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Synthesis and evaluation of new hydrazide derivatives as neuropeptide Y Y_5 receptor antagonists for the treatment of obesity

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Abstract—NPY is the most potent orexigenic agent known to man, with NPY Y1 and NPY Y5 being the receptor subtypes that are most likely responsible for centrally-mediated NPY-induced feeding responses. Based on the aforementioned, novel hydrazide derivatives were prepared for the purpose of searching new NPY Y5 receptor antagonists. Many of the compounds exhibited nanomolar binding affinity for this receptor, affording *trans*-*N*-{4-[*N*′-(3,4-dichlorophenyl)hydrazinocarbonyl]cyclohexylmethyl}-4-fluorobenzenesulfonamide, which showed the best activity (IC_{50}=0.43 nM).

1. Introduction

Obesity is now 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.[1] The exact aetiology of obesity still remains unclear although it appears to be caused mainly by a combination of genetic factors, inappropriate eating, and reduced activity.[2]

Neuropeptide Y (NPY), a 36 amino acid peptide was first isolated and sequenced from porcine brain by Tatemoto et al. in 1982 and belongs to a family of structurally related peptides that includes pancreatid polypeptide (PP) and peptide YY (PYY).[3]

This peptide is one of the most potent feeding stimulating hormones known that can be found in both the peripheral and central nervous system. In the peripheral nervous system, neuropeptide Y is located in postganglionic sympathetic neurons, adrenal medulla, enteric neurons, cardiac nonsympathetic neurons, certain nonadrenergic perivascular neurons and parasympathetic neurons. In sympathetic neurons and adrenal medulla, the peptide is colocalized with the classical sympathetic neurotransmitter noradrenaline. In the brain, neuropeptide Y-containing neuronal cell bodies are found primarily in the locus coeruleus, the nucleus of the solitary tract and the arcuate nucleus of the hypothalamus. In addition, these neuropeptide Y-containing neuronal cell bodies often contain other neurotransmitters, such as noradrenaline, and send projections throughout the brain; hence, neuropeptide Y can be found in most brain regions, particularly in the cortex, hippocampus, thalamus, hypothalamus and brainstem. The physiological effects of NPY are mediated by a series of NPY receptor subtypes (Y_1, Y_2, Y_4, Y_5 and Y_6) that are members of the G-protein coupled receptor (GPCR) family. It is assumed that NPY regulates a variety of physiological processes, including vasoconstriction, nasal congestion, blood pressure, intestinal motility, anxiety, depression, pain, feeding, reproductive endocrinology, neuronal excitability and memory retention. For this reason it is though that NPY receptor-specific ligands may ultimately have value in several therapeutic areas including the treatment of obesity.[4,5] The receptor most likely subypes responsible for centrally-mediated NPY-induced feeding responses are

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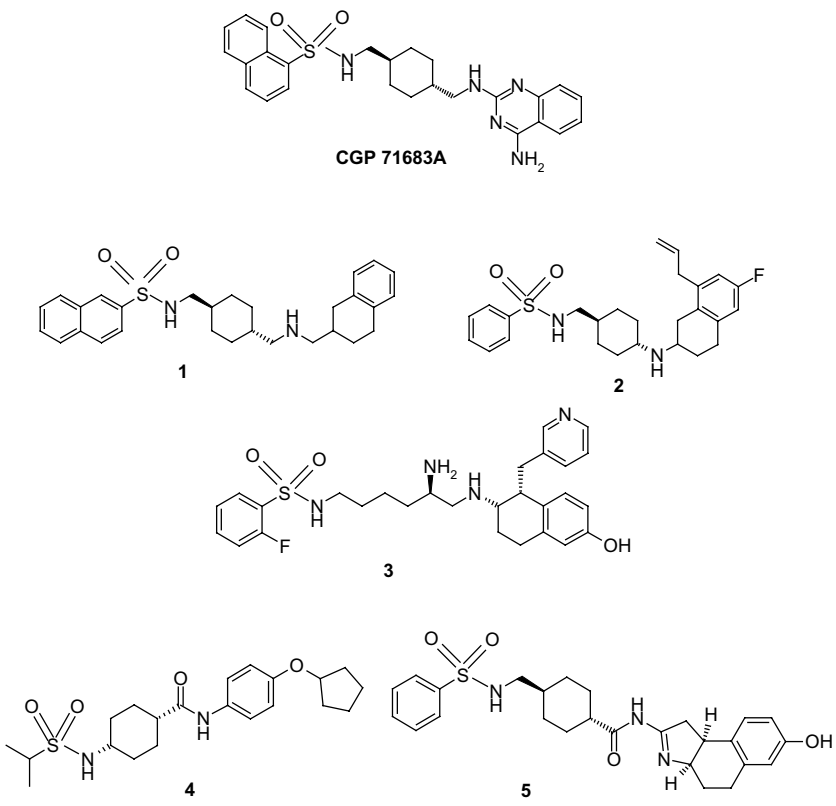


Figure 1. Antecedents.

NPY Y_1 and NPY Y_5. Antagonists at the NPY Y_5 receptor have been shown to be effective in reducing food intake in various animal models of feeding.[6,7]

Recently, it has been reported that there are many diverse structural types (Fig. 1), which antagonize the NYP Y_5 receptor and are considered potential antiobesity agents due to their notable in vitro activity. Compounds such as CGP71683A, Synaptic Pharm's tetralin derivative **1**, α-substituted-β-aminotetralin derivative **2**, **3**, **4** and **5** compounds antagonize the Y5 receptor and are reported to be effective in reducing food intake in *ob*/*ob* mice and Zucker obese rat models.[6,8–10]

Consequently, we attempted to discover a novel compound that possesses antagonist activity on the Y_5 receptor. This led us to synthesize new hydrazide derivatives, all of which have a common arylsulfonamidomethylcyclohexyl nucleus, due to the fact that the sulfonamide group and the cyclohexyl linker were found to be essential in the concession of biological activity.[11]

2. Chemistry

The aforementioned compounds were prepared according to the synthetic route illustrated in Scheme 1.

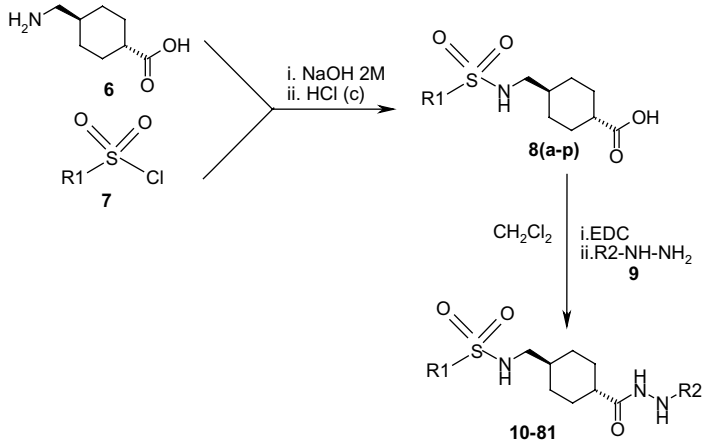
Hydrazide derivatives **10**–**81** were obtained by the formation reaction of the primary sulfonamides **8** (Table 1), which consisted of a nucleophilic attack of amines **6** that was carried out against sulfonyl chlorides **7**,[12] followed by a subsequent acylation of the different hydrazine **9** derivatives previous activation of the carboxylic group with carbodiimide 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide HCl (EDC).[13]

The structures and biological evaluation results of the different hydrazide derivative compounds in terms of potency as Y5 receptor antagonists are shown in Table 2.

All of the compounds were chemically characterized by thin layer chromatography (TLC), melting point, infrared, nuclear magnetic resonance (^{1}H NMR), elemental microanalysis and HPLC.

3. Pharmacology

Binding assays for both receptors, NPY Y_1 and NPY Y_5, were carried out as described by Duhault et al.[14] In brief, for the human Y_1 receptor binding assay, using iodinated Peptide YY (NEN), incubations were performed at 30 °C for 90 min with various competitor concentrations in Buffer A (Hepes/NaOH 20 mM, pH 7.4, NaCl 10 mM, KH_2PO_4 220 μM, $CaCl_2$ 1.26 mM, $MgSO_4$ 0.81 mM and bovine serum albumin 0.1%) with SK-N-MC cell membranes (50 μg of protein/mL of assay) in a total volume of 500 μL. Nonspecific binding was determined in the presence of 1 μM NPY. The reaction was



Scheme 1. Synthetic route.

then stopped by filtration. The filters (GF/B, Whatman, precoated in 0.3% PEI) were washed thoroughly with buffer A and then counted in a gamma counter (Packard). The human Y_5 receptor binding assay was carried out with iodinated peptide YY (NEN) as follows: COS cells transfected with the human Y_5 NPY receptor were lysed and the membranes were prepared by differential centrifugation. These membranes contained about 2 pmol per mg of protein of this receptor. Incubations were performed in a solution of 500 μL comprising, 20 pM final of [^{125}I]PYY in 50 μL, 400 μL of membrane suspension (0.15 mg/mL) and competitor dilutions in 50 μL, at 30 °C for 2 h. The reaction was stopped by filtration using GF/C filters (Whatman). The results in both assays are expressed in IC_{50}.

4. Results and discussion

Seventy-two new hydrazide derivative compounds have been synthesized and their affinities for the NPY Y1 and Y5 receptors have been evaluated in vitro. The compounds presented showed no antagonist activity on the Y1 receptor because their IC_{50} values are greater than 10^5 nM. Several of the compounds reported here show high affinity for the human neuropeptide Y5 receptor. Common to this set of structures are two hydrophobic aromatic groups that are connected via the sulfonamidomethylcyclohexylhydrazide nucleus. The sulfonamide group and the cyclohexyl linker are known to be essential in the concession of biological activity.

A structure–activity relationship study was carried out introducing different aromatic groups, such as R1 and R2.

The most potent compounds among those we prepared were those with the 3,4-dichlorophenyl, 2,4-dichlorophenyl or 2,3,4,5,6-pentafluorophenyl groups as R2. Compounds **46** and **65** have the best affinity for the NPY Y5 receptor, with 0.68 and 0.43 nM IC_{50} values, respectively, with both of them containing 3,4-dichlorophenyl as the R2 group.

In general, when R1 was not a halogenated phenyl group, the compounds were found to be less potent NPY ligands than the halogenated ones, with IC_{50} values >80 nM for all of them. In the same way, the increase of volume and rigidness of the molecule with naphthyl groups as R1 (compounds **72** and **73**) led to a decrease in potency.

In particular, the best affinity was found when R1 was 2-fluorophenyl or 4-fluorophenyl (compounds **46** and **65**).

Nevertheless, the increase of volume of R1 due to the disubstitution with chloro in the benzene ring improved affinity for the NPY Y5 receptor as shown for compounds **33** and **35**, with IC_{50} values of 37.0 and 2.79 nM, respectively.

Variations were also made to R2. Nonsubstituted phenyl as R2 led us to hydrazide derivatives with very low affinity for the NPY Y5 receptor, as observed for **23**, **57** and **79**, among others.

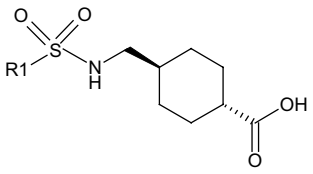
Substitution at the *para*-position of the arylhydrazide moiety with electron-withdrawing groups, such as 4-chloro and 4-bromo, led to a better affinity for the Y5 receptor than the substitution at the *ortho*- or *meta*-position with the same groups. Curiously, this behaviour was not observed when the substituent was 4-fluoro.

Direct comparison between mono- and disubstitution revealed an increase of activity in almost all of the cases when R2 was a disubstituted phenyl group.

5. Conclusion

This publication shows new hydrazide derivatives as antagonist compounds of the NPY Y_5 receptor, characterized by their selectivity on the Y_5 receptor due to their nonaffinity on the receptor Y_1.

Table 1. Sulfonamide compounds



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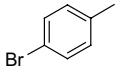
8b	
8c	
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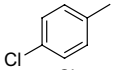
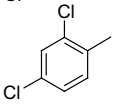
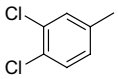
8e	
8f	
8g	
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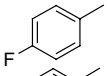
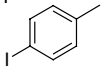
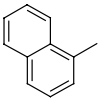
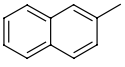
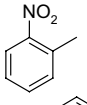
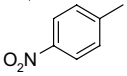
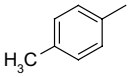
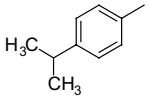
8i	
8j	
8k	
8l	
8m	
8n	
8o	
8p	

Several compounds showed potent antagonistic activity for human NPY Y5 receptors, some of which nanomolar binding affinities. Nevertheless, we are continuing our search for more improved compounds.

6. Experimental protocols

6.1. General

Melting points were determined using a Mettler FP82+FP80 apparatus (Greifense, Switzerland) and have not been corrected. The ^{1}H NMR spectra were recorded on a Bruker AC-200E/AC-400E instrument (Rheinstetten, Germany), using TMS as the internal standard and with DMSO-d_6 as the solvent; the chemical shifts are reported in parts per miilion (δ) and coupling constants (J) values are given in hertz (Hz). Signal multiplicities are represented by: s (singlet), d (doublet), t (triplet), m (multiplet), ds (double singlet), dd (double doublet) and dt (double triplet). The IR spectra were performed on a Perkin–Elmer 1600 FTIR (Norwalk, CT, USA) in KBr pellets; the frequencies are expressed in cm^{-1}. Signal intensities are expressed by: s (strong), m (medium) and w (weak). Elemental microanalyses were obtained on an Elemental Analyzer (Carlo Erba 1106, Milan, Italy) from vacuum-dried samples. The analytical results for C, H and N, were within ±0.4 of the theoretical values.

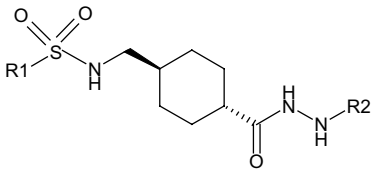
Alugram® SIL G/UV$_{254}$ (layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG. Postfach 101352. D-52313 Düren, Germany) was used for thin layer chromatography and Silica gel 60 (0.040–0.063 mm) was used for column chromatography (Merck). HPLC conditions: Column Nova Pack C18 60 A 4 μm (3.9×150 mm); mobile phase: methanol/water 60/40; flow: 1 mL/min.

Chemicals were purchased from E. Merck (Darmstadt, Germany), Scharlau (F.E.R.O.S.A., Barcelona, Spain), Panreac Química S.A. (Montcada i Reixac, Barcelona, Spain), Sigma–Aldrich Química, S.A., (Alcobendas, Madrid), Acros Organics (Janssen Pharmaceuticalaan 3a, 2440 Geel, België) and Lancaster (Bischheim-Strasbourg, France).

6.1.1. General procedure for the synthesis of trans-4-(R-sulfonylaminomethyl)cyclohexanecarboxylic acid (8). A solution of R-Sulfonyl chloride **7** (1.5 equiv) in a minimum amount of $CHCl_3$ was added, alternately and portionwise, to a solution of *trans*-4-(aminomethyl)-cyclohexanecarboxylic acid **6** (1 equiv) in NaOH 2 M (150 mL), with stirring for 30 min. After the addition, the mixture was stirred at room temperature for 72 h. The aqueous layer was acidified with HCl(c) to pH 1–2. The residue obtained was filtered and washed with H_2O (5×20 mL) and *n*-hexane (5×20 mL) in order to obtain sulfonamides **8**. When necessary, the compounds were purified by recrystallization using the appropriate solvent.

6.1.2. Synthesis of trans-4-(benzenesulfonylaminomethyl)cyclohexanecarboxylic acid (8a). From benzenesulfonyl chloride (6.11 mL, 47.70 mmol), in order to obtain the compound as a white solid (3.46 g, 24%); mp 160–162 °C. IR (KBr): ν 3289 (s, NH); 2929 (m, aliphatic C–H); 1698 (vs, hydrazide C=O); 1326 and 1162 (vs, SO_2–N). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.73–0.90 (m, 2H, H_a of CH–CH_2–CH$_2$–CH–CO); 1.09–1.27

Table 2. IC_{50} (NPY5) results of the hydrazide derivatives



Compd	R1	R2	IC_{50} (nM)
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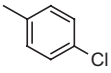
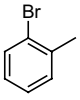
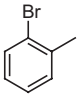
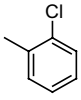
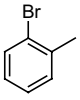
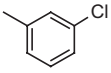
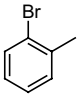
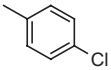
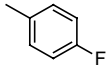
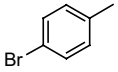
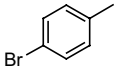
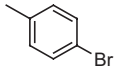
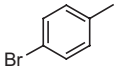
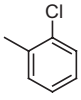
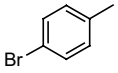
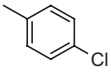
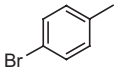
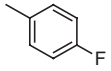
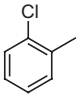
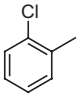
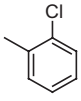
11			751
12			80.5
13			126
14			35.9
15			11.0
16			35.3
17			8520
18			523
19			21.3
20			483
21			32.7
22			—
23			3250
24			33.0

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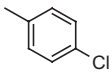
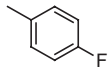
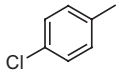
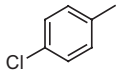
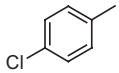
25			—
26			76.2
27			1020
28			—
29			29.0
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34			—
35			2.79
36			10.7
37			55.9
38			251
39			150
40			21.4
41			7.64

(*continued on next page*)

Table 2 (*continued*)

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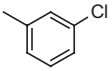
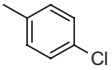
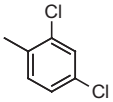
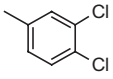
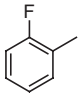
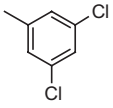
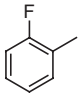
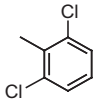
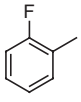
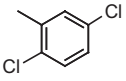
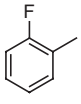
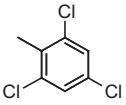
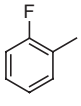
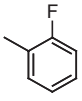
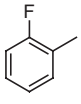
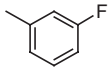
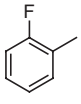
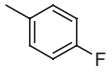
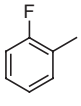
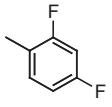
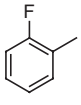
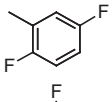
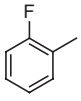
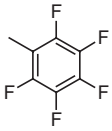
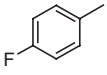
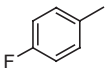
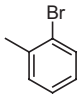
43			38.7
44			13.3
45			3.79
46			0.681
47			57.9
48			954
49			$>10^5$
50			427
51			105
52			235
53			220
54			58.0
55			54.4
56			3.62
57			2150
58			230

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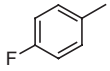
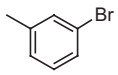
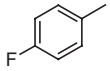
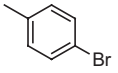
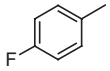
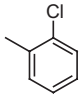
59			40.1
60			17.0
61			429
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65			0.431
66			54.4
67			345
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69			1400
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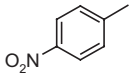
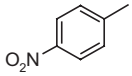
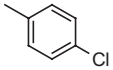
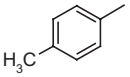
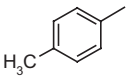
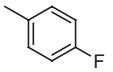
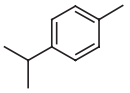
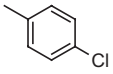
71			405
72			423
73			1450
74			1350
75			109
76			38.7

Table 2 (*continued*)

77			1050
78			696
79			4760
80			712
81			108

(m, 3H, H_a of C*H*–CH_2–CH_2–CH–CO); 1.69 (d, 4H, H_e of CH–CH_2–CH_2–CH–CO); 1.84 (d, 2H, H_e of CH–CH_2–CH_2–CH–CO); 2.07 (t, 1H, CH–CH_2–CH_2–C*H*–CO); 2.56 (t, 2H, SO_2–NH–CH_2); 7.59 (br s, 4H, H_3, H_4 and H_5 of C_6H_5 and SO_2–N*H*); 7.78 (d, 2H, H_2 and H_6 of C_6H_5, J_{2-3}=6.6 Hz); 12.00 (s, 1H, COO*H*) ppm. Anal. $C_{20}H_{22}Cl_2FN_3O_3S$ (C, H, N); C (%): calcd: 50.64; found: 50.41. H (%): calcd: 4.64; found: 4.52. N (%): calcd: 8.86; found: 9.13 (24% yield).

6.1.3. General procedure for the synthesis of trans-N-{4-[N′-(R1-yl)hydrazinocarbonyl]cyclohexylmethyl}-R-sulfonamide (10–81). *trans*-4-(R-Sulfonylaminomethyl)cyclohexanecarboxylic acid **8** (1 equiv) in dry CH_2Cl_2 (150 mL) at 0 °C, under N_2 atmosphere, was treated with EDC (1.13 equiv). After 1 h at 0 °C, the corresponding hydrazine **9** (1.13 equiv) was added. The reaction was stirred at room temperature for 24 h. The solvent was evaporated in vacuo and the residue was taken up with H_2O (20 mL) and diethyl ether (5 mL). The precipitate obtained was filtered and washed with H_2O (5×20 mL) and diethyl ether (1×10 mL) in order to obtain compounds **10–81**. When necessary, the compounds were purified by recrystallization using MeOH/H_2O or by column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH).

6.1.4. Synthesis of trans-N-{4-[N′-(3,4-dichlorophenyl)hydrazinocarbonyl]cyclohexylmethyl}-4-fluorobenzenesulfonamide (65). From *trans*-4-(4-fluorosulfonylaminomethyl)cyclohexanecarboxylic acid (3.17 mmol, 1.00 g), EDC (3.59 mmol, 0.69 g), 3,4-dichlorophenylhydrazine hydrochloride (3.59 mmol, 0.76 g) and triethylamine (3.59 mmol, 0.36 g) in order to obtain **65** as a white solid (0.40 g, 27%); mp 223–225 °C. IR (KBr): *v* 3251 (s, NH); 2937 (m, aliphatic C–H); 1655 (vs, hydrazide C=O); 1322 and 1153 (vs, SO_2–N). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 0.81–0.90 (m, 2H, H_a of CH–CH_2–CH_2–CH–CO); 1.32 (t, 3H, H_a of C*H*–CH_2–CH_2–CH–CO); 1.75 (t, 4H, H_e of CH–CH_2–CH_2–CH–CO); 2.13 (t, 1H, CH–CH_2–CH_2–C*H*–CO); 2.59 (t, 2H, SO_2–NH–CH_2); 6.63 (dd, 1H, H_6 of $C_6H_3Cl_2$, J_{6-5}=8.8 Hz, J_{6-2}=2.5 Hz); 6.78 (ds, 1H, H_2 of $C_6H_3Cl_2$, J_{2-6}=2.5 Hz); 7.33 (d, 1H, H_5 of $C_6H_3Cl_2$, J_{5-6}=8.8 Hz); 7.44 (t, 2H, H_3 and H_5 of C_6H_4F); 7.66 (s, 1H, CO–NH–N*H*–$C_6H_3Cl_2$); 7.85 (dd, 2H, H_2 and H_6 of C_6H_4F, J_{2-3}=6.8 Hz, J_{2-6}=3.1 Hz); 8.11 (s, 1H, SO_2–N*H*); 9.68 (s, 1H, CO–N*H*–NH–$C_6H_3Cl_2$) ppm. Anal. $C_{20}H_{22}Cl_2FN_3O_3S$ (C, H, N); C (%): calcd: 50.64; found: 50.41. H (%): calcd: 4.64; found: 4.52. N (%): calcd: 8.86; found: 9.13 (27% yield).

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2004.06.023.

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