Design, Synthesis and Biological Activity of Rigid Cannabinoid CB₁ Receptor Antagonists

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The design, synthesis and biological activities of potent pyrazole-based tricyclic CB₁ receptor antagonists (2) are described. The key synthetic step involves the ring closure of the lithiated α,γ-keto ester adduct (4). The optimal nitroderivative (28) in this series exhibits a high CB₁ receptor affinity (pKᵢ = 7.2) as well as very potent antagonistic activity (pA₂ = 8.8) in vitro. The regioselectivity of the pyrazole ring closure is shown to depend strongly on the aromatic substitution pattern of the applied arylhydrazine.

Key words benzocycloheptapyrazole; CB₁ receptor antagonist; molecular modeling; regiochemistry

Cannabinoids are present in the Indian hemp Cannabis sativa and have been used as medicinal agents for centuries. However, only within the past ten years the research in the cannabinoid area has revealed pivotal information on CB receptors and their (endogenous) agonists. The discovery and the subsequent cloning of two subtypes of cannabinoid receptor (CB₁ and CB₂) stimulated the search for novel cannabinoid antagonists and triggered the development of cannabinoid drugs for the treatment of diseases.

Several types of CB₁ receptor antagonists are known (Fig. 1). Sanofi disclosed their selective CB₁ receptor antagonist SR141716A (rimonabant) which is currently undergoing clinical development for psychotic disorders and obesity treatment. Iodoprazadoline AM-630 was introduced in 1995. AM-630 is a moderately active CB₁ receptor antagonist, but sometimes behaves as a weak partial agonist. Researchers from Eli Lilly described the selective CB₁ receptor antagonist LY-320135. 3-Alkyl-5,5'-diphenylimidazolidinediones were described as cannabinoid receptor ligands, which were indicated to be cannabinoid antagonists. CP-272871 is a pyrazole derivative, like SR141716A, but less potent and less CB₁ receptor subtype-selective than SR141716A. Recently, Aventis Pharma claimed diarylmethylenazetidine analogs (1) as CB₁ receptor antagonists. Interestingly, many CB₁ receptor antagonists have been reported to behave as inverse agonists in vitro. Several reviews describe the current status in the cannabinoid research area.

In this paper our approach to tricyclic selective CB₁ receptor antagonists of general formula (2) is described. We envisioned that an additional ring constraint from the 4-methyl position in SR141716A to the ortho-position of its 5-aryl substituent would provide a novel class of considerably more rigid benzocycloheptapyrazoles (2), thereby having good prospects as potent CB₁ antagonists. Moreover, three-dimensional comparison of SR141716A with the more rigid counterpart (2) and analysis of their pharmacological results is expected to provide a more detailed insight in the required bioactive conformation of such CB₁ receptor antagonists, which is expected to facilitate their rational structure optimisation.

The four step synthesis route to the CB₁ antagonists (2) is exemplified by the preparation of 8 (Chart 1). Commercially available 1-benzosuberone (3) was deprotonated with lithium bis(trimethylsilyl)amide and subsequently reacted with diethyl oxalate to afford the lithiated keto ester adduct (4). The optimal nitroderivative (28) in this series exhibits a high CB₁ receptor affinity (pKᵢ = 7.2) as well as very potent antagonistic activity (pA₂ = 8.8) in vitro. The regioselectivity of the pyrazole ring closure is shown to depend strongly on the aromatic substitution pattern of the applied arylhydrazine.

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Fig. 1. Chemical Structures of CB₁ Receptor Antagonists

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was formed in 20% yield from 19 via a Sandmeyer reaction. This low yield is due to partial overreduction of 20 to 21.

In this series a number of compounds was prepared for pharmacological evaluation (Table 1).

**Biological Evaluation and Discussion**

The CB1 receptor affinities of thirteen compounds (8, 18, 20—30) were assessed in receptor binding studies (displacement of the specific binding of the CB receptor ligand [H]CP-55,940 to the human CB1 receptor cloned in Chinese hamster ovary (CHO) cells). CB1 receptor antagonistic activities were determined in a functional cell assay (blockade of the CB agonist CP-55,940 induced c-AMP formation in CHO cells, wherein the human CB1 receptor has been cloned). CB1 receptor binding results are expressed as pK\textsubscript{A} values. The CB1 antagonist potencies of the compounds are expressed as pA\textsubscript{2} values.

The pharmacological results of the compounds (8, 18, 20—30) are summarized in Table 2.

Our investigations started with compound (21). This compound is found less active than SR141716A in both assays. Compound (8), wherein the piperidinyl ring of 21 is replaced by the hexahydroazepinyl ring, behaves as a somewhat more potent CB1 receptor antagonist in vitro as compared to 21. However, this compound is still considerably less potent than SR141716A. These findings prompted to design 20, which has exactly the aromatic chloro substitution pattern of SR141716A. Compound (20) exhibits a decreased CB1 receptor binding affinity as compared to the binding of SR141716A. However, its CB1 antagonistic potency is comparable. Compound (25) wherein the piperidinyl ring of 20 is replaced by the hexahydroazepinyl ring shows the same activity profile. Nitro substitution at the 8-position gives rise to 18 and 28, respectively. Both 18 and 28 are very potent CB1 receptor antagonists, showing nanomolar activities in our CB1 functional assay.

Structural comparison by molecular modeling analysis of the closest structural analogue (20) with SR141716A gives some interesting results (Fig. 2). Both 20 and SR141716A were minimized using the MOPAC/PM3 module of the SYBYL package, version 6.3 (Tripos Associates, Inc., St. Louis, U.S.A.) on a Silicon Graphics Indigo2, Impact 10000.

As can be seen the difference in spatial conformation between both molecules is relatively small. This modeling result would predict comparable cannabinoid activities of the compounds. This is in line with the results from our functional CB1 antagonist assay. However, SR141716A is considerably more potent than its tricyclic congeners in the CB1 receptor binding assay.

SR141716A has been reported\(^9\) orally active in vivo. It is
interesting to note that compound (20) showed neither in vivo activity after oral nor i.p. dosing. These results prompted to investigate the bioavailability in our series CB1 antagonists of general formula (2) in more detail. After administration of compound (8), blood plasma levels in rat were found negligible (<50 ng/ml) at 1 h after p.o. (30 mg/kg) and i.p. administration (30 mg/kg), respectively. The low bioavailability of 8 might be ascribed to either its low rate of water dissolution in combination with a poor water solubility or its high lipophilicity (LogP ca. 5.9). This bioavailability issue has to be investigated in more detail.

In conclusion, benzocycloheptapyrazoles (2) constitute a class of very potent CB1 receptor antagonists in vitro. The bioavailability issue in this series of rigid cannabinoid receptor antagonists deserves further attention.

**Experimental**

1H- and 13C-NMR spectra were recorded on a Varian UN400 instrument (400 MHz), using DMSO-d6 or CDCl3 with (CH3)4Si as an internal standard, as solvents. Chemical shifts are given in ppm (δ scale). Thin-layer chromatography was performed on Merck precoated 60 F 254 plates, and spots were visualised with UV light. Column chromatography was performed using silica gel 60 (0.040—0.063 mm, Merck). Melting points were recorded on a Büchi B-545 melting point apparatus and are uncorrected. Mass spectra were recorded with a Micromass GCT or Kratos Concept 1S instrument (compounds (8) and (15)).

1-(2,4-Dichlorophenyl)-N-(hexahydro-1H-azepin-1-yl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide (8)

To a cooled solution (0 °C) of 16 (0.70 g, 1.88 mmol) in dichloromethane (15 ml) was successively added 1-aminohexahydro-1H-azepine (0.24 ml, 2.07 mmol) and EDC (0.4 g, 2.09 mmol). The resulting solution was allowed to attain room temperature and stirred for 48 h. After addition of brine, followed by extraction with CHCl3, the organics were washed with brine and dried over Na2SO4. The product was purified by column chromatography (Et2O/petroleum ether 5:1/v/v) to give 8 (0.60 g, 68%), mp 115—118 °C. 1H-NMR (CDCl3) δ: 1.62—1.68 (m, 4H), 1.72—1.82 (m, 4H), 2.22—2.30 (m, 2H), 2.69 (t, J=5.7 Hz, 2H), 2.80—3.12 (m, 2H), 3.15—3.19 (m, 4H), 6.65 (br d, J=5.8 Hz, 1H), 7.03 (td, J=5.8, 2 Hz, 1H), 7.21 (td, J=5.8, 2 Hz, 1H), 7.30 (br d, J=5.8 Hz, 1H), 7.39 (td, J=5.8, 2 Hz, 1H), 7.44 (d, J=2.2 Hz, 1H), 7.47 (d, J=2.2 Hz, 1H), 7.55 (br s, 1H). 13C-NMR (DMSO-d6) δ: 20.0, 26.7, 26.9, 31.8, 32.1, 57.7, 121.3, 126.5, 127.1, 128.89, 128.92, 129.1, 130.1, 130.2, 132.15, 132.23, 135.2, 136.4, 141.6, 142.9, 144.0, 159.9. Electron impact (EI)-MS m/z: 468 (M+1) (9), 355 (25), 98 (100). High resolution (HR)-EI-MS m/z: 468.1527 (Calcd for C25H26Cl2N4O: 468.1484).
1H). 13C-NMR (DMSO-d6) gave pure 13 (0.25 g, 15%) and 14 (0.28 g, 17%), respectively, followed by elution with MeOH/ethyl acetate (1:4) to give 15 (0.7 g, 46%).

Ethyl 1-(4-Methoxyphenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohept[a,1,2-c]pyrazole-3-carboxylate (17): mp 95—97 °C. H-NMR (DMSO-d6, δ): 1.35 (t, J = 7 Hz, 3H), 2.13—2.22 (m, 2H), 2.68 (t, J = 7 Hz, 2H), 2.75 (t, J = 7 Hz, 2H), 3.80 (s, 3H), 4.34 (q, J = 7 Hz, 2H), 6.71 (d, J = 8 Hz, 1H), 9.67 (d, J = 8 Hz, 2H), 7.08 (t, J = 8 Hz, 1H), 7.22—7.28 (m, 3H), 7.38 (d, J = 8 Hz, 1H). El-MS m/z: 362 (M+) (70), 316 (45), 289 (88), 100 (HR-EL-MS: m/z 362.1637 (Calcd for C21H20N2O2; 362.1630).

1-(2,4-Dichlorophenyl)-4-(1,4,5,6-tetrahydrobenzo[6,7]cyclohept[a,1,2-c]pyrazole-3-carboxylate (14): mp 89 °C. H-NMR (DMSO-d6, δ): 1.14 (t, J = 7 Hz, 3H), 2.02—2.10 (m, 2H), 2.79—2.85 (m, 2H), 3.01 (t, J = 7 Hz, 2H), 3.84 (s, 3H), 4.17 (q, J = 7 Hz, 2H), 7.01 (d, J = 8 Hz, 2H), 7.21—7.26 (m, 3H), 7.37 (d, J = 8 Hz, 2H), 7.79—7.98 (m, 1H). El-MS m/z: 362 (M+) (100), 333 (35), 289 (24). HR-EL-MS: m/z 362.1653 (Calcd for C21H19Cl2N2O2; 362.1650).

Synthesis of 18 and 29 Pure nitric acid (40%) is slowly added to the reaction mixture to obtain 18 (177.6 g, 36.9 mmol) and is added to the resulting mixture is stirred at room temperature overnight and poured onto ice. The formed precipitate is collected by filtration, washed with water and dried to give 17 (15.12 g, 98%) which consists of the 8- and 9-nitro-regiosomer in a molar ratio of 4:3. A suspension of compound 17 (6.27 g, 0.015 mol) in dry acetone (120 ml) is successively added disso-

Cyclohexyl 1-[(2,4-Dichlorophenyl)-4-(1,4,5,6-tetrahydrobenzo[6,7]cyclohept[a,1,2-c]pyrazole-3-carboxamide (22) (Preparation for the compound (8) was given, mp 118 °C. H-NMR (CDCl3) δ: 1.39—1.47 (m, 2H), 1.71—1.79 (m, 4H), 2.21—2.30 (m, 2H), 2.68 (t, J = 7 Hz, 2H), 2.80—3.30 (m, 6H), 6.64 (br d, J = 8 Hz, 1H), 7.01 (td, J = 8, 2 Hz, 1H), 7.20 (td, J = 8, 2 Hz, 1H), 7.29 (br d, J = 8 Hz, 1H), 7.51 (br s, J = 8 Hz, 1H), 7.38 (d, J = 8 Hz, 2H), 7.68 (brs, 1H). EI-MS m/z: 454 (M+) (35), 355 (75), 326 (90), 84 (100). HR-EL-MS: m/z 454.1288 (Calcd for C21H19Cl2N2O2). 1H-NMR (CDCl3) δ: 1.64—1.76 (m, 10H), 2.22—2.31 (m, 2H), 2.68 (t, J = 7 Hz, 2H), 2.76—3.06 (m, 2H), 3.10—3.16 (m, 1H), 6.65 (br d, J = 8 Hz, 1H), 7.02 (dd, J = 8, 2 Hz, 1H), 7.12 (dd, J = 8, 2 Hz, 1H), 7.30 (br d, J = 8 Hz, 1H), 7.38 (d, J = 8 Hz, 2H), 7.68 (brs, 1H). EI-MS m/z: 482 (7), 355 (75), 112 (100). (M+) HR-EL-MS: m/z 482.1616 (Calcd for C21H19Cl2N2O2). 1H-NMR (CDCl3) δ: 1.64—1.76 (m, 10H), 2.22—2.31 (m, 2H), 2.68 (t, J = 7 Hz, 2H), 2.76—3.06 (m, 2H), 3.10—3.16 (m, 1H), 6.65 (br d, J = 8 Hz, 1H), 7.02 (dd, J = 8, 2 Hz, 1H), 7.12 (dd, J = 8, 2 Hz, 1H), 7.30 (br d, J = 8 Hz, 1H), 7.38 (d, J = 8 Hz, 2H), 7.68 (brs, 1H). EI-MS m/z: 482 (7), 355 (75), 112 (100). (M+) HR-EL-MS: m/z 482.1616 (Calcd for C21H19Cl2N2O2). 1H-NMR (CDCl3) δ: 1.64—1.76 (m, 10H), 2.22—2.31 (m, 2H), 2.68 (t, J = 7 Hz, 2H), 2.76—3.06 (m, 2H), 3.10—3.16 (m, 1H), 6.65 (br d, J = 8 Hz, 1H), 7.02 (dd, J = 8, 2 Hz, 1H), 7.12 (dd, J = 8, 2 Hz, 1H), 7.30 (br d, J = 8 Hz, 1H), 7.38 (d, J = 8 Hz, 2H), 7.68 (brs, 1H). EI-MS m/z: 482 (7), 355 (75), 112 (100). (M+) HR-EL-MS: m/z 482.1616 (Calcd for C21H19Cl2N2O2).
2.65 (t, J = 7 Hz, 2H), 2.84—3.40 (m, 6H), 6.61 (d, J = 2 Hz, 1H), 7.18 (dd, J = 8, 2 Hz, 1H), 7.23 (d, J = 8 Hz, 1H), 7.43 (dd, J = 8, 2 Hz, 1H), 7.46 (d, J = 2 Hz, 1H), 7.50 (d, J = 8 Hz, 1H), 7.64—7.74 (m, 1H). EI-MS m/z: 488 (M⁺) (6), 362 (37), 84 (100). HR-EI-MS m/z: 488.0921 (Calcd for C₂₅H₂₅Cl₂N₅O₃: 488.0937).

9-Chloro-1-(2,4-dichlorophenyl)-N-(hexahydro-1H-azepin-1-yl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide (27) Procedure as for the preparation of compound (20) gave 27, mp 150 °C. ¹H-NMR (CDCl₃) δ: 1.62—1.67 (m, 4H), 1.72—1.80 (m, 4H), 2.20—2.28 (m, 2H), 2.65 (t, J = 7 Hz, 2H), 3.14—3.18 (m, 4H), 3.50—3.80 (m, 2H), 6.61 (d, J = 2 Hz, 1H), 7.18 (dd, J = 8, 2 Hz, 1H), 7.23 (d, J = 8 Hz, 1H), 7.43 (dd, J = 8, 2 Hz, 1H), 7.46 (d, J = 2 Hz, 1H), 7.49 (d, J = 8 Hz, 1H), 8.45 (br s, 1H). EI-MS m/z: 502 (M⁺) (6), 389 (62), 98 (100). HR-EI-MS m/z: 502.1049 (Calcd for C₂₅H₂₅Cl₂N₅O₃: 502.1094).

1-(2,4-Dichlorophenyl)-N-(hexahydro-1H-azepin-1-yl)-8-nitro-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide (28) Procedure as for the preparation of compound (18) gave 28 as an amorphous solid. ¹H-NMR (CDCl₃) δ: 1.62—1.68 (m, 4H), 1.70—1.82 (m, 4H), 2.26—2.37 (m, 2H), 2.80 (t, J = 7 Hz, 2H), 3.14—3.18 (m, 4H), 3.22—3.36 (m, 2H), 6.80 (d, J = 8 Hz, 1H), 7.42—7.46 (m, 2H), 7.53 (d, J = 8 Hz, 1H), 7.89 (dd, J = 8, 2 Hz, 1H), 8.12 (br s, 1H), 8.19 (d, J = 2 Hz, 1H). EI-MS m/z: 513 (M⁺) (7), 400 (47), 98 (100). HR-EI-MS m/z: 513.1291 (Calcd for C₂₅H₂₅Cl₂N₅O₃: 513.1334).

1-(2,4-Dichlorophenyl)-N-(hexahydro-1H-azepin-1-yl)-9-nitro-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-c]pyrazole-3-carboxamide (30) Procedure as for the preparation of compound (29) gave 30, mp 191—193 °C. ¹H-NMR (CDCl₃) δ: 1.62—1.70 (m, 4H), 1.72—1.80 (m, 4H), 2.25—2.35 (m, 2H), 2.70—2.90 (m, 4H), 3.14—3.20 (m, 4H), 4.72—7.50 (m, 3H), 7.54 (d, J = 2 Hz, 1H), 7.59 (d, J = 8 Hz, 1H), 8.07 (dd, J = 8, 2 Hz, 1H), 8.11 (br s, 1H). EI-MS m/z: 513 (M⁺) (19), 400 (89), 371 (32), 98 (100). HR-EI-MS m/z: 513.1318 (Calcd for C₂₅H₂₅Cl₂N₅O₃: 513.1334).

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References and Notes
20) During the preparation of this manuscript a group from Sanofi-Synthelabo published a patent application wherein the synthesis of structurally related tricyclic compounds is mentioned. Barth F., Congy C., Martinez S., Rinaldi M., Patent WO 01 32,663 (2001) [Chem. Abstr., 134, 340504 (2001)].