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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 4077–4084
Bioorganic & Medicinal Chemistry

Scaffold hopping, synthesis and structure–activity relationships of 5,6-diaryl-pyrazine-2-amide derivatives: A novel series of CB1 receptor antagonists

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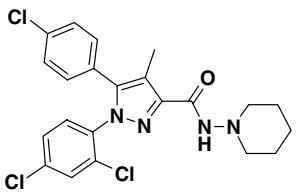
Received 7 September 2006; revised 23 February 2007; accepted 27 March 2007
Available online 30 March 2007

Abstract—A scaffold hopping approach has been exploited to design a novel class of cannabinoid (CB1) receptor antagonists for the treatment of obesity. On the basis of shape-complementarity and synthetic feasibility the central fragment, a methylpyrazole, in Rimonabant was replaced by a pyrazine. The synthesis and CB1 antagonistic activities of a new series of 5,6-diaryl-pyrazine-2-amide derivatives are described. Several compounds showed antagonist potency below 10 nM for the CB1 receptor.

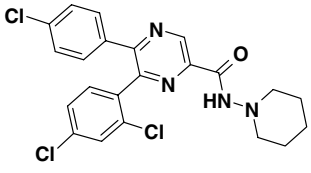
1. Introduction

Cannabis is well known for stimulating appetite and the short bursts of extreme hunger are commonly known as the munchies. This characteristic effect of cannabis is believed to occur because Δ^9-THC activates the cannabinoid CB1 receptor in the brain. The crux of the CB1 hypothesis is that antagonism of CB1 receptors would have the opposite effect and it has been postulated that cannabinoid CB1 modulators are useful in the treatment of obesity.[1–3] The cannabinoid CB1 receptor belongs to the large superfamily of G-protein coupled receptors, which have a seven transmembrane helix structure. As is the case for most transmembrane proteins, cannabinoid receptors do not readily crystallize and no receptor structures have yet been experimentally obtained. Hence, in the quest for designing high-affinity CB1 receptor antagonists with a superior clinical profile for the treatment of obesity a ligand-based strategy has to be employed.

Sanofi-Aventis is currently launching their cannabinoid CB1 receptor antagonist Rimonabant (Acomplia™), to treat obesity. However, there is a need for novel cannabinoid CB1 modulators with improved physicochemical properties, DMPK and/or pharmacodynamic properties. Consequently, a scaffold hopping approach aiming at replacing the central fragment, the methylpyrazole, in Rimonabant was initiated. Several different compound classes, such as thiazoles, pyrroles and pyrazines, were scrutinized for their chemical feasibility and their shape-complementarity to the putative bioactive conformation of Rimonabant.[4] Mini-libraries of around 20 compounds per substance class were synthesized to evaluate the different classes. Among the most interesting of the classes was the pyrazine series.[5] In the current study, we present the synthesis and the CB1 antagonistic activities of a new series of 5,6-diaryl-pyrazine-2-amide derivatives.



1: Rimonabant



2a

Keywords: Scaffold hopping; Shape-matching; Cannabinoid; Antagonist.
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doi:10.1016/j.bmc.2007.03.075

2. Results and discussion

2.1. Molecular design

A putative bioactive conformation of Rimonabant was deduced on the basis of SAR, small molecule X-ray data and conformational analysis. It largely agrees with the later published proposal from Shim and co-workers.[6] Taking into account that bioactive conformation, we embarked on a scaffold hopping route steered by shape similarity. With the aim of retaining the global shape of Rimonabant, significant molecular modifications were performed to obtain the designed compounds. Using this strategy in combination with ease of synthesis, several new scaffolds were ranked and selected. ROCS was used to calculate the shape-overlays.[7,8] Evidently, aromatic five- and six-membered rings are structurally different and do not, for example, share the same optimal bond-angles. This was reflected in a slightly lower shape overlay for the pyrazine containing compound **2a**, as compared to the other selected five-membered counterparts, the pyrrole (shape Tanimoto: 0.94) and thiazole (shape Tanimoto: 0.92) derivatives. Nevertheless, the shape Tanimoto for **2a** was high (0.90), meaning that it is virtually identical to Rimonabant with respect to shape. In addition, the shape-based alignment shows that not only is the global shape retained, but it also imposes the ring nitrogens to occupy the same region in space. The molecular alignment of **1** and the corresponding pyrazine compound **2a** is shown in Figure 1. Mini-libraries of the two other highly ranked scaffolds (pyrrole and thiazole) were also synthesized. These series have been reported upon elsewhere.[9,10]

Moreover, conformational energy penalties may significantly influence the affinity of a ligand.[11] Thus, a standard approach to conformational analysis is to apply conformational energy cut-offs.[11–13] This pragmatic approach reduces the number of conformations in a calculated ensemble, while at the same time removing energetically unrealistic conformations. The calculated conformational energy penalties of the proposed bioactive conformations for compounds **2a–b**, **5a–f**, **6a–e**, **7a–e** were obtained as described in the Section 4.3. The calculated conformational energies are all low (<3.0 kcal/mol) for the proposed bioactive conformations of **2a–b**, **5a–f**, **6a–e**, **7a–e**, see Figure 2. Such low values are compatible with ligands showing high potencies for the CB1 receptor.

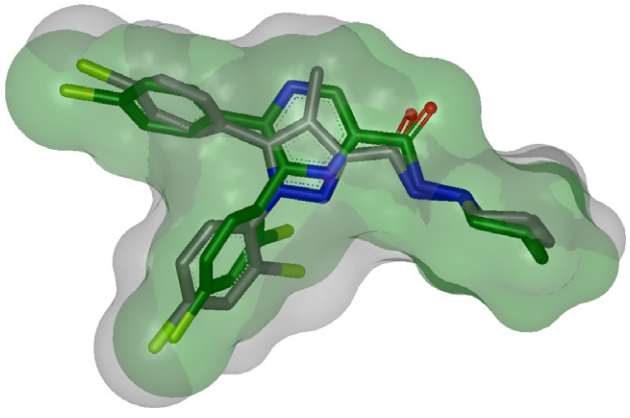


Figure 1. Molecular superimposition of compounds **1** and **2a**, illustrating the scaffold hopping. The methylpyrazole moiety in Rimonabant (**1**) is exchanged for a pyrazine moiety in our compound **2a**. The hydrogens have been removed for clarity. The shape Tanimoto value is 0.9 meaning that they are virtually identical with respect to shape. The carbon atoms and the molecular shape are visualized grey for **1** and green for **2a**.

2.2. Chemistry

Six commercially available symmetrical diketones of varying size and electronic properties were selected to gain knowledge on how the substitution pattern on the phenyl rings affected affinity as compared to the substitution pattern seen in the corresponding Rimonabant derivatives. The symmetrical diketones were condensed with monohydrochloride of 2,3-diaminopropionic acid (**3**), Scheme 1, under basic conditions in refluxing methanol.[14] The reaction proved to be dependent upon the ketone, and the reaction times varied between 20 min and 16 h to yield the dihydrointermediates. Subsequent to the condensation, air was bubbled through the reaction mixture until the desired pyrazines, **4a–f**, were formed. We decided to make three different amides and therefore compounds **4a–f** were further coupled with 1-aminopiperidine, cyclohexylamine and aniline, respectively, by using EDC and DMAP in DCM at room temperature overnight to give the final products **5a–f**, **6a–e** and **7a–e**, respectively.

The synthesis of **2a** and **2b** is described in Scheme 2. Friedel-Crafts acylation of dichlorobenzene **9** with 4-chlorophenylacetyl chloride **8** yielded **10**,[15] which was then oxidized by PCC in 1,2-dichloroethane to give the 1,2-diketone **11**. Condensation of **11** with 2,3-diaminopropionic acid (**3**) furnished the two regioisomers **12a** and **12b**, which were transformed into the corresponding acid chlorides by treatment with thionyl chloride in toluene at reflux temperature for 3 h. The acid chlorides were then reacted with 1-aminopiperidine in DCM at room temperature overnight yielding a mixture of **2a** and **2b**. The final compounds **2a** and **2b** were purified by preparative chromatography.

2.3. Receptor functional studies

A GTPγS CB1 functional assay was used to assess the potency of the pyrazine derivatives described above. Receptor inhibition potencies for the compounds modified at the R1–R4 position, with different *N*-substituted carboxamides, are reported in Table 1 (piperidylamide), 2 (cyclohexylamide) and 3 (aniline). For all three series of carboxamides the di-*para*-substituted analogues (**5a–e**, **6a–d** and **7b–d**) showed higher potency for CB1 than the corresponding *ortho*-substituted derivatives (**5f**, **6e** and **7e**) (Tables 2 and 3).

The SAR of different *N*-substituted carboxamides at the 2-position of the pyrazine ring was also investigated. For example, the bis(*para*-chloro-phenyl) *N*-piperidylamide

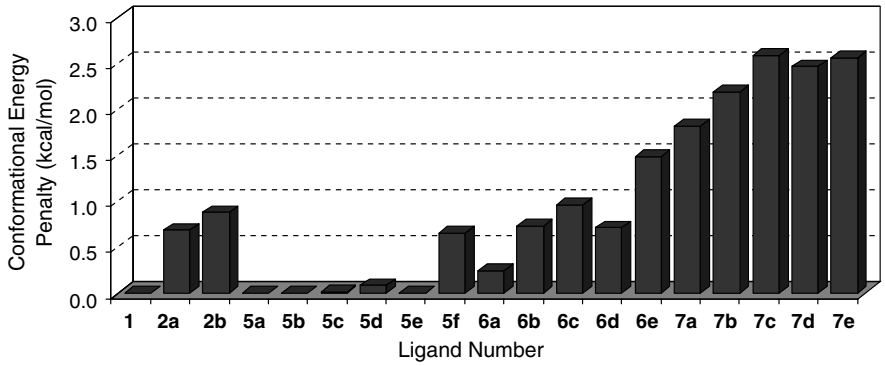
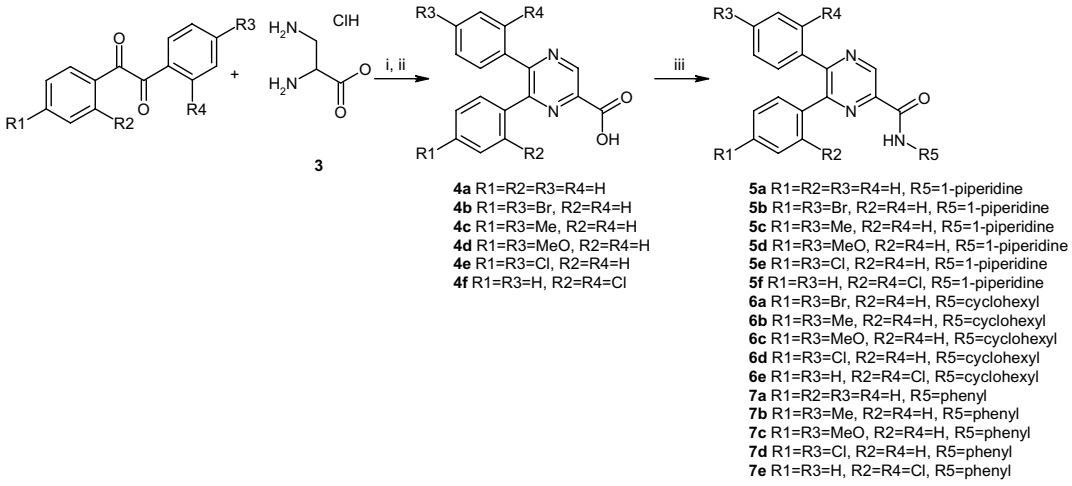
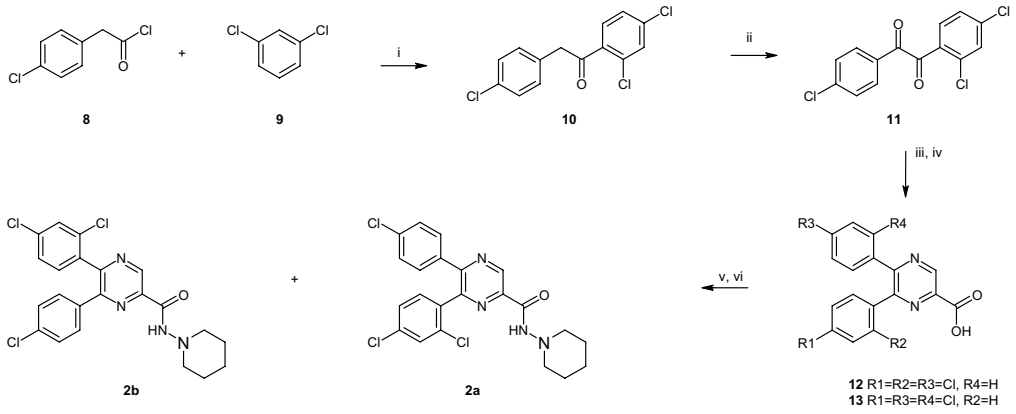


Figure 2. Calculated conformational energy penalties of the proposed bioactive conformations of ligands **1**, **2a–b**, **5a–f**, **6a–e**, **7a–e**. All ligands display values less than 3 kcal/mol. However, the conformational energy penalties are slightly higher for the *N*-phenyl substituted compounds **7a–e**, providing a possible rationale for their somewhat lower CB1 receptor affinities.



Scheme 1. Reagents and condition: (i) NaOH, MeOH, reflux; (ii) air bubbling; (iii) EDC, DMAP, DCM, rt.

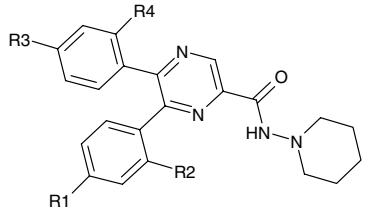


Scheme 2. Reagents and conditions: (i) $AlCl_3$, neat, 0 °C, ⩾rt; (ii) PCC, pyridine, 1,2-dichloroethane, reflux; (iii) **3**, NaOH, MeOH, reflux; (iv) air bubbling; (v) $SOCl_2$, toluene, reflux; (vi) 1-aminopiperidine, TEA, DCM, rt.

analogue (**5e**) gave the optimal potency for CB1 (IC_{50} of 4 nM) followed by the *N*-cyclohexylamide derivative (**6d**), whereas the corresponding aromatic *N*-carboxamide (**7d**) showed somewhat lower potency. This general trend of lower potencies for the aromatic *N*-carboxamide compounds (**7a–e**), as compared to the *N*-piperidylamide (**5a–f**) and *N*-cyclohexylamide analogues (**6a–e**), may be rationalized by their somewhat higher conformational energy penalties (Fig. 2).[11–13]

Compound **5b**, a piperidino derivative incorporating bromine atoms at both the *para*-substituted phenyl rings, was found to be the most potent antagonist for the cannabinoid CB1 receptor (IC_{50}: 1 nM), whereas

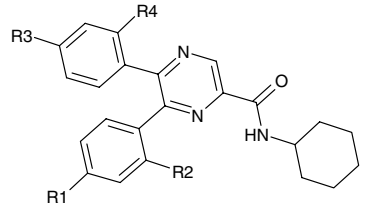
Table 1. Structure and activity for compounds with *N*-piperidylamide substituents



Compound	R1	R2	R3	R4	GTPγS IC_{50} values (nM)
1 (Rimonbant)					3
2a	Cl	Cl	Cl	H	114
2b	Cl	H	Cl	Cl	10
5a	H	H	H	H	766
5b	Br	H	Br	H	1
5c	Me	H	Me	H	6
5d	OMe	H	OMe	H	21
5e	Cl	H	Cl	H	4
5f	H	Cl	H	Cl	174

The experimental potency data are given as IC_{50} values, for the CB1 antagonists.

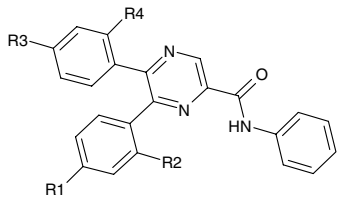
Table 2. Structure and activity for compounds with *N*-cyclohexylamide substituents



Compound	R1	R2	R3	R4	GTPγS IC_{50} values (nM)
6a	Br	H	Br	H	11
6b	Me	H	Me	H	13
6c	OMe	H	OMe	H	8
6d	Cl	H	Cl	H	19
6e	H	Cl	H	Cl	1578

The experimental potency data are given as IC_{50} values, for the CB1 antagonists.

Table 3. Structure and activity for compounds with *N*-phenyl substituents



Compound	R1	R2	R3	R4	GTPγS IC_{50} values (nM)
7a	H	H	H	H	2790
7b	Me	H	Me	H	326
7c	OMe	H	OMe	H	119
7d	Cl	H	Cl	H	80
7e	H	Cl	H	Cl	763

The experimental potency data are given as IC_{50} values, for the CB1 antagonists.

the unsubstituted aromatic carboxamide (**7a**) displayed the poorest activity (IC_{50}: 2790 nM).

In our in vitro functional assays, the pyrazine derivatives show common characteristics in SAR with that previously reported for the pyrazole series,[4] providing support for the shape-based molecular alignment shown in Figure 1. For example, the bis(*para*-phenyl) pyrazoles substituted analogues to ligand **1** also display significantly higher potency compared with the bis(*ortho*)phenyl substituted pyrazoles. Furthermore, the aromatic carboxamide pyrazole analogues also showed decreased potency.[4] This suggests a common mode of binding for the pyrazole and pyrazine series at cannabinoid receptors.

Recently, several other CB1 receptor antagonists, with variations in the central scaffold, have been published. For example, the pyrazole ring in **1** has been exchanged with the corresponding thiazole,[9] pyrrole[10] and pyridine.[16,17] All these classes include ligands with high potencies for the CB1 receptor. Since these fragments are electronically quite different we believe that the central scaffold is mainly essential for geometrical reasons. That is, the function of the central scaffold is not to make any direct interactions with the cannabinoid receptor, but rather to place the substituted phenyl rings and the *N*-carboxyamide fragments in an optimal 3D orientation. This hypothesis is in line with previous work in the CB1 field.[18]

3. Conclusions

A scaffold hopping approach has been exploited for the design of a novel class of CB1 antagonists for the treatment of obesity. The synthesis and CB1 antagonistic activities of a new series of 5,6-diaryl-pyrazine-2-amide derivatives have been described. Several compounds showed in vitro potency below 10 nM for the CB1 receptor. Bis *para*-substitution improves potency as compared with bis *ortho*-substituted pyrazines. We believe that the scaffold hopping was successful because the global shape was preserved and that the central scaffold seems mainly to be essential for geometric reasons.

4. Experimental

4.1. General synthetic methods

Mass spectra were recorded on either a Micromass ZQ single quadrupole or a Micromass LCZ single quadrupole mass spectrometer both equipped with a pneumatically assisted electrospray interface (LC-MS). ^{1}H NMR measurements were performed on either a Varian Mercury 300 or a Varian Inova 500, operating at ^{1}H frequencies of 300 and 500 MHz, respectively. Chemical shifts are given in ppm with $CDCl_3$ as internal standard. Purification was performed on a semipreparative HPLC with a mass triggered fraction collector, Shimadzu QP 8000 single quadrupole mass spectrometer equipped with 19×100 mm C8 column. The mobile phase used was, if nothing else stated, acetonitrile and buffer (0.1 M NH_4Ac:acetonitrile 95/5).

For isolation of isomers a kromasil-CN E9344 (250 × 20 mm id) column was used. As mobile phase heptane:ethyl acetate:DEA 95/5/0.1 was used. Fractions were collected by UV.

4.1.1. 5,6-Diphenyl-pyrazine-2-carboxylic acid (4a). Compound **3** (500 mg, 3.56 mmol) and benzil (890 mg, 4.23 mmol) were added to a solution of sodium hydroxide (677 mg, 16.93 mmol) in methanol (10 mL). An extra portion of methanol was added (5 mL) and the reaction mixture was refluxed for 20 min. The mixture was cooled to 25 °C and air was bubbled through for 30 min. Hydrochloric acid (aq, 2 M) was added until the reaction mixture reached pH 2. The solution was extracted with diethyl ether. The combined diethyl ether phases were dried ($MgSO_4$), filter and the solvent was evaporated under reduced pressure to give the crude product. The crude product was used in steps described below without further purification.

MS *m*/*z* 277 $(M+H)^+$.

4.1.2. 5,6-Bis-(4-bromo-phenyl)-pyrazine-2-carboxylic acid (4b). The title compound was prepared essentially as described in Section 4.1.1, using **3** (600 mg, 4.26 mmol) and 4,4′-dibromobenzil (1.745 g, 4.26 mmol, 90%) as starting materials. The reaction mixture was refluxed for 2 h and air was bubbled through for 1 h. Hydrochloric acid (aq, 2 M) was added until reaching pH 2. The mixture was evaporated under reduced pressure and the residue was dissolved in water. The solution was extracted with diethyl ether, the combined diethyl ether phases were dried ($MgSO_4$), filter and the solvent was evaporated under reduced pressure. The crude product (500 mg, 27%) was used in steps described below without further purification.

MS *m*/*z* 435, 437, 439 $(M+H)^+$.

4.1.3. 5,6-Di-*p*-tolyl-pyrazine-2-carboxylic acid (4c). The compound **4c** was prepared as described in Section 4.1.1 using 4,4′-dimethylbenzil (848 mg, 3.56 mmol). The reaction mixture was however refluxed for 1 h and air was bubbled through the reaction mixture for about 7 h. The mixture was evaporated and the residue was dissolved water. Hydrochloric acid (aq, 2 M) was added until reaching pH 2. The solution was extracted with diethyl ether, the combined diethyl ether phases were dried ($MgSO_4$), filter and the solvent was evaporated under reduced pressure. The crude product (918 mg, 85%) was used in steps described below without further purification.

MS *m*/*z* 305 $(M+H)^+$.

4.1.4. 5,6-Bis-(4-methoxy-phenyl)-pyrazine-2-carboxylic acid (4d). The title compound was prepared as described in Section 4.1.3 using 4,4′-dimethoxybenzil (961 mg, 3.56 mmol) as starting material. The reaction mixture was refluxed overnight and air was bubbled through the mixture for 8 h. The crude product (435 mg, 36%) was used in steps described below without further purification.

MS *m*/*z* 335 $(M+H)^+$.

4.1.5. 5,6-Bis-(4-chloro-phenyl)-pyrazine-2-carboxylic acid (4e). The compound **4e** was prepared as described in Section 4.1.3 using 4,4′-dichlorobenzil (993 mg, 3.56 mmol). Reflux for 1 h gave the oxidised pyrazine directly. The crude product (923 mg, 75%) was used in steps described below without further purification.

MS *m*/*z* 343, 345, 347 $(M-H)^-$.

4.1.6. 5,6-Bis-(2-chloro-phenyl)-pyrazine-2-carboxylic acid (4f). The title compound was prepared as described in Section 4.1.3 using 2,2′-dichlorobenzil (993 mg, 3.56 mmol). The crude product (895 mg, 73%) was used in steps described below without further purification.

MS *m*/*z* 343, 345, 347 $(M-H)^-$.

4.1.7. 5,6-Diphenyl-pyrazine-2-carboxylic acid piperidin-1-ylamide (5a). Compound **4a** (500 mg, 1.81 mmol) was dissolved in DCM (4 mL) and DMF (150 μL). DMAP (22 mg, 0.18 mmol) and 1-aminopiperidine (218 mg, 2.17 mmol) were added and the solution was cooled to 0 °C. A slurry of EDC (1.99 mmol, in 2 mL DCM and 100 μL DMF) was added dropwise. The reaction mixture was stirred at 25 °C. After 17 h, additional 1-aminopiperidin (40 mg, 0.40 mmol) and EDC (76 mg, 0.40 mmol) were added and the mixture was stirred for another 3 h. The crude was diluted with DCM (5 mL) and washed with a saturated solution of $NaHCO_3$. The organic phase was dried ($MgSO_4$), filtered and the solvent was evaporated. Flash chromatography (SiO_2, ethyl acetate:hexane 2/1) gave the title compound (160 mg, 25%) as a white solid.

^1H NMR (300 MHz) δ 9.41 (s, 1H), 8.52 (s, 1H), 7.50–7.29 (m, 10H), 2.94 (t, 4H), 1.81 (m, 4H), 1.50 (m, 2H). MS *m*/*z* 359 $(M+H)^+$.

4.1.8. 5,6-Bis-(4-bromo-phenyl)-pyrazine-2-carboxylic acid piperidin-1-ylamide (5b). To compound **4b** (108 mg, 0.25 mmol), DMAP (0.025 mmol, in 500 μL DCM), 1-aminopiperidine (0.25 mmol, in 1100 μL DCM), and EDC (0.27 mmol, in 1100 μL DCM and cooled to 8 °C) were added. The reaction mixture was stirred at 25 °C for 20 h, then washed with saturated $NaHCO_3$ solution, dried ($MgSO_4$), filtered and the solvent was evaporated. Semipreparative HPLC (0.01% TEA in the buffer phase) gave the title compound (6.7 mg, 5.4%).

^1H NMR (300 MHz) d 9.41 (s, 1H), 8.48 (s, 1H), 7.54 (d, 2H), 7.51 (d, 2H), 7.36 (d, 2H), 7.34 (d, 2H), 2.94 (t, 4H), 1.81 (m, 4H), 1.55–1.45 (m, 2H).

MS *m*/*z* 515, 517, 518 $(M+H)^+$.

4.1.9. 5,6-Di-*p*-tolyl-pyrazine-2-carboxylic acid piperidin-1-ylamide (5c). Compound **4c** (76 mg, 0.25 mmol) was used as described in Section 4.1.8 to give the title compound (27 mg, 28%).

^{1}H NMR (300 MHz) δ 9.35 (s, 1H), 8.57 (s, 1H), 7.38 (d, 4H), 7.18 (d, 2H), 7.13 (d, 2H), 2.92 (t, 4H), 2.40 (s, 3H), 2.37 (s, 3H), 1.86–1.75 (m, 4H), 1.54–1.44 (m, 2H).

MS *m*/*z* 387 $(M+H)^+$.

4.1.10. 5,6-Bis-(4-methoxy-phenyl)-pyrazine-2-carboxylic acid piperidin-1-ylamide (5d). Compound **4d** (84 mg, 0.25 mmol) was used as described in Section 4.1.8 to give the title compound (20 mg, 19%).

^{1}H NMR (300 MHz) δ 9.31 (s, 1H), 8.57 (s, 1H), 7.46 (d, 2H), 7.44 (d, 2H), 6.90 (d, 2H), 6.86 (d, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 2.93 (t, 4H), 1.80 (m, 4H), 1.54–1.45 (m, 2H).

MS *m*/*z* 419 $(M+H)^+$.

4.1.11. 5,6-Bis-(4-chloro-phenyl)-pyrazine-2-carboxylic acid piperidin-1-ylamide (5e). Compound **4e** (86 mg, 0.25 mmol) was used as described in Section 4.1.8 to give the title compound (16 mg, 15%).

^{1}H NMR (300 MHz) δ 9.40 (s, 1H), 8.49 (s, 1H), 7.45–7.31 (m, 8H), 2.94 (t, 4H), 1.80 (m, 4H), 1.54–1.45 (m, 2H).

MS *m*/*z* 427, 429, 431 $(M+H)^+$.

4.1.12. 5,6-Bis-(2-chloro-phenyl)-pyrazine-2-carboxylic acid piperidin-1-ylamide (5f). Compound **4f** (86 mg, 0.25 mmol) was used as described in Section 4.1.8 to give the title compound (6 mg, 6%).

^{1}H NMR (300 MHz) δ 9.52 (s, 1H), 8.52 (s, 1H), 7.44–7.17 (d, 8H), 2.94–2.88 (t, 4H), 1.85–1.70 (m, 4H), 1.52–1.44 (m, 2H).

MS *m*/*z* 427, 429, 431 $(M+H)^+$.

4.1.13. 5,6-Bis-(4-bromo-phenyl)-pyrazine-2-carboxylic acid cyclohexylamide (6a). Compound **4b** (109 mg, 0.25 mmol) was reacted essentially as described in Section 4.1.8 but with cyclohexylamine (0.25 mmol in 1 mL DCM) instead. DMF (100 μL) was also added. Semipreparative HPLC (0.15% TFA/water:acetonitrile 95/5 instead of the buffer phase) gave the title compound (7 mg, 8%) after washing with Na_2CO_3.

^{1}H NMR (300 MHz) δ 9.41 (s, 1H), 7.68 (s, 1H), 7.54 (d, 2H), 7.50 (d, 2H), 7.36 (d, 2H), 7.34 (d, 2H), 4.11–3.96 (m, 1H), 2.12–1.20 (m, 10H).

MS *m*/*z* 514, 516, 518 $(M+H)^+$.

4.1.14. 5,6-Di-*p*-tolyl-pyrazine-2-carboxylic acid cyclohexylamide (6b). Compound **4c** (76 mg, 0.25 mmol) was used as described in Section 4.1.13. Semipreparative HPLC (0.01% TEA in the buffer phase) gave the title compound (4 mg, 4%).

^{1}H NMR (300 MHz) δ 9.36 (s, 1H), 7.77 (d, 1H), 7.39 (d, 4H), 7.18 (d, 2H), 7.13 (d, 2H), 4.10–3.96 (m, 1H), 2.40 (s, 3H), 2.37 (s, 3H), 2.09–1.20 (m, 10H).

MS *m*/*z* 386 $(M+H)^+$.

4.1.15. 5,6-Bis-(4-methoxy-phenyl)-pyrazine-2-carboxylic acid cyclohexylamide (6c). Compound **4d** (76 mg, 0.25 mmol) was used essentially as described in Section 4.1.13 but the reaction mixture was first stirred overnight, then more cyclohexylamine (25 mg, 0.25 mmol) was added and the mixture was stirred for another two days prior to workup. Semipreparative HPLC (0.15% TFA in the buffer phase) gave the title compound (12 mg, 11%).

^{1}H NMR (300 MHz) δ 9.32 (s, 1H), 7.76 (d, 1H), 7.47 (d, 2H), 7.45 (d, 2H), 6.90 (d, 2H), 6.86 (d, 2H), 4.10–3.96 (m, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 2.09–1.17 (m, 10H).

MS *m*/*z* 418 $(M+H)^+$.

4.1.16. 5,6-Bis-(4-chloro-phenyl)-pyrazine-2-carboxylic acid cyclohexylamide (6d). Compound **4e** (86 mg, 0.25 mmol) was used as described in Section 4.1.15 to give the title compound (7 mg, 8%) after washing with Na_2CO_3.

^{1}H NMR (300 MHz) δ 9.41 (s, 1H), 7.69 (s, 1H), 7.47–7.30 (m, 8H), 4.10–3.97 (m, 1H), 2.10–1.18 (m, 10H).

MS *m*/*z* 426, 428, 430 $(M+H)^+$.

4.1.17. 5,6-Bis-(2-chloro-phenyl)-pyrazine-2-carboxylic acid cyclohexylamide (6e). Compound **4f** (86 mg, 0.25 mmol) was used as described in Section 4.1.15 to give the title compound (14 mg, 13%).

^{1}H NMR (300 MHz) δ 9.51 (s, 1H), 7.74 (s, 1H), 7.41–7.18 (m, 8H), 4.10–3.97 (m, 1H), 2.07–1.14 (m, 10H).

MS *m*/*z* 426, 428, 430 $(M+H)^+$.

4.1.18. 5,6-Diphenyl-pyrazine-2-carboxylic acid phenylamide (7a). Compound **4a** (70 mg, 0.25 mmol) was used as described in Section 4.1.13 but aniline (0.25 mmol in 1 mL DCM) was used instead. The reaction mixture was worked up as described in Section 4.1.8. Semipreparative HPLC (0.15% TFA/water:acetonitrile 95/5 instead of the buffer phase) gave the title compound (27 mg, 30%) after washing with Na_2CO_3.

^{1}H NMR (300 MHz) δ 9.75 (s, 1H), 9.52 (d, 1H), 7.80 (d, 2H), 7.55–7.32 (m, 12H), 7.20 (t, 1H).

MS *m*/*z* 352 $(M+H)^+$.

4.1.19. 5,6-Di-*p*-tolyl-pyrazine-2-carboxylic acid phenylamide (7b). Compound **4c** (77 mg, 0.25 mmol) was used as described in Section 4.1.18 to give the title compound (28 mg, 29%).

^{1}H NMR (500 MHz) δ 9.78 (s, 1H), 9.49 (s, 1H), 7.81 (d, 2H), 7.47–7.43 (m, 6H), 7.25–7.17 (m, 5H), 2.45 (s, 3H), 2.41 (s, 3H).

MS *m*/*z* 380 $(M+H)^+$.

4.1.20. 5,6-Bis-(4-methoxy-phenyl)-pyrazine-2-carboxylic acid phenylamide (7c). Compound **4d** (85 mg, 0.25 mmol) was used as described in Section 4.1.18 to give the title compound (33 mg, 32%).

^{1}H NMR (300 MHz) δ 9.74 (s, 1H), 9.42 (s, 1H), 7.79 (d, 2H), 7.50 (d, 4H), 7.42 (t, 2H), 7.19 (t, 1H), 6.94 (d, 2H), 6.89 (d, 2H), 3.88 (s, 3H), 3.85 (s, 3H).

MS *m*/*z* 412 $(M+H)^+$.

4.1.21. 5,6-Bis-(4-chloro-phenyl)-pyrazine-2-carboxylic acid phenylamide (7d). Compound **4e** (87 mg, 0.25 mmol) was used as described in Section 4.1.18 to give the title compound (6 mg, 6%).

^{1}H NMR (300 MHz) δ 9.66 (s, 1H), 9.52 (s, 1H), 7.79 (d, 2H), 7.48–7.35 (m, 10H), 7.21 (t, 1H).

MS *m*/*z* 420, 422, 424 $(M+H)^+$.

4.1.22. 5,6-Bis-(2-chloro-phenyl)-pyrazine-2-carboxylic acid phenylamide (7e). Compound **4f** (87 mg, 0.25 mmol) was treated as described in Section 4.1.18 to give the title compound (27 mg, 25%).

^{1}H NMR (500 MHz) δ 9.73 (s, 1H), 9.66 (s, 1H), 7.81(d, 2H), 7.46–7.22 (m, 11H).

MS *m*/*z* 420, 422, 424 $(M+H)^+$.

4.1.23. 2-(4-Chloro-phenyl)-1-(2,4-dichloro-phenyl)-ethanone (10). To a dried round-bottomed flask, $AlCl_3$ (4.94 g, 37.0 mmol) and (**11**) (38.9 g, 264.5 mmol) were added under nitrogen. The slurry was cooled to 0 °C by an ice bath. (**10**) (5.0 g, 26.5 mmol) was added dropwise during 10 min. The ice bath was removed and the reaction mixture was left to warm to room temperature while stirring during night. The excess of reagent was removed by rotatory evaporation under reduced pressure. To the remaining, ice (100 mL) in HCl (concd 10 mL) was added and the aqueous phase was extracted with DCM. The organic phase was washed with NaOH (2 M, aq), brine, water, dried ($MgSO_4$), filtered and the solvent was evaporated. Flash chromatography (SiO_2, toluene:heptane 1/1) gave the title compound (3.12 g, 39%).

^{1}H NMR (500 MHz) δ 7.46 (d, 1H), 7.38 (d, 1H), 7.28–7.34 (m, 3H), 7.17 (d, 2H), 4.23 (s, 2H).

MS *m*/*z* 297, 299, 301, 303 $(M+H)^-$.

4.1.24. 1-(4-Chloro-phenyl)-2-(2,4-dichloro-phenyl)-ethane-1,2-dione (11). Compound **10** (2.7 g, 9.01 mmol) was dissolved in 1,2-dichloroethane (25 mL) and freshly made PCC (3.89 g, 18.02 mmol), pyridine (1.43 g, 18.02 mmol) and molecular sieves were added. The reaction mixture was refluxed under inert atmosphere overnight. The solution was cooled to 25 °C, filtered through silica and the solvent was evaporated under reduced pressure. The crude product (1.9 g, 66%) was used directly in the next step.

^{1}H NMR (500 MHz) δ 7.97 (d, 2H), 7.84 (d, 1H), 7.52 (d, 2H), 7.46 (s, 1H), 7.44 (d, 1H).

4.1.25. 5-(4-Chloro-phenyl)-6-(2,4-dichloro-phenyl)-pyrazine-2-carboxylic acid (12a) and 6-(4-chloro-phenyl)-5-(2,4-dichloro-phenyl)-pyrazine-2-carboxylic acid (12b). The title compounds were prepared as described in (**4a**) using **11** (1.85 g, 5.90 mmol) and **3** (0.61 g, 5.90 mmol) as starting materials. The mixture was refluxed for 30 min and then directly worked up. The crude product was allowed to stand overnight to spontaneously aromatize. Flash chromatography (SiO_2, DCM:methanol 10/1, 1% acetic acid) gave the isomer mixture (0.2 g, 10%). MS *m*/*z* 377, 379, 381 $(M-H)^-$.

4.1.26. 5-(4-Chloro-phenyl)-6-(2,4-dichloro-phenyl)-pyrazine-2-carboxylic acid piperidin-1-ylamide (2a) and 6-(4-chloro-phenyl)-5-(2,4-dichloro-phenyl)-pyrazine-2-carboxylic acid piperidin-1-ylamide (2b). The mixture of **12a** and **12b** (78 mg, 0.205 mmol) and thionyl chloride (147 mg, 1.23 mmol) was refluxed in toluene (2 mL) for 3 h. The solvent and the excess of reagent were evaporated under reduced pressure. The intermediates were dissolved in DCM (1 mL). TEA (42 mg, 0.41 mmol) and 1-aminopiperidine (21 mg, 0.205 mmol) were both dissolved in DCM (1 mL) and added. The reaction mixture was stirred at 25 °C overnight. The solvent was evaporated under reduced pressure and the crude product was directly purified by flash chromatography (SiO_2, heptane:ethyl acetate 1/1) which gave the isomers (45 mg, 47%). ^{1}H NMR (300 MHz) δ 9.46 (s, 1H), 8.39 (s, 1H), 7.47–7.28 (m, 7H), 3.02–2.84 (m, 4H), 1.89–1.73 (m, 4H), 1.57–1.41 (m, 2H) and 9.42 (s, 1H), 8.51 (s, 1H), 7.47–7.28 (m, 7H), 3.02–2.84 (m, 4H), 1.89–1.73 (m, 4H), 1.57–1.41 (m, 2H).

4.1.27. Compound 2a was isolated from its isomer by preparative chromatography (9 mg). ^{1}H NMR (300 MHz) δ 9.46 (s, 1H), 8.38 (s, 1H), 7.46–7.24 (m, 7H), 2.89 (t, 4H), 1.78 (p, 4H), 1.52–1.40 (m, 2H).

4.1.28. Compound 2b was isolated from its isomer by preparative chromatography (11 mg). ^{1}H NMR (300 MHz) δ 9.42 (s, 1H), 8.50 (s, 1H), 7.39–7.30 (m, 7H), 2.93 (t, 4H), 1.80 (p, 4H), 1.54–1.43 (m, 2H).

4.2. GTPγS assay

[^{35}S] GTPγS binding assays were performed at 30 °C for 45 min in membrane buffer (100 mM NaCl, 5 mM, 1 mM EDTA, and 50 mM HEPES, pH 7.4) containing 0.025 μg/μL of membrane protein with 0.01% bovine serum albumin (fatty acid free), 10 μM GDP, 100 μM DTT and 0.53 nM [^{35}S] GTPγS (Amersham Pharmacia Biotech) in a final volume of 200 μL. Non specific binding was determined in the presence of 20 μM GTPγS. For antagonist experiments the EC80 for CP55940 was determined and used to activate the receptor. The reaction was terminated by addition of ice cold wash buffer (50 mM Tris–HCl, 5 mM $MgCl_2$, and 50 mM NaCl, pH 7.4) followed by rapid filtration under vacuum through Printed Filtermat A glass fibre filters (Wallac) (0.05% PEI treated) using a Micro 96 Harvester (Skatron

Instruments). The filters were dried for 30 min at 50 °C, then a paraffin scintillate pad was melted onto the filters and the bound radioactivity was determined using a 1450 Microbeta Trilux (Wallac) scintillation counter.

4.3. Computational methods

Tanimoto values were calculated to assess the shape similarity of compounds **2a**–**b**, **5a**–**f**, **6a**–**e**, **7a**–**e** as compared to the proposed bioactive conformation of Rimonabant. The shape complementary program ROCS[8] was used. Shape Tanimoto values above 0.8 correspond to structures of visually very similar shape. The shape Tanimoto values were calculated as follows. A multi conformational database was generated for compounds **2a**–**b**, **5a**–**f**, **6a**–**e**, **7a**–**e** according to standard procedure.[19] That is, the SMILES codes were converted to 3D by using Corina.[20] The structures obtained from Corina were subjected to rapid geometry optimization (25 iterations) using the MMFF94s force field as implemented in Szybki.[21] A conformational ensemble was generated using the OMEGA program.[22] The compounds were aligned onto the proposed bioactive conformation of Rimonabant using ROCS. The bioactive conformation for each compound was deduced on the basis of the highest shape Tanimoto.

The affinity of a ligand may be significantly influenced by the conformational energy component.[11] Thus, conformational energy penalties were calculated, as follows. First, the global minimum for each ligand was obtained from an exhaustive conformational analysis using the Mixed Torsional/Low Mode search, implemented in MacroModel v9.0.[23] The MMFFs force field[24,25] and the Generalized Born/Surface Area (GB/SA)[26] solvent model was used. Second, for each ligand the structure obtained by shape-matching was energy minimized with 'flat bottomed' Cartesian constraints. This procedure is to make bond lengths and bond-angles consistent with the MMFFs force field, ensuring that deviations in these terms do not make spurious contributions to the conformational energy.[11] Finally the conformational energy penalty was calculated from the difference in the global energy minimum and the conformational energy after constrained optimization.

To facilitate new ideas and the chemist's awareness of 3D the above-described superposition procedure was automated and an interactive web interface was designed, using dynamic HTML documents. For an input structure, conformation ensembles were generated by OMEGA, ROCS was used to identify the most similar conformations according to shape and the aligned structures and the shape Tanimoto similarity value were reported.

Acknowledgments

The authors would like to thank Maria Lindskog, for purification work, Christina Löwendahl and Hanna Nelander for separating isomers **2a** and **2b** and Gunnar Grönberg for structrual elucidation of isomers **2a** and **2b**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.03.075.

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