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Novel Potent Antagonists of Human Neuropeptide Y Y5 Receptor. Part 1: 2-Oxobenzothiazolin-3-acetic Acid Derivatives

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Abstract—Novel NPY-Y5 antagonist **FR73966** was discovered by screening of our in-house chemical library. The analogues were prepared by application of parallel synthesis techniques. Some of the resulting 2-oxobenzothiazolin-3-acetic acid derivatives exhibited nanomolar binding affinity for human NPY-Y5 receptors.

Neuropeptide Y (NPY) is a 36 amino acid peptide that was first isolated from porcine brain[1] and is found abundantly in the central and peripheral nervous system.[2,3] NPY is involved in a number of physiological responses, such as food intake,[4–6] blood pressure regulation,[7,8] hormone secretion,[9] sexual behavior,[10] and circadian rhythm[7] and is implicated in the pathophysiology of several disorders. For example, it has been reported that chronic injection of NPY in rats leads to severe overeating resulting to the development of obesity.[6] Obesity is becoming a major health problem in advanced nations and even mild obesity enhances the risk of premature death, hypertension, diabetes mellitus, hyperlipidaemia, atherosclerosis, coronary heart disease, arthritis, sleep apnea and certain types of cancer.[11,12] A strong association between obesity and non-insulin dependent diabetes mellitus (NIDDM) has been claimed, and more than 80% of NIDDM patients are known to be clinically obese.[13] It is known that NPY-Y1 and -Y5 receptor subtypes in already cloned five different receptor subtypes are activated according to centrally mediated NPY-induced feeding responses.[14–17]

Recently, it has been reported that compounds which antagonize the Y5 receptor, for example CGP 71683A,[18] Synaptic Pharm's tetralin derivative **1**,[19] and α-substituted-β-aminotetralin **2**[20] (Fig. 1) is reported to be effective in reducing food intake in *ob*/*ob* mice and Zucker obese rat models.[21]

Consequently, we initiated a program to identify a novel chemical entity possessing Y5 receptor antagonistic activity for the treatment of obesity and eating disorders.

By screening of our in-house chemical library, we discovered 5-chloro-2-oxobenzothiazolin-3-acetic acid derivative **3** (**FR73966**) (Fig. 2), which showed high affinity ($IC_{50} = 53$ nM) for human Y5 receptors. This compound was attractive owing to the simple and novel

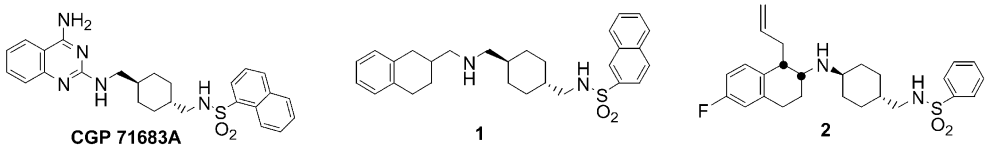

Figure 1.

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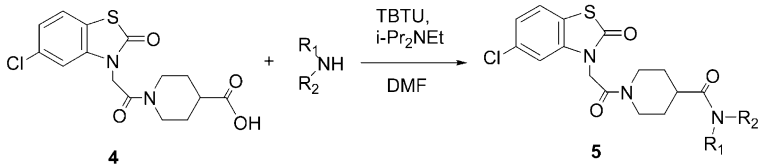
3. FR73966 : hY5r : IC_{50}=53 nM

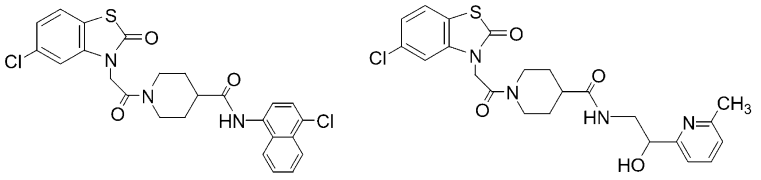
5a. FR235656 : hY5r : IC_{50}=4.4 nM

Figure 2.



4 **5**

Scheme 1.



FR236478 : hY5r : IC_{50}=0.23 nM

FR235208 : hY5r : IC_{50}=3.3 nM

Figure 3.

chemical structure, and the preparation of its analogues might be possible by application of parallel synthesis techniques using amide formation reactions with many kinds of amines.

This letter describes the terminal amide formation by parallel synthesis techniques and the structure–activity relationships of the products in terms of potency as Y5 receptor antagonists.

We prepared various new derivatives **5** by reacting acid **4**, obtained easily by reaction of 5-chloro-2-oxobenzothiazolin-3-acetic acid with isonipecotinic acid ester followed by alkali hydrolysis, with a variety of amines, as shown in Scheme 1.

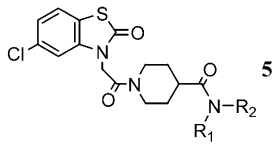
The prepared derivatives were obtained as described below. Each reaction mixture was used directly for the assay after dilution with DMSO, because the yields in this amide formation were high and none of the starting materials, reagents and solvent DMF showed affinities for Y5 receptors.

1. Preparation of a 0.1 M solution of **4** in DMF for use in *n* parts, each containing 0.05 mM of **4**
2. Addition of TBTU (0.05 mM×1.5 equiv×*n*)
3. Addition of *i*-Pr_2NEt (0.05 mM×1.5 equiv×*n*)
4. Stirring for 1 h at ambient temperature
5. Separation of the obtained activated acid solution into *n* test tubes equipped with a sealed cap by using an Eppendorf pipetter
6. Addition of a 0.5 M solution of the desired amine in DMF (0.11 mL/0.05 mM×1.1 equiv) into the test tubes
7. Heating each mixture at 50 °C overnight
8. Identification of the products by thin-layer chromatography and mass spectrometers
9. Transfer of each reaction mixture into a 96-well plate, dilution of each to 10^{-2} M with DMSO and preservation in a refrigerator
10. Binding tests for Y5 receptor were performed after resolution of the preservated samples and further dilution with DMSO to $10^{-6}\sim10^{-10}$ M concentrations.

About 300 derivatives were prepared by this parallel synthesis technique and compounds which showed IC_{50} values less than 10 nM were synthesized by manual methods separately for confirming evaluation. The structures and biological evaluation results of the products in terms of potency as Y5 receptor antagonist are shown in Table 1, accompanied with those of manual synthesis products.

Unsubstituted anilide (**5a**) (Fig. 2) was more potent affinity for human Y5 receptors than the lead **FR73966**. Other primary anilides and naphthilides (**5b**–**5e**) showed also potent affinities but secondary cyclic anilides (**5f**, **5g**) were found to be weak. Furthermore, 4-substituted anilines, even when the substituents were hydrophilic or lipophilic, were more effective than the sterically hindered 2-substituted anilines (**5h**, **5i** > **5j**, **5k**). Meanwhile, heteroaromatic primary amides (**5l**–**5n**) generally showed potent affinities. In the case of using 4-methoxybenzyl, 4-sulfamoyllphenethyl, 2-phenylethanol amines, the primary amide (**5o**–**5q**) were also found to have high affinities, but secondary amides (**5r**, **5s**), prepared with even a benzyl or a phenethyl type amines, showed little affinities, as similar to the structure–activity relationships of the anilides. It is conceivable that there is a

Table 1. Structure–activity relationships of **5**



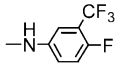
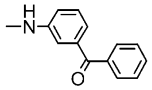
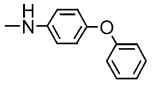
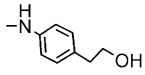
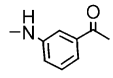
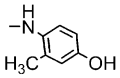
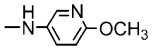
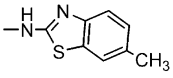
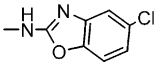
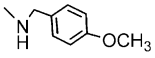
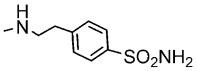
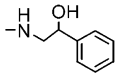
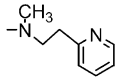
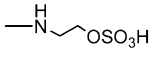
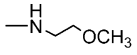
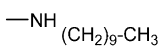
Compd		Inhibition % at				IC_{50} (nM)[a]
		10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	
5b		108.3	99.0	83.4	49.3	0.71[b] (17.0)[c]
5c		103.8	97.7	93.2	52.6	0.70[b] (0.59)[c]
5d		102.3	101.2	80.5	45.3	1.4[b] (0.89)[c]
5e		97.2	99.4	94.5	53.2	0.85[b] (1.5)[c]
5f		51.4	59.8	−3.5	−4.5	—
5g		43.1	ND	43.1	ND	—
5h		84.7	101.3	74.6	28.7	2.9[b] (1.7)[c]
5i		100.5	98.5	76.8	41.5	2.0[b] (0.59)[c]
5j		95.0	91.4	41.1	5.6	1.4[b] (4.7)[c]
5k		88.8	67.8	−5.3	−16.9	—
5l		94.0	88.5	70.3	29.9	2.6[b] (2.3)[c]
5m		107.0	86.6	68.1	31.9	2.8[b] (10.0)[c]
5n		86.2	101.7	68.4	16.5	4.7[b] (3.8)[c]
5o		98.0	105	61.6	11.5	1.4[b] (2.3)[c]
5p		102.0	88.8	44.7	19.5	9.0[b] (6.1)[c]
5q		107.9	97.1	79.4	47.8	1.1[b] (1.2)[c]
5r		77.2	15.6	ND	ND	—

Table 1 (*continued*)

Compd		Inhibition % at				IC_{50} (nM)[a]
		10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	
5s		87.9	−3.9	ND	ND	—
5t		19.9	ND	ND	ND	—
5u		ND	14.5	ND	ND	—
5v		ND	106.2	89.6	ND	2.6[b] (1.3)[c]

[a]Concentration of compound that inhibited 50% of total specific binding of ^{125}I-PYY as a ligand to HEK 293 cells stably transfected with the human NPY-Y5 cDNA; obtained from the mean value of two experiments at each concentration (10^{-6}–10^{-10} M).
[b]IC_{50} value of compound prepared by parallel synthesis technique.
[c]IC_{50} value of compound prepared by manual synthesis method.

hydrogen bond interaction between Y5 receptor and –CONH moiety of the ligand. Several compounds, amidated with aliphatic amines possessing a sulfonic acid or a methoxyethyl moiety (**5t**, **5u**), showed little affinity for Y5 receptors. The other type of compounds, amidated with an amine containing an alkyl long chain, which are considered to have sufficient lipophilicity (**5v**), were surprisingly found to have potent affinities.

The most potent compound amongst those we prepared was **FR236478** (Fig. 3), with an IC_{50} value of 0.23 nM. Unfortunately, this compound was poorly absorbed by po administration. We speculated that the poor absorption is due to its low solubility in water by high lipophilicity.

FR235208 (Fig. 3) showed one tenth potency ($IC_{50} = 3.3$ nM) when compared with **FR236478**. Nevertheless it was sufficiently absorbed from a judgment of its plasma concentration ($C_{max} = 15.7$ μg/mL). But its poor permeability to brain was shown by *exo vivo* binding assay when orally administered at 100 mg/kg to rats.

Summary

Many 2-oxobenzothiazolin-3-acetic acid derivatives were prepared by parallel synthesis techniques around **FR73966**, novel structural lead compound discovered by screening of our in-house chemical library.

They showed potent antagonistic activity for human NPY-Y5 receptors. Some of them showed nanomolar binding affinities. Nevertheless, we are continuing our search for improved compounds, because the derivatives described in this letter have poor absorption and permeability to brain when orally administered.

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