 Hz); 4.52, t, 1H, (J = 10.8 Hz); 4.38, qn, 1H, (J = 5.7 Hz); 4.14, br s, 2H; 3.98, d, 1H, (J = 12.9 Hz); 3.84, br s, 1H; 3.40–3.16, m, 2H; 2.93, bq, 2H, (J = 15.9 Hz).

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