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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1864–1868

Optimization of 2,4-diaminopyrimidines as GHS-R antagonists: Side chain exploration

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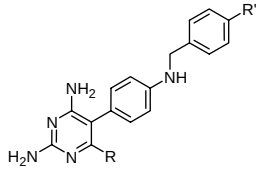
Received 16 November 2005; revised 27 December 2005; accepted 4 January 2006
Available online 25 January 2006

Abstract—The synthesis and structure–activity relationships of the 4- and 6-substituents of 2,4-diaminopyrimidine-based growth hormone secretagogue receptor (GHS-R) antagonists are described. Diaminopyrimidines with 6-norbornenyl (**4n**) and 6-tetrahydrofuranyl (**4p**) substitutents were found to exhibit potent GHS-R antagonism and good selectivity (~1000-fold) against dihydrofolate reductase.

Obesity has become an epidemic disease in developed countries and is also increasing at an alarming rate in developing nations.[1] Existing FDA approved anti-obesity drugs have only moderate efficacy.[2] Ghrelin, a 28 amino acid peptide with a unique *n*-octanoyl modification on Ser 3, has attracted tremendous attention since its discovery as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R).[3] Besides being a potent growth hormone secretagogue and regulator of other endocrine functions, ghrelin is also implicated in the short and long-term regulation of energy balance.[4] A small molecule GHS-R antagonist could potentially be a novel anti-obesity therapy.

Recently, we reported the discovery of novel isoxazole carboxamides[5] and tetralin carboxamides[6] as potent and selective human GHS-R antagonists. Continuing the pursuit of additional pharmacologically active GHS-R antagonists, we identified 2,4-diaminopyrimidine derivative **1** as a potent, competitive, intraperitoneally bioavailable small molecule GHS-R antagonist with in vivo efficacy for decreasing food intake and body weight in several rat studies (Fig. 1).[7] Extensive structure–activity relationship (SAR) studies on the aminobenzyl group of **1** revealed that certain electron-withdrawing groups could provide activity improvement, and good oral bioavailability, as exemplified by **2** (Fig. 1).[7]



1 (R = -CH_2OBn, R' = Cl)
IC_{50}s = 12 nM (Binding), 170 nM (FLIPR)
K_i = 42 nM (FLIPR)
F (i.p.) = 68% (rats)

2 (R = Et, R' = -SO_2Me)
IC_{50}s = 9.7 nM (Binding), 37 nM (FLIPR)
F (p.o.) = 52% (rats)

Figure 1. Representative first-generation 2,4-diaminopyrimidine-based GHS-R antagonists.

Unfortunately, compounds **1** and **2** were also found to be potent human dihydrofolate reductase (DHFR) inhibitors,[7] consistent with the fact that 2,4-diaminopyrimidine is a privileged pharmacophore for DHFR inhibitors, such as trimethoprim and pyrimethamine.[8] So our SAR efforts focused on achieving greater DHFR selectivity via pyrimidine core replacement with other heterocycles. Unfortunately, any deviation from the pyrimidine template, such as 2,6-diaminopyridine, 3-aminopyrazole, 3,5-diamino[1,2,4]triazine, etc., led to complete abolishment of GHS-R activity (data not shown). In the meantime, modification of the 2-amino

Keywords: Ghrelin; GHS-R antagonist; Diaminopyrimidine; DHFR selectivity.
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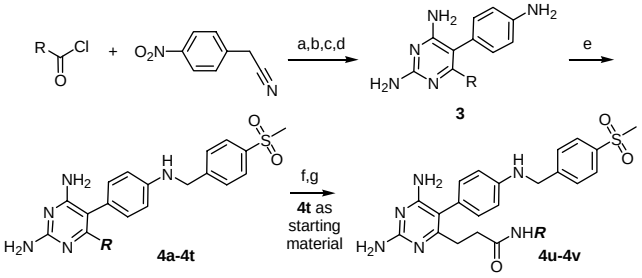
doi:10.1016/j.bmcl.2006.01.012

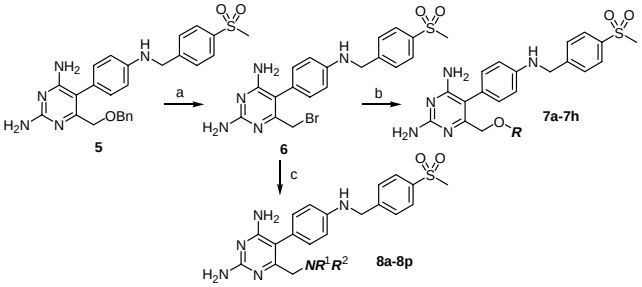
group of **2** to other groups (hydroxyl, methylamino, methyl, etc.) also resulted in inactive analogs (data not shown). In this letter, we will describe the SAR studies on the diaminopyrimidine 4- and 6-position side chain modification to achieve high ghrelin antagonist potency and good DHFR selectivity.

The diaminopyrimidine 6-position side chain modifications (**4a**–**4t**) were prepared in a similar fashion as described previously.[7] Briefly, 4-nitrophenyl-acetonitrile was first acylated with appropriate acid chlorides (Scheme 1). Subsequent methylation, cyclization with guanidine, and reduction of the nitro group yielded aniline **3**. Reductive amination of **3** with *p*-methylsulfonyl benzaldehyde provided the ghrelin antagonists **4a**–**4t**. Carboxamides **4u** and **4v** were synthesized from basic hydrolysis of ethyl ester in **4t**, followed by condensing the resulting acid with corresponding benzyl amines.

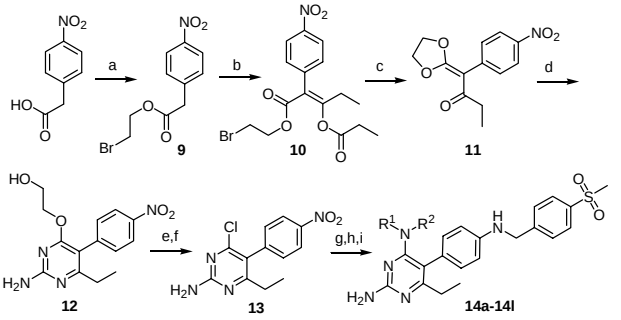
The 6-ether side chain modifications (**7a**–**7h**) were achieved starting from the previously described benzyl ether **5**.[7] Debenzylation and bromination of **5** generated benzyl bromide **6** (Scheme 2). A small amount of water in the reaction mixture prevented the formation of aniline acetamide during this process. Williamson ether synthesis using **6** with various alcohols provided 6-ether analogs **7a**–**7h**. Analogously, 6-aminoalkyl-substituted pyrimidines **8a**–**8p** could be synthesized through displacement of benzyl bromide **6** with different amines.



Scheme 1. Reagents and conditions: (a) Et_3N, DMAP, CH_2Cl_2, rt; (b) $TMSCHN_2$, CH_2Cl_2, rt; (c) guanidine, EtOH, reflux, 45–80% over three steps; (d) H_2, $Pd(OH)_2$, MeOH, 95%; (e) *p*-methanesulfonylbenzaldehyde, $NaBH_3CN$, MeOH/HOAc/NaOAc, rt, 70%; (f) LiOH, THF/MeOH, rt; (g) H_2NR, TBTU, *i*-Pr_2NEt, DMF, rt, 82% over two steps.



Scheme 2. Reagents and conditions: (a) 30% HBr/AcOH with 5% H_2O, reflux, 4 h, 72–93%; (b) ROH, NaH, THF, DMPU, rt, 12–25%; (c) HNR_1R_2, *i*-Pr_2NEt, THF, rt, 25–32%.



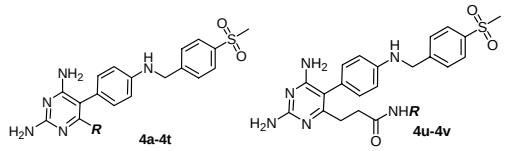
Scheme 3. Reagents and conditions: (a) 1—$SOCl_2$, 2—$BrCH_2CH_2OH$; (b) CH_3CH_2COCl, Et_3N, 0 °C-rt, 43% over 3 steps; (c) NaH, DMF, rt; (d) guanidine, EtONa, reflux, 70% over 2 steps; (e) TMSBr, dioxane, 150 °C; (f) $POCl_3$, 100 °C, 85% over 2 steps; (g) HNR_1R_2, dioxane, 170 °C, 20 min; (h) 10% Pd/C, MeOH, rt; (i) 4-methanesulfonylbenzaldehyde, $NaBH_3CN$, MeOH/AcOH/NaOAc, rt, ca. 35% over three steps.

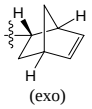
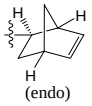
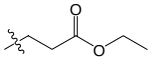
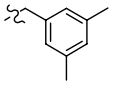
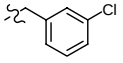
The 4-alkylaminopyrimidine analogs **14a**–**14l** were prepared as described in Scheme 3. Condensation of bromoethyl alcohol and an acid chloride made in situ from *p*-nitrophenyl acetic acid and thionyl chloride led to ester **9**. Acylation of **9** with propionyl chloride provided enol ester **10**. Treatment of **10** with NaH in DMF produced a dienolate intermediate, which underwent an intramolecular O-alkylation reaction followed by a facile cleavage of the enol ester to provide ketene acetal **11**. Condensation between **11** and guanidine gave pyrimidine **12**, which was converted to 4-chloropyrimidine **13** in a 2-step sequence. Reaction of chloropyrimidine **13** with a variety of primary and secondary amines gave 4-akylamino pyrimidines. Reduction of the nitro group, followed by a reductive amination step, produced final analogs **14a**–**14l**.

Speculating that the 6-ethyl group of diaminopyrimidine **2** might occupy the same binding site as the octanoyl group of ghrelin, we replaced the ethyl group of **2** with different lipophilic chains and found that this position is quite tolerant of different substitution. As shown in Table 1, both straight and branched aliphatic chain (3–8 carbons) substituents led to analogs (**4a**–**4h**) with low nanomolar binding IC_{50} values comparable to those of lead compound **2**.[9] Unfortunately, the longer aliphatic chains (**4f**–**4h**) did not provide any FLIPR potency boost as expected. Only 6-isopropyl diaminopyrimidine **4a** was 2-fold more potent in both binding and FLIPR assays than **2**. Consistent with the beneficial effect of α-branching, all the 6-cycloalkyl-substituted diaminopyrimidines (**4i**–**4l**) exhibited equal or a slightly better FLIPR activity. In contrast, 6-bicycloalkyl substituents led to compounds (**4m**–**4o**) with 2- to 8-fold-loss in FLIPR potency. Further functionalization of the aliphatic chain provided some highly potent analogs, such as propionyl benzyl amides **4u** and **4v**, both of which have single-digit nanomolar potency in FLIPR assay and sub-nanomolar binding IC_{50} for **4u**.

Previous SAR studies have illustrated the importance of the 6-benzyloxymethyl ether in **1** for maintaining ghrelin activity.[7] Not surprisingly, this ether in combination with 4-methylsulfonyl benzyl amine

Table 1. SAR of 6-alkyl-substituted diaminopyrimidines

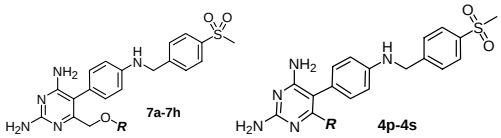


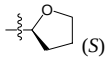
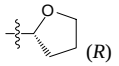
Compound	R	Binding IC_{50}[a] (nM)	FLIPR IC_{50}[a] (nM)
2	Ethyl	9.7	37
4a	Isopropyl	5.4	14
4b	*n*-Butyl	11	32
4c	Isobutyl	10	40
4d	*t*-Butyl	ND[b]	11
4e	*n*-Pentyl	19	211
4f	*n*-Hexyl	6.0	62
4g	*n*-Heptyl	6.0	38
4h	*n*-Octyl	5.2	57
4i	Cyclopropyl	ND[b]	19
4j	Cyclobutyl	ND[b]	14
4k	Cyclopentyl	ND[b]	21
4l	Cyclohexyl	ND[b]	36
4m[c]	 (endo & exo)	ND[b]	137
4n[c]	 (exo)	ND[b]	66
4o[c]	 (endo)	ND[b]	283
4t		ND[b]	82
4u		0.4	3.0
4v		2.0	5.4

[a] Values are averages of triplicate from one assay against human GHS-R.
[b] ND, not determined.
[c] A racemic mixture.

moiety generated compound **5**, one of the more potent GHS-R antagonists in both binding and FLIPR. Replacing the benzyl group of **5** with an isopentenyl group led to **7a** with sub-nanomolar binding potency and low nanomolar functional activity (Table 2). Saturation of the double bond led to **7b** with a 3- to 6-fold loss of potency, while the much shorter methyl ether **7c** was substantially weaker. Smaller furanylmethyl ethers (**7d**–**7e**) were equipotent in the FLIPR assay to **5**, but the more basic 3-pyridylmethyl ether (**7f**) was much less potent in the functional assay. In the meantime, cyclic ethers directly linked to the 6-position of diaminopyrimidine core were also investigated. Non-aromatic, cyclic tetrahydrofuran-substituted diaminopyrimidines **4p** and **4q** exhibited comparable FLIPR activity to that of **2**, without any chirality preference. When the phenyl ring was re-introduced to the 6-position side chain as dihydrobenzofuran, a slightly better FLIPR activity

Table 2. SAR of 6-cyclic ether/6-methyl ether-substituted diaminopyrimidines



Compound	R	Binding IC_{50}[a] (nM)	FLIPR IC_{50}[a] (nM)
5	Not applicable	0.3	7.1
7a		0.4	1.9
7b		2.8	6.8
7c	$-CH_3$	7.5	53
7d		2.7	9.6
7e		3.4	9.7
7f		38	187
4p	 (*S*)	ND[b]	54
4q	 (*R*)	ND[b]	57
4r[c]		ND[b]	38
4s[c]		ND[b]	180

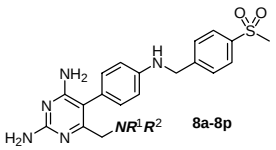
[a] Values are averages of triplicate from one assay against human GHS-R.
[b] ND, not determined.
[c] A racemic mixture.

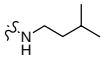
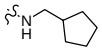
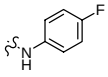
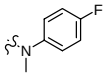
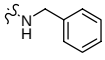
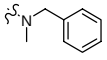
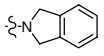
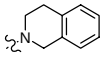
was observed with compound **4r**. The slightly larger dihydrobenzoxine-substituted **4s** fared much worse in the functional assay.

We also explored amino replacements for the 6-benzyloxymethyl ether side chain of **5**. In general, *N*-alkylaminomethyl-substituted diaminopyrimidines (**8a**–**8g**) were substantially less potent than the ether analogs in both binding and FLIPR, as illustrated by the direct comparison between amine **8d** and ether **7b** (Table 3). In contrast, when an *N*-arylaminomethyl group was introduced in the diaminopyrimidine 6-position, the resulting compounds (**8h**–**8k**) displayed large potency improvement. Particularly, both *sec*-amine **8h** and *tert*-amine **8i** showed single-digit nanomolar IC_{50}s in both binding and FLIPR assays. In comparison, *N*-benzylaminomethyl substituents (**8l**–**8m**) were 5- to 10-fold worse than the corresponding *N*-aryl analogs. Conformationally restricted amines, such as isoindoline, indoline, and tetrahydroisoquinoline, provided equipotent functional antagonists to compound **2**.

Previous SAR studies indicated that 2-amino group of the diaminopyrimidine core is critical for maintaining the intrinsic ghrelin antagonist activity. Using the chemistry developed in Scheme 3, we were able to examine substitu-

Table 3. SAR of 6-aminoalkyl-substituted diaminopyrimidines



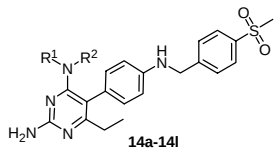
Compound	–NR^1R^2	Binding IC_{50}[a] (nM)	FLIPR IC_{50}[a] (nM)
8a	–NH_2	634	7160
8b	–$NH(CH_3)$	279	1380
8c	–$N(CH_3)_2$	186	2580
8d		61	1420
8e		65	945
8f		122	3430
8g		16	316
8h		5.5	9.1
8i		2.5	9.0
8j		1.5	12
8k		ND[a]	10
8l		96	49
8m		4	69
8n		ND[b]	34
8o		ND[b]	13
8p		ND[b]	41

[a] Values are averages of triplicate from one assay against human GHS-R.
[b] ND, not determined.

tions on the 4-amino group of the diaminopyrimidine ring. As shown in Table 4, only 4-methylaminopyrimidine **14a** has the same level of potency as **2** in both binding and FLIPR. Other larger substituents (**14c**, **14e**–**14k**) and N,N-disubstitution (**14b**, **14d**, and **14l**) resulted in substantial binding and FLIPR potency decreases. An unsubstituted amino group at 4-position of pyrimidine ring is necessary for optimal ghrelin antagonism.

As indicated earlier, 2,4-diaminopyrimidine is a privileged pharmacophore for DHFR inhibitors, and compounds **1** and **2** were found to be potent DHFR inhibitors. Therefore, the impact of diaminopyrimidine 4,6-position modification on DHFR selectivity was examined. As shown in Table 5, many of the preferred

Table 4. SAR of diaminopyrimidine 4-amino modification



Compound	R^1	R^2	Binding IC_{50}[a] (nM)	FLIPR IC_{50}[a] (nM)
14a	Me	H	37	69
14b	Me	Me	192	772
14c	Et	H	244	>10,000
14d	Et	Et	450	380
14e	*n*-Pr	H	151	237
14f	*n*-Bu	H	276	736
14g	*i*-Amyl	H	199	2480
14h	2-Hydroxyethyl	H	354	3080
14i	Cyclopropyl	H	601	>10,000
14j	Ph	H	700	>10,000
14k	Bn	H	248	354
14l	Morpholin-4-yl		3250	>10,000

[a] Values are averages of triplicate from one assay against human GHS-R.

Table 5. DHFR selectivity of representative diaminopyrimidine analogs

Compound	FLIPR IC_{50} (nM)	% hDHFR inhibition at 1 μM	% hDHFR inhibition at 10 μM
2	37	>99	>99
4d	11	47	87
4k	21	73	97
4l	36	16	50
4m	137	3	21
4n	66	ND[a]	38 *(55% inh. at 30 μM)*
4o	283	ND[a]	45
4p	54	ND[a]	25 *(38% inh. at 30 μM)*
4q	57	ND[a]	84
4u	3.0	82	93
7b	6.8	88	97
7g	38	34	93
8i	9.0	42	84
8p	41	29	58
14a	69	41	82

[a] ND, not determined.

6-position variations (**4d**, **4k**, **4u**, **7b**, and **8i**) led to minimal DHFR selectivity improvement.[10] However, a clear trend for achieving DHFR selectivity emerged from data shown in Table 5. When the diaminopyrimidine 6-position was substituted with a bulkier cyclohexyl group (**4l**), moderate DHFR selectivity improvement was observed. Further increase of the steric bulkiness of the cyclohexane ring with bicyclic norbornane (**4m**) and norbornene (**4n**) resulted in compounds with ca. 1000-fold separation of ghrelin and DHFR potencies. Equally encouraging, when the diaminopyrimidine 6-position was substituted with a (*S*)-tetrahydrofuranyl group, the resulting compound **4p** also exhibited ~1000-fold DHFR selectivity.

In summary, extensive SAR exploration has revealed that the 2,4-diaminopyrimidine core is optimal for

ghrelin antagonist activity, and the diaminopyrimidine 6-position is a key region for GHS-R potency improvement. Most importantly, 6-norbornenyl (**4n**) and 6-tetrahydrofuranyl (**4p**)-substituted diaminopyrimidines exhibited potent GHS-R antagonism with good selectivity (~1000-fold) against DHFR. Evaluation of ADME/PK properties of these analogs and further optimization of them in their DHFR selectivity could provide valuable tool compounds for elucidating the roles ghrelin plays in obesity.[11]

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9. In vitro (human GHS-R binding and FLIPR) assay were performed using the conditions described in Ref. 5a.
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