Bioorganic & Medicinal Chemistry Letters 20 (2010) 6103–6107

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Discovery and structure–activity relationship of a novel spirocarbamate series of NPY Y5 antagonists

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ARTICLE INFO

*Article history:*
Received 13 July 2010
Revised 6 August 2010
Accepted 7 August 2010
Available online 12 August 2010

*Keyword:*
NPY Y5

ABSTRACT

A novel series of *trans*-8-aminomethyl-1-oxa-3-azaspiro[4.5]decan-2-one derivatives was identified with potent NPY Y5 antagonist activity. Optimization of the original lead furnished compounds **23p** and **23u**, which combine sub-nanomolar Y5 activity with metabolic stability, oral bioavailability, brain penetration and strong preclinical profile for development. Both compounds significantly inhibited the food intake induced by a Y5 selective agonist with minimal effective doses of 3 mg/kg po.



Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter discovered in 1982.[1] A member of the pancreatic polypeptide family, it is abundant in both the central and the peripheral nervous systems[2] and it is implicated in a variety of physiological functions, notably the central regulation of feeding behavior and energy homeostasis.[3]

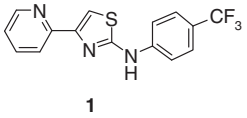
The actions of NPY are mediated through five G-protein coupled receptors (Y1, Y2, Y4, Y5, y6).[4] A number of pharmacological studies conducted with transgenic mice and/or sub-type selective agonists and antagonists have suggested that the Y5 receptors are involved in the regulation of food intake.[5] Additionally, small molecule Y5 antagonists have proven efficacious in animal models of addiction to drugs of abuse[6] and in animal models of depression.[7] To date, clinical studies reported with small molecule Y5 antagonists have demonstrated modest, albeit significant, anti-obesity effects in obese human subjects.[8] However, considering the potential implication of NPY Y5 in regulating addiction and depression there is ample scope for Y5 antagonists to offer greater efficacy in psychiatric eating disorders with stress and/or compulsive/impulsive components. Hence, antagonism of the Y5 receptor represents an attractive pharmacological approach with potential therapeutic applications for the treatment of eating disorders, drug dependency and depression.

We report here our efforts to identify a proprietary series of Y5 antagonists that led to the discovery of a novel spirocarbamate scaffold, endowed with potent Y5 antagonist activity and a strong preclinical profile for development.

A screening campaign of our corporate compound collection generated a number of promising chemotypes from which the aminothiazolepyridine **1** was identified as an efficient, orally bioavailable and brain penetrant Y5 antagonist (Fig. 1).

A direct comparison of compound **1** with the structures of known Y5 antagonists[9] revealed some recurrent pharmacophoric features and suggested that the opportunity to further optimize this molecule lay with the modification of the *N*-phenyl side chain. Accordingly, a number of prospective compound arrays were prepared from commercially available 4-(2-pyridinyl)-1,3-thiazol-2-amine **2**. From amongst the derivatives synthesized, attention was drawn to the spirocyclic urea derivative **3**, which was characterized by potent Y5 antagonist activity and promising in vitro criteria for development (Fig. 2).

It is worthy to note that a number of highly potent NPY Y5 antagonists containing other spiropiperidine substructures have




**1**

hNPY Y5 fpKi 7.8
hNPY Y1/2 fpKi<4.5
CYP450 1A2 <0.1 uM
$Cl_b$ 27 ml/min/kg
F= 30%
B/B 4.2

**Figure 1.** Structure of hit compound **1**.

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doi:10.1016/j.bmcl.2010.08.041

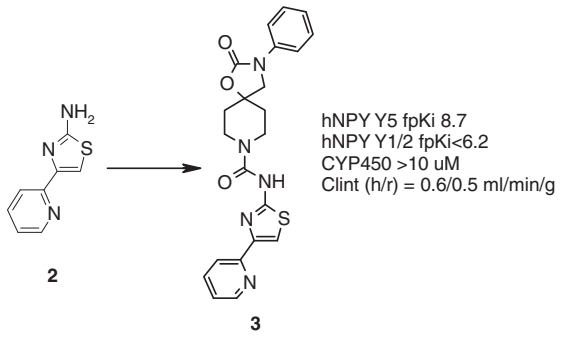



**Figure 2.** Structure of lead compound **3**.

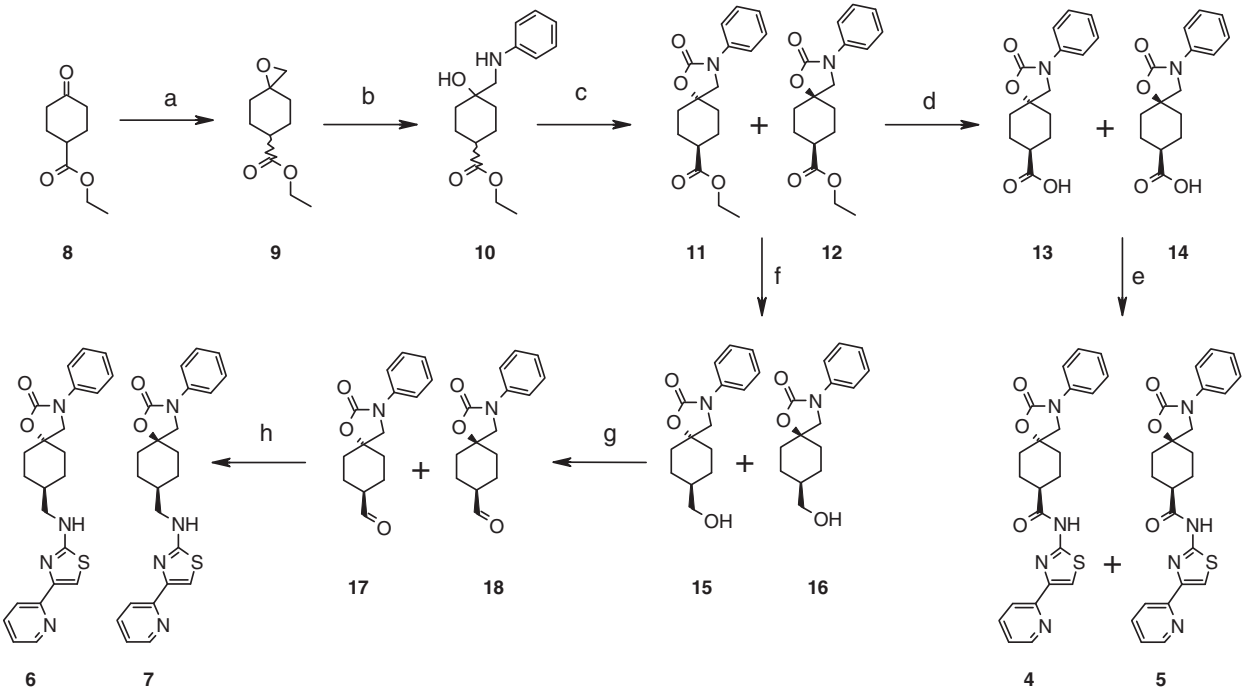
been disclosed in the literature[10] suggesting that such a spirocyclic system is particularly efficacious in presenting the relevant pharmacophoric features of the molecules in a suitable orientation for interaction with the receptor.

Considering its attractive in vitro profile, compound **3** was further characterized for its pharmacokinetic profile in rat.[11] In agreement with its low in vitro intrinsic clearance ($Cl_{int}$ = 0.5 ml/min/g liver) compound **3** showed low blood clearance ($Cl_b$ = 9 ml/min/kg) and high bioavailability (*F* = 49%) but unfortunately it did not exhibit any appreciable brain penetration (B/B <0.1). Hypothesizing that the low brain penetration may have been a consequence of the high polar surface area (PSA) of **3** (PSA = 88), the initial optimization strategy focused on lowering PSA via removal of heteroatoms. Initial attempts to replace the oxazolidinone ring with less polar five membered heterocycles led to a dramatic loss of Y5 activity (data not shown) suggesting that the carbamate enters into crucial pharmacophoric interactions with the Y5 receptor. Attention was therefore focused on the urea linker, which proved more amenable to modification. The chemistry used to access the desired analogs will be described next. The initial synthetic route used to access the amide and amine analogues **4**–**7** is outlined in Scheme 1.

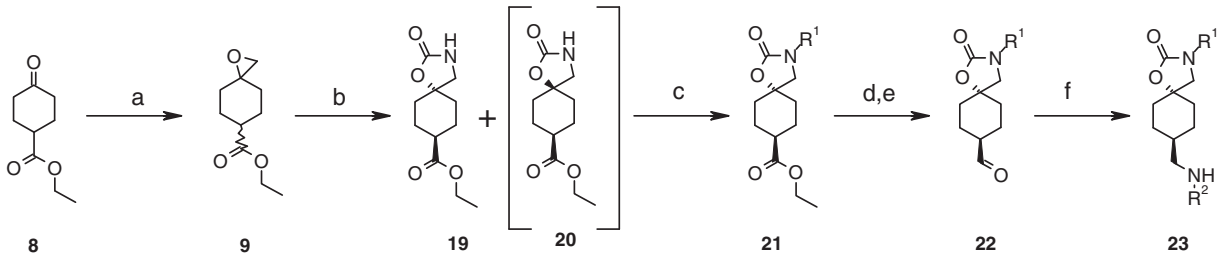
Commercially available ethyl 4-oxocyclohexanecarboxylate (**8**) was reacted with a trimethylsulfoxonium iodide derived ylide to give an inseparable diastereoisomeric mixture of epoxides **9** that favoured the *cis* diastereoisomer (*cis*/*trans* ratio ~85:15). The mixture of epoxides was opened with an excess of aniline to give a similar diastereomeric mixture of the β-hydroxyanilines **10**, that was subsequently cyclized with triphosgene at low temperature to give, after chromatographic separation on silica gel, the *trans* and *cis* spirocarbamates **11** and **12**. Ester saponification and amide coupling gave the *trans* and *cis* amide derivatives **4** and **5**, respectively.

Alternatively, ester reduction with lithium aluminium hydride followed by oxidation with Dess–Martin periodinane gave the *trans* and *cis* aldehydes **17** and **18** which underwent reductive amination to give the *N*-alkylated derivatives **6** and **7**, respectively. The amide derivatives **4** and **5** were found to be poorly active at Y5 (fpKi = 6.8 and <5.3, respectively) but the *N*-alkylated derivates **6** and **7** showed excellent target activity (fpKi = 10.0 and 8.8, respectively). In both pairs of products a notable preference was evident for the *trans* diastereoisomer.

Bolstered by this result, the potent *trans N*-alkyl derivative **6** was progressed into rat pharmacokinetic studies, where it was found to have moderate blood clearance ($Cl_b$ = 47 ml/min/kg) and moderate bioavailability (*F* = 19%) but more importantly a greatly improved brain penetration (B/B = 1.2) compared to **3**. Before embarking on a full blown lead optimization campaign around lead **6** it was necessary to develop an alternative synthetic sequence giving more efficient access to the more potent *trans* diastereoisomer (Scheme 2). Improved diastereoselectivity was achieved by exploiting the known propensity of trimethylsulfonium derived ylides to attack cyclohexanones with axial methylene transfer,[12] leading to a complementary stereoselectivity with respect to the trimethylsulfoxonium derived ylide used in our initial synthesis. In this specific case, the choice of base employed to generate the ylide proved fundamental. Several bases promoted the reaction, all giving epoxide **9** with ~60:40 *trans*/*cis* diastereoselectivity, but high yields were only observed with a Verkade nonionic super-




**Scheme 1.** Reagents and conditions: (a) $Me_3SOI$, tBuOK, DMSO, rt; (b) $PhNH_2$, *t*BuOH, 150 °C, microwave irradiation; (c) triphosgene, $Et_3N$, $CH_2Cl_2$, −50 °C; (d) LiOH, MeOH, rt; (e) amine **2**, HOBt, EDC, MeCN, rt; (f) $LiAlH_4$, THF, −20 °C; (g) Dess–Martin periodinane, $CH_2Cl_2$, rt; (h) amine **2**, $TiCl(OiPr)_3$, $CH_2Cl_2$, 0 °C to rt, then $NaBH(OAc)_3$, AcOH.




**Scheme 2.** Reagents and conditions: (a) $Me_3SI$, 2,8,9-triisobutyl-2,5,8,9-tetraaza-1-phosphabicyclo[3.3.3]undecane, MeCN, rt; (b) ethyl carbamate, *t*BuOK, DMPU or DMF, 130 °C; (c) $R^1Br$ or $R^1I$, CuI, *trans*-1,2-diaminocyclohexane, $Cs_2CO_4$ or $K_3PO_4$, toluene or 1,4-dioxane, 80–120 °C; (d) $LiAlH_4$, THF, −20 °C; (e) Dess–Martin periodinane, $CH_2Cl_2$, rt; (h) amine $R^2NH_2$, $Ti(OiPr)_4$, $CH_2Cl_2$, rt, then $NaBH_4$.

base whereas inorganic bases such as NaH and *t*BuOK gave low yields. A convenient one step formation of the oxazolidinone ring using ethyl carbamate and *t*BuOK was preferred as it afforded a more convergent sequence and hence facilitated SAR exploration. After chromatographic separation from the *cis* diastereoisomer **20**, the desired spirocarbamate intermediate **19** was arylated via an Ullmann–Goldberg type coupling to give **21**. The synthetic route was completed with similar functional group modification and reductive amination as before. When opportune the order of the steps could be modified, postponing the Ullmann–Goldberg coupling to the end of the sequence. Furthermore, in some instances the amine $RNH_2$ utilized for the reductive amination step was a halogenated arylamine, which was subjected to an additional Suzuki coupling with an appropriate boronic acid to prepare the final compounds.

The optimization of lead **6** was initially divided into two parallel explorations (i) substitution on the pyridinethiazole biaryl portion or replacement with alternative biaryl groups (ii) substitution on the N-phenyl ring or replacement of the phenyl with isosteric heterocycles. In actual fact, the biaryl exploration was performed with a 2-pyridyl on the carbamate as the analogue **23a** showed identical activity at Y5 and similar pharmacokinetics to **6** but presents a much preferable lipophilicity (*c* log *P* = 2.9 vs 4.4).

A representative selection of compounds prepared during the explorations is shown in Tables 1 and 2.

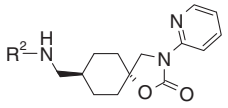
Examples of simple substitution, such as methylation or fluorination, of the pyridinethiazole are not shown as they did not lead to any appreciable improvement in the CYP450 inhibition profile or the protein binding—the unbound fraction in the brain ($Fu_{br}$) was deemed an important parameter to increase during the optimization process as experience gained previously across multiple leads series for numerous CNS targets has taught that pharmacodynamic efficacy most closely correlates to the free concentration of test compound in the brain. A selection of alternative biaryl groups was identified that maintain high target potency; of these **23b**, **23e** and **23i** offered somewhat improved CYP450 profiles and/or higher $Fu_{br}$.

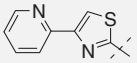
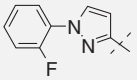
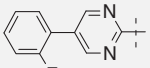
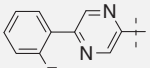
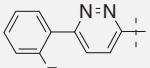
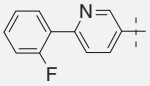
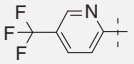
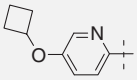
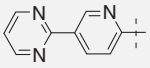
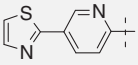
The exploration of the carbamate *N*-aryl group was undertaken with the phenylpyrazole biaryl group from **23b**. Again, in this case simple substitution was unproductive (data not shown) and again a wide variety of heterocycles were found to be tolerated by the target receptor (Table 2). Modification of this part of the molecule proved especially effective in improving the CYP450 profile and the $Fu_{br}$. In particular, derivative **23p** presented an in vitro profile suitable for progression into in vivo studies.

Building on the learning's from the initial exploration, further iterative rounds of optimization were conducted.

Indeed, the propensity of the 3-pyridazinyl substituent to simultaneously improve CYP450 and $Fu_{br}$ was confirmed with other biaryl groups (Table 3) and a number of further derivatives suitable for progression into in vivo studies (**23t–x**) were identified.

**Table 1**
NPY Y5 activities,[a] CYP450 inhibition,[b] *c* log *P*[c] and $Fu_{br}$[d] of *N*-pyridyl derivatives **23a–j**



| Compds | $R^2$ | NPY Y5 fpKi[a] | CYP450 Inh. (μM)[b] | *c* log *P*[c] | $Fu_{br}$[d] (%) |
|---|---|---|---|---|---|
| **23a** |  | 10.1 | >10/3/6/>10/3 | 2.9 | 1.0 |
| **23b** |  | 10.5 | >10/4/2/>10/ >10 | 3.4 | — |
| **23c** |  | 9.5 | 6/4/1/>10/5 | 3.5 | <1.0 |
| **23d** |  | 10.6 | >10/2/0.3/>10/ >10 | 3.7 | 0.2 |
| **23e** |  | 10.2 | 5/2/1/2/>10 | 3.6 | 1.6 |
| **23f** |  | 8.3 | —/9/1/2/9 | 4.3 | — |
| **23g** |  | 9.0 | >10/5/2/>10/ >10 | 3.2 | 0.5 |
| **23h** |  | 9.8 | >10/7/2/2/3 | 3.4 | — |
| **23i** |  | 9.8 | >10/10/5/>10/ >10 | 1.9 | 3.5 |
| **23j** |  | 9.9 | >10/3/0.3/1/7 | 2.7 | — |

[a] The functional activity (fpKi) at the human NPY Y5 receptor stably expressed in HEK293 cells was assessed using FLIPR/$Ca^{2+}$ methodology in a 384 well format. Each determination lies within 0.3 log units of the mean with a minimum of two replicates.

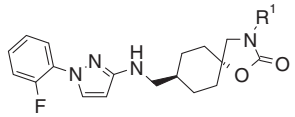
[b] CYP450 inhibition potential at 1A2/2C19/2C9/2D6/3A4 isoforms, respectively, determined in Cypex bactosomes.

[c] Calculated octanol/water partition coefficient using Daylight® 4.81 software.[13]

[d] Fraction unbound determined in an equilibrium dialysis assay using homogenized rat brain expressed as a percentage.

Intrinsic clearance ($Cl_{int}$) values for rat and human, and pharmacokinetic parameters from in vivo cassette studies in rat for compounds **23a,p,t,u,x** are illustrated in Table 4. Blood clearance was

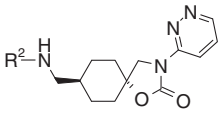
**Table 2**
NPY Y5 activities,[a] CYP450 inhibition,[b] *c* log *P*[c] and $Fu_{br}$[d] of aminopyrazole derivatives **23k–r**

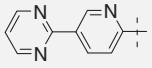
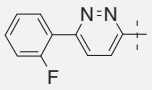
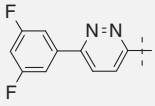
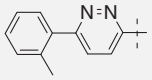
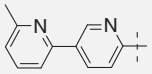
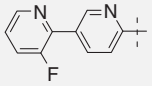


| Compds | $R^1$ | NPY Y5 fpKi[a] | CYP450 Inh. (μM) [b] | *c* log *P*[c] | $Fu_{br}$[d] (%) |
|---|---|---|---|---|---|
| **23k** | 3-Pyridinyl | 10.0 | >10/8/7/>10/1 | 3.3 | 2.5 |
| **23l** | 2-Methyl-3-pyridinyl | 9.2 | All >10 | 3.8 | — |
| **23m** | 5-Pyrimidinyl | 10.4 | >10/6/>10/>10/2 | 2.4 | 4.4 |
| **23n** | 2-Fluoro-3-pyridinyl | 10.0 | >10/8/6/>10/5 | 3.6 | 1.9 |
| **23o** | 2-Pyrazinyl | 10.2 | >10/2/3/2/0.6 | 2.4 | 1.7 |
| **23p** | 3-Pyridazinyl | 10.0 | All >10 | 2.1 | 3.1 |
| **23q** | 4-Pyridazinyl | 9.7 | >10/>10/>10/>10/6 | 2.1 | 4.4 |
| **23r** | 5-Methyl-1,3,4-thiadiazol-2-yl | 9.7 | >10/>10/>10/>10/6 | 2.6 | 2.6 |

[a,b,c,d] See Table 1.

**Table 3**
NPY Y5 activities,[a] CYP450 inhibition,[b] *c* log *P*[c] and $Fu_{br}$[d] of *N*-pyridazinyl derivatives **23s–x**



| Compds | $R^2$ | NPY Y5 fpKi[a] | CYP450 Inh. (μM) [b] | *c* log *P*[c] | $Fu_{br}$[d] (%) |
|---|---|---|---|---|---|
| **23s** |  | 8.8 | All >10 | 0.7 | — |
| **23t** |  | 9.8 | All >10 | 2.3 | 4.6 |
| **23u** |  | 10.0 | >10/10/8/>10/>10 | 2.5 | 1.6 |
| **23v** |  | 10.2 | All >10 | 2.4 | 5.1 |
| **23w** |  | 9.6 | All >10 | 2.1 | 5.6 |
| **23x** |  | 9.5 | All >10 | 1.8 | 5.2 |

[a,b,c,d] See Table 1.

moderate except for the aminopyridine derivatives **23u,x**, which showed higher clearance. Oral bioavailability was good to excellent in all cases however brain penetration varied widely. The low brain/blood ratio observed with compound **23t** was verified to be a consequence of P-glycoprotein (P-gp) efflux (B/B increased five-fold when a P-gp inhibitor was co-administered), but this issue could be mitigated through minor structural modifications giving compound **23u** which has a good brain/blood ratio.

Compounds **23p** and **23u** emerged as potential preclinical candidates and were subjected to further characterization. Comprehensive selectivity screening performed within GSK and at CEREP[14] (General Safety Profile) evidenced an excellent selectivity

**Table 4**
Selected DMPK parameters[a] for compounds **23a**,**p**,**t**,**u**,**x**

| Compds | $Cl_{int}$ r/h[b] (ml/min/g liver) | $Cl_b$ (ml/min/kg) | F% Rat | B/B rat | PD model MED[c] (mg/kg po) |
|---|---|---|---|---|---|
| **23a** | 3.1/4.0 | 30 | 35 | 0.7 | 10 |
| **23p** | <0.5/<0.5 | 13 | 52 | 0.9 | 3 |
| **23t** | <0.5/<0.5 | 18 | 90 | 0.25 | — |
| **23u** | <0.5/<0.5 | 41 | 75 | 1.6 | 3 |
| **23x** | —/— | 70 | 50 | 0.4 | — |

[a] In vivo data determined by 0.5 mg/kg iv and 1 mg/kg po administration in rat. Brain/Blood ratio (B:B) determined from $AUC_{0-\infty}$ following po dosing.
[b] Intrinsic clearance values ($Cl_{int}$) expressed as ml/min/g liver were determined in human (h) and rat (r) liver microsomes.
[c] Minimal effective dose in NPY Y5 agonist driven pharmacodynamic model in rat.

of at least 1000-fold against all of the ~200 targets tested. Additionally, both derivatives demonstrated potent in vivo antagonist activity in a pharmacodynamic model, each reversing the food intake induced by icv administration of 0.6 nmol of the NPY Y5 selective agonist, [cPP1-7,NPY19-23,Ala31,Aib32,Gln34]hPP, to male rat; **23p** and **23u** caused similar dose-dependent reductions of food intake with minimal effective doses of 3 mg/kg po.

In conclusion, a novel series of highly potent, functional NPY Y5 antagonists has been prepared based around a spirocarbamate scaffold. An extensive SAR exploration allowed the shortcomings of the original lead to be addressed and led to the identification of compounds **23p** and **23u** which have been progressed into preclinical disease models and toxicological studies which will be the subject of a separate publication.

## Acknowledgements

The authors thank Dr. Carla Marchioro and members of the Analytical Chemistry department, Verona for support in the analytical characterization of the compounds described. The authors also thank Francesca Graziani and Alberto Buson of Molecular Discovery Research, Verona for support in the in vitro characterization of the compounds described.

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