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Synthesis, biological evaluation, and structural studies on N1 and C5 substituted cycloalkyl analogues of the pyrazole class of CB1 and CB2 ligands

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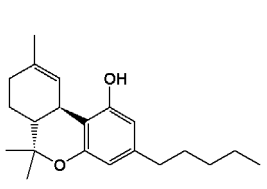
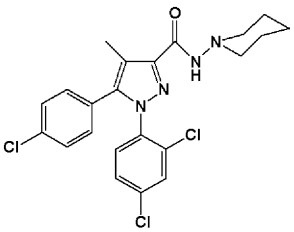
Abstract—A series of N1 and C5 substituted cycloalkyl and C5 4-methylphenyl analogues of the *N*-(piperidin-1-yl)-4-methyl-1H-pyrazole-3-carboxamide class of cannabinoid ligands were synthesized. The analogues were evaluated for CB1 and CB2 receptor binding affinities and receptor subtype selectivity. The effects of pyrazole substitution on ligand conformation and as such receptor affinities was not readily apparent; therefore, the geometries of the N1 and C5 substituents relative to the pyrazole ring were studied using high field NMR spectroscopy and systematic molecular mechanics geometry searches. An analysis of the relative ring geometries and functional group orientations provides new insight into the structural requirements of the CB1 and CB2 ligand binding pocket.

1. Introduction

Obesity is rapidly becoming one of the major health threats facing industrialized nations. In the United States it is estimated that over one third of the population is overweight with a statewide average of 20 percent being obese.[1,2] This condition is a complicating factor in diabetes and cardiovascular disease as well as causing an increased risk of certain types of cancer.[3,4] Overall, obesity ranks second to smoking in preventable deaths contributing to an estimated 300,000 deaths annually.[5] Diet and exercise is the first line therapy for this disease but this often fails due to non-compliance or poor education. Ultimately, many individuals afflicted with obesity seek chemotherapeutic intervention as a means of weight reduction. Stimulant drugs such as amphetamine, fenfluramine, and ephedrine have been used to treat obesity, however adverse effects and addiction can severely reduce the efficacy of these treatments.[6] Agonists of the β_3-adrenergic receptor have been shown to regulate fat cell thermogenesis and are under investigation for obesity therapy, however to date there are no clinically relevant drugs.[7–9] Similarly, discovery of leptin[10] and the leptin receptor[11] opened the possibility of a new therapeutic target for the treatment of obesity but molecules designed to target this pathway have yet to emerge. One aspect of the leptin pathway that provides another target for treating obesity is the receptor mediated regulation of the release of endocannabinoids thus regulating food intake.[12] This combined with the demonstrated appetite stimulation associated with other cannabinoid receptor 1 (CB1) agonists,[13,14] e.g., Δ^9-THC; **1**, indicated that antagonists of the receptor provided a novel target for treating obesity. In fact, this hypothesis is being tested by Sanofi-Synthelabo in Phase III clinical trails utilizing the diaryl pyrazole CB1 antagonist SR141716 (**2**; rimonabant).[15] The apparent success of SR141716 suggests that the development of new selective CB1 antagonists holds promise for the identification of novel anti-obesity drugs.



1, Δ^9-THC



2, SR141716

Keywords: Cannabinoid; Pyrazole; Cycloalkyl; NMR; Molecular modelling.
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The CB1 antagonist SR141716[16] is one of a large number of analogues that have been developed around the pyrazole scaffold.[17,16,18–21] Efforts to develop the SAR of this class of CB1 ligands have focused on the substitution and orientation of the N1 and C5 aryl functionalities and the substituent at the 3-carboxamide position. Structure activity studies on 3-carboxamide derivatives indicate that one or more hydrogen-bond donor-acceptor pairs between the ligand and the ligand binding pocket (LBP) exist;[22,23] furthermore, functional groups that occupy the aminopiperidine region of the LBP have an optimum length of 3 Å.[21] Synthetic analogues developed to probe the C5 and N1 positions have generally retained the aminopiperidine of SR141716 and one aromatic ring at either the C5 or N1 positions. The overall trend observed for CB1 activity shows that a 4-substituted phenyl ring at the C5 position is associated with high affinity receptor binding. In the N1 position 2,4-dichlorophenyl is the optimum substituent for both high CB1 affinity and receptor subtype selectivity.[24,20] The inclusion of an N1 4-butylphenyl, 4-pentylphenyl or phenyl group significantly reduces affinity (K_is = 150–433 nM)[18] although the introduction of *n*-pentyl, *n*-hexyl, and *n*-heptyl retains affinity (K_is = 23–47 nM)[23] comparable to the N1 4-chlorophenyl analogue. Unfortunately, it is not possible to make a comparison relative to the CB1 versus CB2 selectivity since CB2 receptor binding affinity is not reported for many of the analogues.

It is interesting to note that linear chain alkyl substituents in the N1 position cause only a modest reduction in binding.[23] This prompted us to question the effects of substituting cycloalkyl groups in either or both the C5 and N1 positions based on the following: 1) we have previously demonstrated that there is a pocket in both the CB1 and CB2 receptors capable of accommodating a cycloalkyl substituent at the C3 position of classical cannabinoids (CCBs);[25] 2) Shim et al. has suggested that the N1 substituent on the diaryl pyrazoles may occupy the same receptor subsite as the C3 side chain of CCBs;[23] and 3) it has been proposed that a cyclohexyl ring can serve as an isosteric replacement for a phenyl ring.[26] Based on these points a series of N1 and C5 substituted cycloalkyl 4-chlorophenylpryazoles and a dicycloalkylpyrazole were designed and synthesized. Additionally, the C5 4-methylphenyl N1 2,4-dichlorophenyl was synthesized to study if a halogen is required for the high affinity and selectivity reported for SR141716 and AM-251.[20] In this study we report the binding affinities of these compounds with the CB1 and CB2 receptors as well as 1D and 2D NMR studies directed at determining the solution conformation of these compounds relative to the diarylpryazoles analogues.

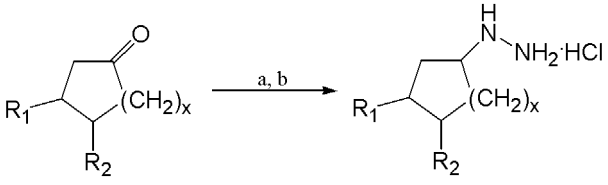
2. Results and discussion

2.1. Chemistry

The synthesis of the cycloalkyl substituted pyrazoles was carried out via the condensation of the appropriately 5 position substituted 3-methyl-2,4-dioxo-pentanoic acid ethyl ester with the required alkyl or aryl hydrazines.[20] The intermediate cycloalkyl hydrazines were prepared by reacting ketones 3-7 with *t*-butyl carbazate followed by the reduction of the N′-cycloalkylidene-hydrazinecarboxylic acid ethyl ester with diborane in 6N HCl yielding compounds **8**–**12** as the hydrochloride salts (Scheme 1).[27] The 5-aryl-3-methyl-2,4-dioxo-pentanoic acid ethyl esters were prepared as previously described by reacting the 4-chloro-1-phenyl-propan-1-one **13** with diethyl oxalate in the presence of lithium bis(trimethylsilyl)amide to provide **14** as the lithium salt (Scheme 2).[28] Compound **14** was then allowed to react with the hydrazine salts of **8**–**12** yielding the substituted 4-methyl-1H-pyrazole-3-carboxylic acid ethyl esters **15**–**19** (Scheme 2).[29] The resulting esters were hydrolyzed, converted to their acid chlorides utilizing $SOCl_2$ then reacted with 1-amino-piperidine in the presence of triethylamine to yield the corresponding carboxamides **20**–**24** of which **23** was isolated as a diastereomeric mixture.[20] In a similar fashion carboxamide **27** was synthesized from 4-methyl-1-phenyl-propan-1-one and 2,4-dichlorophenyl hydrazine (Scheme 3). The requisite 4-cycloalkyl-3-methyl-2,4-dioxo-butyric acid ethyl esters for the C5 cycloalkyl pyrazoles were prepared from either aldehyde **28** via reaction with ethyl magnesium bromide, oxidation of the resulting alcohol with PCC[30] or by the reaction of the Grignard of **29** with propionaldehyde and then oxidation with PCC (Scheme 4). The ketones **30** and **31** were converted to **32** and **33** as previously described then allowed to react with 4-chloro-phenyl hydrazine each yielding the C5 and N1 substituted cycloalkyl pyrazoles which were resolved via flash chromatography. Utilizing the aforementioned chemistry compounds **34** and **35** were converted to the aminopiperidine carboxamides **36** and **37**. The dicyclohexyl pyrazole analogue **39** was prepared from **32** and **9** (Scheme 5).

2.2. Receptor binding assays

Cell membranes from HEK293 cells transfected with the human CB1 receptor and membranes from CHO-K1 cells transfected with the human CB2 receptor were used in the receptor binding assays.[25] Binding affinities of compounds **20**–**24**, **27**, **36**, **37**, and **39** were determined by assaying the displacement of [^{3}H]-CP 55,940 from the CB1 and CB2 receptor preparations by increasing concentrations of the pyrazole analogues (Table 1). The receptor affinities of our analogues displayed unique affinities for the CB1 and CB2 receptors;

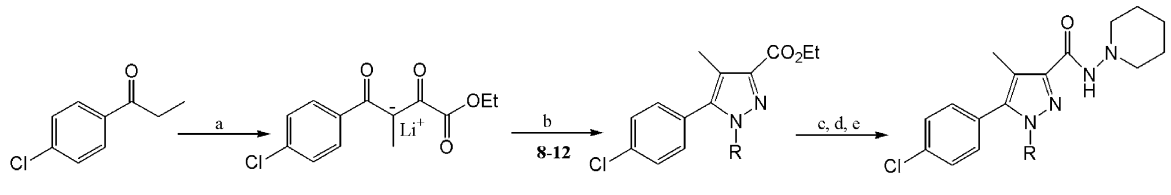


3, R_1 = R_2 = H, x=1
4, R_1 = R_2 = H, x=2
5, R_1 = R_2 = H, x=3
6, R_1 = CH_3, R_2 = H, x=2
7, R_1 = H, R_2 = CH_3, x=2

8, R_1 = R_2 = H, x=1
9, R_1 = R_2 = H, x=2
10, R_1 = R_2 = H, x=3
11, R_1 = CH_3, R_2 = H, x=2
12, R_1 = H, R_2 = CH_3, x=2

(a) *t*-butyl carbazate, hexane; (b) B_2H_6, 6N HCl

Scheme 1.



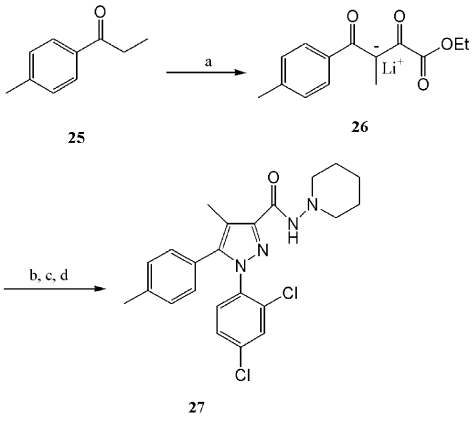
13 **14**

15, R = cyclopentyl
16, R = cyclohexyl
17, R = cycloheptyl
18, R = 3-methyl-cyclohexyl
19, R = 4-methyl-cyclohexyl

20, R = cyclopentyl
21, R = cyclohexyl
22, R = cycloheptyl
23, R = 3-methyl-cyclohexyl
24, R = 4-methyl-cyclohexyl

(a) LiHMDS, diethyl oxylate, ether; (b) EtOH, TEA; (c) MeOH, KOH; (d) toluene, $SOCl_2$; (e) 1-amino piperidine, DCM, TEA

Scheme 2.



25 **26** **27**

(a) LiHMDS, diethyl oxylate, ether; (b) EtOH, TEA, 2,4-dichlorophenyl hydrazine hydrochloride; (c) MeOH, KOH; (d) toluene, $SOCl_2$; (e) 1-amino piperidine, DCM, TEA

Scheme 3.

however, only compound **27** had an affinity and selectivity (CB1 $K_i = 39$ nM, CB1–CB2 = 1:64) comparable to the benchmark diarylpryazole SR141716 (CB1 K_i range = 1.98–12.3 nM, CB1–CB2 ratio range = 1:57–1:505)[31–33,16,20] and AM-251 (CB1 $K_i = 7.49$ nM, CB1–CB2 = 1:306).[20] Within the C5 substituted cycloalkyl series, the cyclohexyl, **21**, and cycloheptyl, **22**, derivatives had a modest 9.1 and 8.0 CB1–CB2 ratio, respectively, with CB1 affinities of 351 and 275 nM. Interestingly, substitution of the C5 cyclohexyl group for 3-methyl- and 4-methyl cyclohexyl had little effect on the CB1 affinity but did result in an 11.4- and 4.6-fold increases in CB2 affinity for the 3-methyl, **23**, and 4-methyl, **24**, compounds, respectively. Despite the modest affinities of the C5 substituted analogues for both receptor subtypes it is interesting to note the that the C5 phenyl N1 2,4-dichlorophenyl pyrazole (CB1 $K_i = 123$ nM, CB1–CB2 ratio = 1:1.8) reported by Lan et al. had modestly higher affinities, i.e., 1.3–4-fold,[20] compared to those observed for the methylcycloalkyl anaologues. This suggests that under certain conditions the cyclohexyl group can be substituted for a phenyl ring.

Substitution of the N1 aryl group for cyclohexyl, **36**, and cycloheptyl, **37**, resulted in compounds with 133–410 nM affinities that were relatively non-selective for the receptors subtypes. With respect to the equivalent C5 substituted analogues **21** and **22**, the CB1 affinities are essentially the same; however, analogue **36** has a 24-fold higher affinity for the CB2 receptor relative to **21** while the affinity of **37** for the same receptor shows a 5-fold increase. These data suggest that CB2 selective pyrazole analogues can be developed utilizing **36** as the lead structure. Substitution of the pyrazole scaffold with two cyclohexyl groups, compound **39**, drastically reduced the CB1 affinity and totally abolished binding to the CB2 receptor. In combination these data suggested that interesting information regarding the ligand–receptor interactions could be derived; therefore, these compounds were studies by high field NMR spectroscopy and molecular modeling studies.

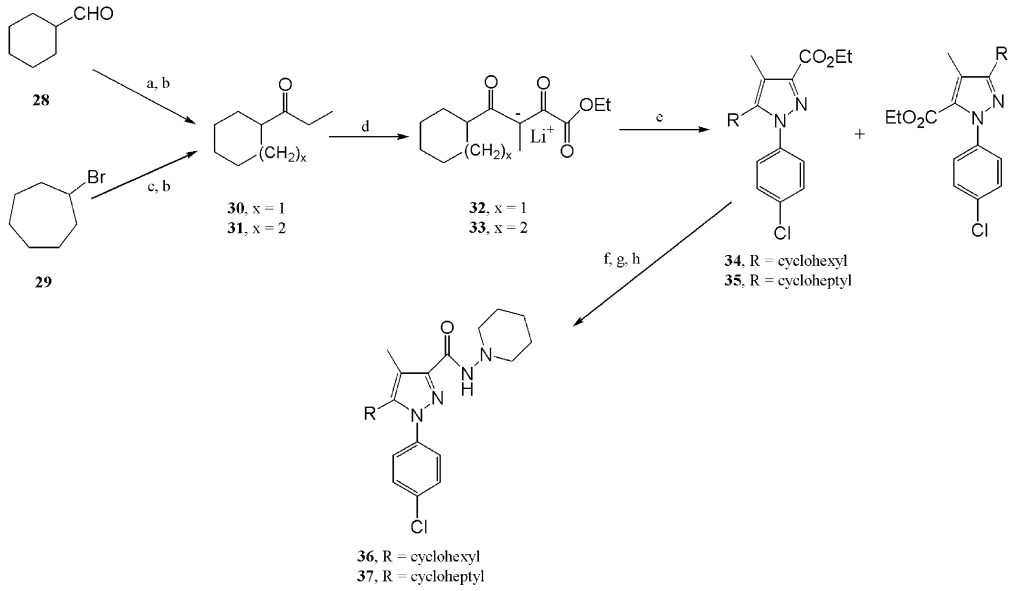
Table 1. Binding affinities of the pyrazole analogues **20–24**, **27**, **36**, **37**, and **39** for the CB1 and CB2 receptors

Compd	CB_1 K_i (nM)[a]	CB_2 K_i (nM)	Ratio CB_1/CB_2
20	1560 (±77)	1020 (±22)	1 : 0.65
21	351 (±1.5)	3210 (±45)	1 : 9.1
22	275 (±67)	2197 (±21)	1 : 8.0
23	494 (±57)	281 (±11)	1 : 0.56
24	264 (±26)	479 (±50)	1 : 1.8
27	39 (±2.0)	2490 (±102)	1 : 64
36	318 (±8.5)	133 (±30)	1 : 0.42
37	273 (±19)	410 (±10)	1 : 1.5
39	5110 (±110)	$>2.5\times10^5$	1 : 49

[a] The K_i values for the analogues were obtained from $n \geq 3$ independent experiments run in triplicate showing the standard error of the mean in parentheses.

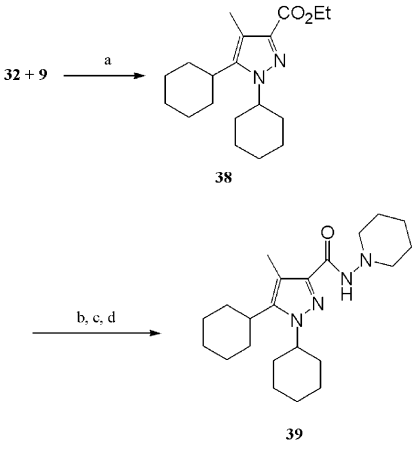
2.3. NMR and molecular modeling studies

The affinity spectrum observed for our compounds suggested that conformational studies on the series could provide insight into the structural elements underlying the activity. It was predicted that the increased steric bulk of the cycloalkyl group in either the N1 or C5 position would affect the orientation of the remaining aromatic ring relative to the pyrazole ring. Due to the fact that the substitution of the 4-methylphenyl of **27** for the 4-iodophenyl of AM-251 for cycloalkyl groups has negligible effects on the calculated logP, it was proposed that the spatial orientation of the N1 and C5 sub-



(a) EtMgBr, THF; (b) PCC, DCM; (c) Mg, THF, propionaldehyde; (d) LiHMDS, diethyl oxylate, ether; (e) EtOH, TEA, 4-chlorophenyl hydrazine hydrochloride; (f) MeOH, KOH; (g) toluene, $SOCl_2$; (h) 1-amino piperidine, DCM, TEA

Scheme 4.



(a) EtOH, TEA; (b) MeOH, KOH; (c) toluene, $SOCl_2$; (d) 1-amino piperidine, DCM, TEA

Scheme 5.

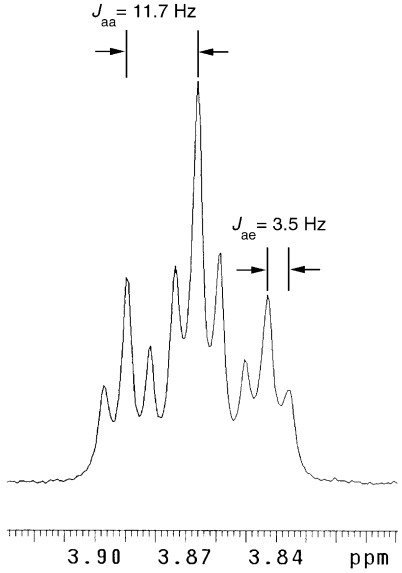


Figure 1. Partial 1D NMR spectrum of 21 showing the axial and equatorial coupling constants of the cyclohexyl methyne proton.

stituents played a major role in binding affinity. To examine this effect 1D and 2D high field NMR experiments were conducted on compounds **21**, **24**, **27**, **36**, **39**, and AM-251. The chemical shifts of the protons in the aforementioned compounds were first assigned using 1D, *g*HSQC, *g*HMBC, and *g*COSY experiments. An analysis of the methyne proton signals in the 1D spectra of **21**, **24**, **36**, and **39** showed a clear triplet of triplets (Fig. 1) indicating that the methyne proton was in the axial position for all the cycloalkyl derivatives. To understand the relative spatial orientation of all the assigned protons 2D NOESY experiments were conducted on the analogues. In all cases a network of NOEs was observed interconnecting the C4 methyl, the C5 substituent and the N1 substituent (Fig. 2). This figure only shows the NOEs that were used to establish the relative orientation of the pyrazole substituents and not intra-functional group signals. Interestingly, in compounds **21**, **24**, and **39** weak signals between the cyclohexyl protons and the NH of the carboxamide were observed with a clear absence of a NH to C3 methyl NOE. This suggests that, in chloroform and for the compounds showing the NOE, the geometry of the carboxamide is the T_s and T_g conformation as previously defined by Shim et al. (Fig. 3).[23] In addition, the degeneracy of the methylene protons in the piperidine ring, that is, no resolvable axial equatorial coupling, suggest that the piperidine ring is rapidly inter-converting between the s and g form in the NMR time scale.

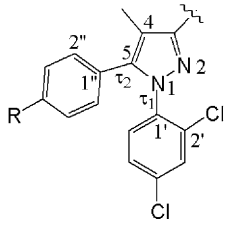
A qualitative analysis of the NOE intensities as well as the presence or absence of NOEs suggested that introduction of a cycloalkyl at either or both the N1 and C5

positions resulted in geometries unique to each class of the analogues. In order to more accurately define the relative orientation of the N1 and C5 substitutents with respect to the pyrazole ring a quantitative analysis of the NOESY spectra was conducted. This was accomplished by integrating of the non-overlapping unambiguously assigned peaks utilizing TRIPOS TRIAD followed by constraint generation utilizing MARDIGRAS.[34] Due to the degeneracy of the piperidine ring, no constraints were generated for NOEs between this ring and the carboxamide NH. The number of constraints generated and their location are shown in Figure 2. Utilizing these constraints the models were subjected to 10 cycles of simulated annealing to determine the populations of conformers associated with the NMR data. Compounds **36** and **39** converged on a single conformer while compounds **27**, **21**, **24**, and AM-251 had two conformers that could be found on the potential energy surface derived from a systematic molecular mechanics search of τ_1 and τ_2 (Table 2). The torsional values for **27** and AM-251 are consistent with the low energy conformations of unprotonated SR141716 reported by Shim et al.[23] Taken together these data support the NMR derived structures thus identifying the predominant conformation of the compounds in chloroform; furthermore, the conformations may reflect the aqueous structure when considering the limited steric space available to cyclic substituents appended from the N1 and C5 positions of the pyrazole ring.

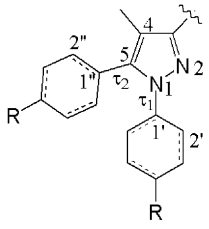
The NMR derived structures combined with the receptor affinity data suggested that information on the ligand binding pockets of the CB1 and CB2 receptors could be derived from a structural analysis. A detailed analysis of the receptor interactions utilizing CoMFA or a related method was not deemed appropriate due to a lack of functional data; however, a qualitative analysis of the molecular surfaces could identify basic elements that give rise to the observed binding affinities. To this end, the NMR derived average structures were aligned by overlaying the pyrazole atoms of each compound followed by Connolly molecular surface generation utilizing MOLCAD (Fig. 2).[34] In general, **27** and AM-251 are identical with the exception of a 21 $Å^3$ increase in volume arising from the iodo group and slight increase in clogP from 6.19 to 6.28 for the unprotonated forms of 27 and AM-251, respectively. However, the differences in the receptor affinities and selectively are significant suggesting that there are electronic effects that cannot be studied via molecular mechanics based calculations. This possible role is presently being investigated using quantum mechanical calculations to assess potential electronic effects.

The role of hydrophobic interactions between the receptor and the pyrazole N1 and C5 substitutents has been studied by several groups.[18–20,23] Compounds containing hydrophobic groups, such as aryl, alkylaryl, and *N*-alkyl, at the N1 and C5 positions and analogues containing a 1,4-dihydroindeno[35] (Fig. 4) group generally bind to both CB receptor types; however, the spectrum of affinities as well as CB1 and CB2 selectivity appear to be dependent on the spatial orientation of the groups relative to the pyrazole ring. In the context of

Table 2. Low energy pyrazole analogue conformations of τ_1 and τ_2 as determined by NMR and systematic conformational searches



27 and AM-251



21, **24**, **36**, and **39**

	Simulated Annealing		Systematic search		
Compd	Rotamers[a]	τ_1, τ_2	Local minima	Unique rotomers[b]	τ_1, τ_2
27 and AM-251	2	48, 234 310, 305	8	4	40, 230 120, 320 230, 230 310, 310
21 and **24**	2	10, 249 61, 285	6	4	10, 250 50, 300 60, 240 110, 300
36	1	98, 290	4	2	80, 120 90, 290
39	1	80, 325	4	4	60, 110 80, 330 250, 280 270, 270

[a] Average minimized structure of rotomer populations.
[b] Conformations not arising from the 2-fold symmetry of the cyclohexyl 4-methylphenyl, 4-iodophenyl, and 4-chlorophenyl group are defined as unique.

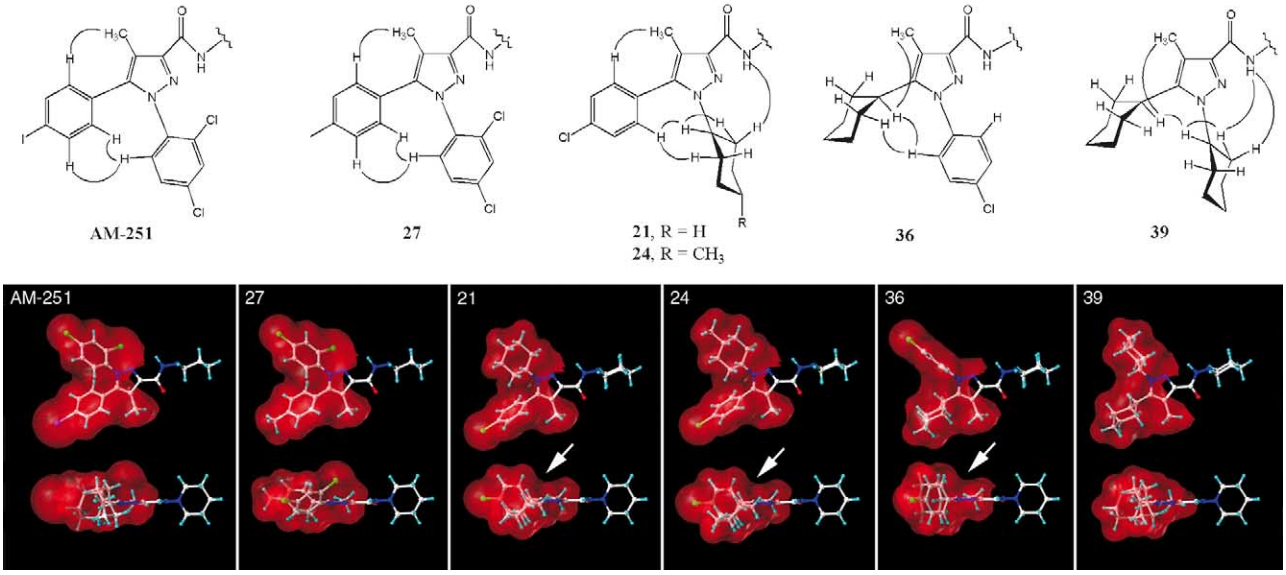


Figure 2. The upper panel shows the inter-proton NOEs (curved lines) that were utilized to calculate the inter-proton distance constraints. The lower panel shows the average structures calculated utilizing simulated annealing studies that are surrounded by the molecular surfaces of the pyrazole, N1, and C5 substituents. White areas indicate potential volume excluded regions in the CB2 receptor.

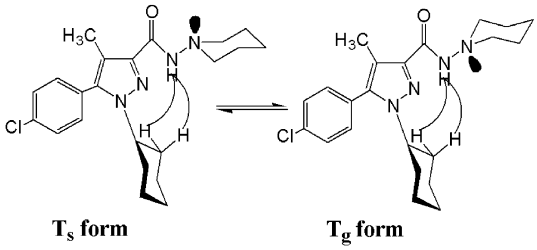


Figure 3. Proposed conformations of the *N*-piperidinyl carboxamide moiety in compounds **21**, **24**, and **39**. The NOEs between the carboxamide NH and the cyclohexyl protons combined with the degenerate *N*-α methylene protons suggests that the *N*-piperidinyl carboxamide is interconverting between the T_s and T_g forms.

the present study, the orientation of the cycloalkyl groups and directing effects of the cycloalkyl groups on the aromatic ring were of particular interest. In the report by Shim et al.[23] the N1 cyclohexyl C5 phenyl analogue had comparable affinity to our N1 cyclohexyl C5 4-chlorophenyl analogue **21** for the CB1 receptor (391 versus 351 nM); however, the affinity of the N1 *n*-hexyl and *n*-heptyl C5 phenyl analogues reported by Shim et al. are, on average, 10-fold higher for the CB1 receptor. The aforementioned compounds are the carbon equivalents of **21** and **22** with one distinct difference; the conformational space available is drastically reduced in the cyclic analogues. In combination these results support the hypothesis that the N1 linear sidechains adopt a conformation in the receptor that approximates the geometry of an N1 aryl group. Additionally, for compounds **27**, AM-251, **21**, and **22** that exhibit high to modest receptor selectivity, the structural data suggest that a τ_2 in the range of 230 degrees is optimum for selectivity. This result may suggest that the high CB2 selectivity observed for the 1,4-dihydroindeno is in part due to locking τ_2 into a low energy torsional well between −10 and 10 degrees.

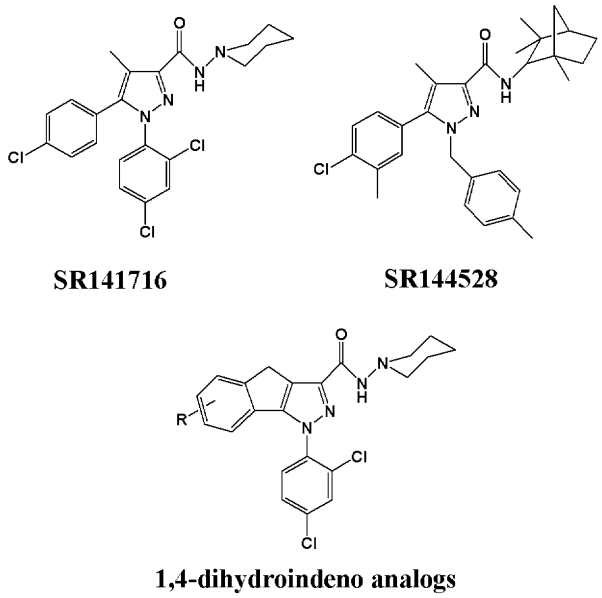


Figure 4. Molecular structures of related pyrazole CB1 and CB2 receptor ligands.

It is clear that the angle of τ_2 is not the sole determinate of subtype selectivity when considering that SR144528 is a CB2 selective pyrazole analogue.[36] Two important modifications in SR144528 are the replacement of the aminopiperidine by (1*S*)-endo-fenchylamine and the introduction of a 4-methylbenzyl group in the N1 position. Although the (1*S*)-endo-fenchylamine functionality likely contributes significantly to CB2 selectivity, the introduction of the 4-methylbenzyl is of particular interest in this study since: (1) introduction of the methyl groups in compounds **23** and **24** increased the CB2 affinity relative to **21** and; (2) a comparison of the molecular surfaces in Figure 2 suggest that there is a receptor excluded volume in the CB2 receptor. This excluded volume may be, in part, responsible for the

selectivity of SR144528 wherein the methylene spacer effectively projects the 4-methylphenyl past the sterically restricted region of the CB2 receptor. Interestingly, the increased steric bulk of the 4-methylphenyl and analogue **23** does correlate with the reported decreased affinity for the CB1 receptor when hydrophobic groups are extended beyond the N1 2,4-dichlorophenyl moieties in SR141716 related compounds.[23]

The 5- to 24-fold higher CB2 affinities of the C5 cycloalkyl derivatives **34** and **35** relative to the equivalent N1 congeners **21** and **22** was surprising when considering that the CB1 affinities were essentially equivalent. The solution structure models suggest that the steric bulk of the cyclohexyl ring directs both the N1 and C5 substituents into a geometry that is orthogonal to the pyrazole ring (Fig. 2). This finding is significant in that, of the compounds that have affinity for either receptor, only compound **34** is predicted to be restricted to two doubly orthogonal rotomers of which only one satisfies the NMR data. It is difficult to assess the significance of this result since ligands do not necessarily bind to receptors in low energy conformations; however, this geometry does provide a novel template molecule from which QSAR studies can be based. To this end, the relative lack of affinity of **39** may further aid in defining the steric limitations of the CB1 and CB2 receptor ligand binding pocket.

3. Conclusions

A series of N1 and C5 cycloalkyl substituted pyrazole aminopiperizine carboxamides as well as the C5 4-methylphenyl analogue of SR141716 were synthesized and evaluated for CB1 and CB2 affinity. Within this series the 4-methylphenyl derivative **25** had an affinity and subtype selectivity comparable to the benchmark compound SR141716 while the N1 cyclohexyl and cycloheptyl analogues had modest selectivity that is comparable to several diaryl pyrazoles. The qualitative analysis of the NMR derived structures has identified structural features that are consistent with and supportive of previous QSAR studies. Our studies have also identified additional elements in the pyrazole structures that may provide new insights into the requirements of the ligand binding pockets of the CB1 and CB2 receptors. We believe that the results of this study, in combination with the recently reported 1,4-dihydroindeno pyrazole analogues, will enable researchers to develop and refine the pyrazole QSAR models of the ligand binding pockets of the CB1 and CB2 receptors.

4. Experimental

All chemicals and reagents were purchased from Sigma-Aldrich or Fisher Scientific Inc. AM-251 was purchased form Tocris. Anhydrous solvents were prepared by distillation over sodium metal or calcium hydride just prior to use. All reactions were carried out under dry conditions and under an argon atmosphere. Silica Gel 60, 200–425 mesh was used for flash chromatography. Routine NMR spectra were obtained on a Bruker ARX-300MHz NMR and were consistent with the assigned structure, high resolution 1D and 2D spectra were obtained on a Varian Inova 500 MHz NMR. All NMR were recorded in $CDCl_3$ unless otherwise specified. Routine mass spectra were determined on a Bruker ESQUIRE Ion Trap LC/MS(n) system. Elemental analyses were performed by Atlantic Microlabs. Corrected melting points were determined on an Electrothermal IA9000 digital melting point apparatus. IR spectra were measured on a Perkin–Elmer Model 1605 FT infrared spectrophotometer. Thin layer chromatography was performed on silica gel plates (Merck TLC plates, silica gel 60, F_{254}).

4.1. Cyclopentylidene carbazate (3)

Cyclopentanone (2.65 mL, d = 0.951, 30 mmol) and tert-butyl carbazate (3.96 g, 30 mmol) in 33 mL hexane were heated to reflux for 20 min. The reaction mixture was then cooled to room temperature and the precipitate filtered and dried to afford the carbazate as a white solid 5.28 g (88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.51 (s, 9H), 1.84 (m, 4H), 2.19 (t, J = 7.2 Hz, 2H), 2.49 (t, J = 7.2 Hz, 2H), 7.23 (brs, 1H); MS: (ESI, Pos) m/z 221.1 [$(M+23)^+$].

Utilizing the appropriate cycloalkyl ketones the following cycloalkyl carbazates were similarly prepared.

4.1.1. Cyclohexylidene carbazate (4). Yield 5.92 g (92.2%) as a white solid. ^{1}H NMR (300MHz, $CDCl_3$): δ (ppm) 1.52 (s, 9H), 1.66 (m, 6H), 2.20 (t, J = 6 Hz, 2H), 2.36 (t, J = 6 Hz, 2H), 7.52 (brs, 1H); MS: (ESI, Pos) m/z 235.1 [$(M+23)^+$].

4.1.2. Cycloheptylidene carbazate (5). Yield 12.37 g (91.1%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.50 (s, 9H), 1.59 (m, 6H), 1.73 (m, 2H), 2.28 (t, J = 6 Hz, 2H), 2.53 (t, J = 6.3 Hz, 2H), 7.38 (brs, 1H); MS: (ESI, Pos) m/z 249.1 [$(M+23)^+$].

4.1.3. 3-Methyl-cyclohexylidene carbazate (6). Yield 6.12 g (90.0%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.00 (m, 3H), 1.18 (m, 1H), 1.51 (s, 9H), 1.64 (m, 2H), 1.82 (m, 3H), 2.10 (m, 1H), 2.58 (m, 2H), 7.59 (brs, 1H); MS: (ESI, Pos) m/z 249.1 [$(M+23)^+$].

4.1.4. 4-Methyl-cyclohexylidene carbazate (7). Yield 6.42 g (94.7%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 0.95 (d, J = 6.6 Hz, 3H), 1.20 (m, 2H), 1.50 (s, 9H), 1.69 (m, 1H), 1.88 (m, 3H), 2.21 (m, 1H), 2.55 (m, 2H), 7.58 (brs, 1H); MS: (ESI, Pos) m/z 249.1 [$(M+23)^+$].

4.1.5. Cyclopentyl hydrazine hydrochloride (8). Diborane (25 mL of 1M solution in THF, 25 mmol) was added to compound 3 (5.0 g, 25.3 mmol) and the reaction mixture was allowed to stir at room temperature for 15 min after which 6N HCl (13 mL) was added dropwise. The reaction mixture was then heated over a steam bath for 15 min and the solvent removed under

reduced pressure. The residue was then dissolved in THF and boric acid was filtered off. The solvent was then evaporated under reduced pressure and the residue recrystallized from THF/ether to afford 2.64 g (76.7%) of compound **6** as white crystalline solid. Mp 129 °C. ^{1}H NMR (300 MHz, DMSO): δ (ppm) 1.70 (m, 8H), 3.51 (m, 1H), 5.02 (brs, 4H); MS: (ESI, Pos) *m*/*z* 101.4 [(M + 1)$^+$].

Using the appropriate cycloalkyl carbazates the following cycloalkyl hydrazine hydrochlorides were similarly prepared.

4.1.6. Cyclohexyl hydrazine hydrochloride (9). Yield 1.64 g (46.5%). Mp 112–113 °C. ^{1}H NMR (300 MHz, DMSO): δ (ppm) 1.12 (m, 5H), 1.59 (m, 1H), 1.74 (m, 2H), 1.96 (s, 2H), 2.83 (m, 1H), 5.00 (brs, 4H); MS: (ESI, Pos) *m*/*z* 115.2 [(M + 1)$^+$].

4.1.7. Cycloheptyl hydrazine hydrochloride (10). Yield 2.30 g (46.7%) as white crystalline solid. Mp 80 °C ^{1}H NMR (300 MHz, DMSO): δ (ppm) 1.53 (m, 10H), 2.02 (m, 2H), 3.04 (m, 1H), 5.42 (brs, 4H); MS: (ESI, Pos) *m*/*z* 129.1 [(M + 1)$^+$].

4.1.8. 3-Methyl-cyclohexyl-hydrazine hydrochloride (11). Diborane (26.6 mL of 1M solution in THF, 26.6 mmol) was added to compound 6 (6.00 g, 26.6 mmol) and the reaction mixture was allowed to stir at room temperature for 15 min after which 6N HCl (14 mL) was added to it dropwise. The reaction mixture was then heated over a steam bath for 15 min and the solvent removed under reduced pressure. The residue was then dissolved in THF and boric acid was filtered off. The solvent was then evaporated under reduced pressure and the residue was taken to the next step without further purification.

4.1.9. 4-Methyl-cyclohexyl-hydrazine hydrochloride (12). Diborane (26.6 mL of 1M solution in THF, 26.6 mmol) was added to compound **7** (6.01 g, 26.6 mmol) and the reaction mixture was allowed to stir at room temperature for 15 min after which 6N HCl (14 mL) was added to it dropwise. The reaction mixture was then heated over a steam bath for 15 min and the solvent removed under reduced pressure. The residue was then dissolved in THF and boric acid was filtered off. The solvent was then evaporated under reduced pressure and the residue was taken to the next step without further purification.

4.1.10. 1-cyclohexyl-propanone (30). Cyclohexane carboxaldehye (**28**, 12.1 mL, d = 0.926, 100 mmol) was dissolved in dry THF (250 mL) and cooled to −20 °C. To this solution ethyl magnesium bromide (40 mL of 3M solution in ether, 120 mmol) was added slowly and the reaction mixture was then allowed to stir at the same temperature for 5 h. 1N HCl was then added to the reaction mixture and it was extracted with ether. The organic layer was separated, dried over sodium sulfate and then evaporated under reduced pressure to afford the 12 g of crude alcohol as a colorless oil. The crude alcohol was dissolved in methylene chloride and to this a mixture of Celite (43 g) and PCC (43 g, 200 mmol) was added and the reaction mixture was allowed to stir at room temperature for 18 h. It was then diluted with ether and stirred for 30 min, then filtered over a pad of silica gel, washed with ether and the ether evaporated under reduced pressure to give the crude product. The crude product was then purified by column chromatography using EtOAc–hexane (4:96) to yield the ketone as a yellow oil (8.08 g, 68.3%). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.03 (t, *J* = 7.2 Hz, 3H), 1.28 (m, 5H) 1.71 (m, 5H), 2.35 (m, 1H), 2.46 (q, *J* = 7.2 Hz, 2H); IR (KBr, neat): υ 1712 cm^{-1} (C=O); MS: (ESI, Pos) *m*/*z* 163.1 [(M + 23)$^+$].

4.1.11. 1-cycloheptyl-propanone (31). Cycloheptyl bromide (**29**, 10 mL, d = 1.289, 72.8 mmol) was added to dried magnesium turnings in 90 mL of dry THF and the reaction mixture was heated to reflux for 30 min to form the Grignard reagent. Propionaldehyde (2.08 g, 35.9 mmol) was added to dry THF (56 mL) and the solution was cooled to −20 °C. To this solution, the Grignard reagent was added dropwise and the reaction mixture was allowed to stir at the same temperature for 6 h. 1N HCl was then added to the reaction mixture and it was extracted with ether. The organic layer was separated, dried over sodium sulfate and then evaporated under reduced pressure to afford 1 g of the crude alcohol as colorless oil. The crude alcohol was then oxidized to the corresponding ketone using PCC as described above. Yield 0.84 g (85%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.05 (t, *J* = 6 Hz, 3H), 1.45 (m, 8H), 1.77 (m, 4H), 2.45 (q, *J* = 6 Hz, 2H), 2.53 (m, 1H); IR (KBr, neat): υ 1713 cm^{-1} (C=O); MS: (ESI, Pos) *m*/*z* 177.3 [(M + 23)$^+$].

4.1.12. 1-(Cyclopentyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (15). 4′-chloropropiophenone (**13**, 1.55 g, 9.23 mmol) was dissolved in anhydrous THF (24 mL) and cooled to −78 °C. To this LiHMDS (9.3 mL of 1M solution in hexane, 9.3 mmol) was added and the reaction mixture was stirred at the same temperature for 45 min after which it was warmed to −20 °C and then cooled back to −78 °C. Then, diethyl oxalate (1.46 mL, 10.4 mmol) in dry THF (3 mL) was added to it and the reaction mixture was allowed to stir at room temperature for 20 h. It was then diluted with ether and washed with 1N HCl. The organic layer was then separated, dried over sodium sulfate and then evaporated under reduced pressure to give 2.43 of crude ethyl-2,4-dioxo-3-methyl-4- (4-chlorophenyl)-butanoate as yellow oil. The crude product (2.43 g, 8.99 mmol) was dissolved in ethanol (24.3 mL) and to this compound 8 (1.27 g, 8.99 mmol) and triethyl amine (0.91 g, 8.99 mmol) were added. The reaction mixture was then heated to reflux for 9 h. It was then cooled, washed with brine and extracted with tert-butyl ethyl ether. The organic layer was then separated, dried over sodium sulfate and evaporated. The crude product was then purified by column chromatography with petroleum ether–ethyl acetate (95:5) to give compound **15** as a yellow solid (697 mg, 23.4%). R_f = 0.3 (ethyl acetate–petroleum ether 5:95). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.40 (m, 4H), 1.56 (m, 2H), 1.69 (s, 1H), 1.93 (m, 4H), 2.13 (s, 3H), 4.41 (m, 3H), 7.22 (d, *J* = 8.1 Hz, 2H), 7.48 (d, *J* = 8.4 Hz, 2H); MS: (ESI, Pos) *m*/*z* 333.4 [(M + 1)$^+$] and 355.4 [(M + 23)$^+$].

Utilizing the appropriate cycloalkyl or substituted phenyl hydrazine hydrochlorides and the appropriate diketo esters the following pyrazole esters were similarly prepared.

4.1.13. 1-(Cyclohexyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (16). Yield 554 mg (24.9%) as a white solid. $R_f = 0.39$ (ethyl acetate–petroleum ether 5:95) ^{1}H NMR (300MHz, $CDCl_3$): δ (ppm) 1.22 (m, 3H), 1.41 (t, $J = 7.2$ Hz, 3H), 1.57 (s, 3H), 1.83 (m, 4H), 2.13 (s, 3H), 3.91 (m, 1H), 4.42 (q, $J = 7.2$ Hz, 2H), 7.19 (d, $J = 8.4$ Hz, 2H), 7.48 (d, $J = 8.1$ Hz, 2H); MS: (ESI, Pos) m/z 347.4 $[(M+1)^+]$ and 369.4 $[(M+23)^+]$.

4.1.14. 1-(Cycloheptyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (17). Yield 1.91 g (39.3%) as a white solid. $R_f = 0.52$ (petroleum ether–ethyl acetate 95:5) ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.41 (t, $J = 7.2$ Hz, 3H), 1.58 (m, 6H), 1.82 (m, 4H), 2.12 (s, 3H), 2.21 (m, 2H), 4.09 (m, 1H), 4.42 (q, $J = 7.2$ Hz, 2H), 7.19 (d, $J = 8.4$ Hz, 2H), 7.48 (d, $J = 8.7$ Hz, 2H); MS: (ESI, Pos) m/z 361.4 $[(M+1)^+]$ and 383.3 $[(M+23)^+]$.

4.1.15. 1-(3-Methyl-cyclohexyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (18). Yield 1.04 g (31.3%) as a yellow oil. $R_f = 0.49$ (hexane/ethyl acetate 9:1) ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 0.90 (m, 3H), 1.38 (t, $J = 6.9$ Hz, 3H), 1.67 (m, 1H), 1.80 (m, 2H), 2.02 (m, 2H), 2.13 (s, 3H), 2.24 (m, 2H), 2.36 (m, 1H), 3.97 (m, 1H), 4.21 (m, 1H), 4.36 (q, $J = 6.9$ Hz, 2H), 7.20 (d, $J = 8.1$ Hz, 2H), 7.48 (d, $J = 8.1$ Hz, 2H); MS: (ESI, Pos) m/z 361.4 $[(M+1)^+]$ and 383.4 $[(M+23)^+]$.

4.1.16. 1-(4-Methyl-cyclohexyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (19). Yield 1.22 g (36.6%) as a white solid. $R_f = 0.49$ (hexane–ethyl acetate 92:8) ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.04 (d, $J = 7.2$ Hz, 3H), 1.41 (t, $J = 6.9$ Hz, 3H), 1.62 (m, 6H), 1.88 (m, 1H), 2.12 (s, 3H), 2.20 (m, 2H), 3.93 (m, 1H), 4.41 (q, $J = 7.2$ ppm, 2H), 7.20 (d, $J = 8.7$ Hz, 2H), 7.47 (d, $J = 8.7$ Hz, 2H); MS: (ESI, Pos) m/z 361.4 $[(M+1)^+]$ and 383.4 $[(M+23)^+]$.

4.1.17. 1-(4-Chlorophenyl)-5-(cyclohexyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (34). Yield 525 mg (12.1%) as a yellow oil. $R_f = 0.45$ (ethyl acetate–petroleum ether 1:3) ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.17 (m, 2H), 1.39 (t, $J = 7.2$ Hz, 3H), 1.75 (m, 8H), 2.43 (s, 3H), 2.59 (m, 1H), 4.40 (q, $J = 7.2$ Hz, 2H), 7.30 (d, $J = 8.4$ Hz, 2H), 7.45 (d, $J = 8.4$ Hz, 2H); MS: (ESI, Pos) m/z 347.4 $[(M+1)^+]$ and 369.4 $[(M+23)^+]$.

4.1.18. 1-(4-Chlorophenyl)-5-(cycloheptyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (35). Yield 302 mg (15.8%) as yellow oil. $R_f = 0.24$ (ethyl acetate–hexane 1:9) ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.39 (t, $J = 6.9$ Hz, 3H), 1.61 (m, 7H), 1.79 (m, 5H), 2.38 (s, 3H), 2.75 (m, 1H), 4.39 (q, $J = 6.9$ Hz, 2H), 7.30 (d, $J = 8.7$ Hz, 2H), 7.45 (d, $J = 8.7$ Hz, 2H); MS: (ESI, Pos) m/z 361.5$[(M+1)^+]$ and 383.4 $[(M+23)^+]$.

4.1.19. 1,5-Dicyclohexyl-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (38). Yield 286 mg (10.2%) as a yellow oil. $R_f = 0.29$ (ethyl acetate–petroleum ether 5:95). ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.38 (m, 7H), 1.85 (m, 16H), 2.31 (s, 3H), 2.71 (m, 1H), 4.11 (m, 1H), 4.38 (q, $J = 7.2$ Hz, 2H); MS: (ESI, Pos) m/z 319.4 $[(M+1)^+]$ and 341.4 $[(M+23)^+]$.

4.1.20. 1-(2,4-Dichloro phenyl)-5-(4-methylphenyl)-4-methyl-1H-pyrazole-3-carboxylic acid, ethyl ester (27). Yield 521 mg (20.8%) as cream colored solid. $R_f = 0.33$ (ethyl acetate–hexane 6:94) ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.32 (t, $J = 7.2$ Hz, 3H), 2.34 (s, 3H), 2.40 (s, 3H), 4.29 (q, $J = 7.2$ Hz, 2H), 7.02 (d, $J = 8.1$ Hz, 2H), 7.13 (d, $J = 8.4$ Hz, 2H), 7.28 (m, 2H), 7.44 (m, 1H); MS: (ESI, Pos) m/z 390.4 $[(M+1)^+]$ and 412.3 $[(M+23)^+]$.

4.1.21. *N*-piperidinyl-1-(cyclopentyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (20). Compound **15** (0.697 g, 2.1 mmol) was dissolved in methanol (14 mL) and to this a solution of KOH (0.24 g, 4.2 mmol) in methanol (5 mL) was added. The reaction mixture was heated to reflux for 3 h, cooled and poured into water. It was acidified with 10% HCl and the precipitate was filtered, washed with water and dried under vacuum to give the crude acid (0.581 g, 91%). The crude acid (0.581 g, 1.91 mmol) was dissolved in toluene (18 mL) and to this solution thionyl chloride (0.52 mL, d = 1.631, 7.05 mmol) was added and the reaction mixture was heated to reflux for 3 h. It was then cooled and the solvent evaporated under reduced pressure. The residue was then dissolved in toluene and solvent was again evaporated under reduced pressure to yield the crude acid chloride (0.391 g, 63.3%).

The crude acid chloride (0.391 g, 1.21 mmol) was dissolved in dry methylene chloride (5.5 mL) and this solution was added drop wise to a solution of triethyl amine (0.19 g, 1.87 mmol) and 1-amino piperidine (0.2 mL, d = 0.928, 1.87 mmol) in methylene chloride (4.3 mL) maintained at 0 °C. The reaction mixture was stirred at room temperature for 8 h after which it was added to brine and extracted with methylene chloride. The organic layer was separated, dried over sodium sulfate and purified by column chromatography with petroleum ether–ethyl acetate (3:1) to yield a white solid which was then recrystallised with methylene chloride/hexane to yield the amide as a white crystalline solid (110 mg, 23.6%). $R_f = 0.34$ (ethyl acetate–petroleum ether 1:3), mp: 185 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.52 (m, 2H), 1.64 (m, 2H), 1.82 (p, $J = 5.5$ Hz, 4H), 1.97 (m, 4H), 2.10 (m, 2H), 2.25 (s, 3H), 2.98 (t, $J = 5$ Hz, 4H), 4.49 (p, $J = 7$ Hz, 1H), 7.26 (d, $J = 9$ Hz, 2H), 7.52 (d, $J = 8.5$ Hz, 2H), 7.74 (s, 1H); MS: m/z 387.4 (M + 1). Anal. (calcd for $C_{21}H_{27}ClN_4O$) C, H, N (Theoretical: C-65.19%, H-7.03%, N-14.48% Found: C-65.23%, H-6.96%, N-14.47).

Utilizing the appropriate pyrazole esters the following pyrazole carboxamides were similarly prepared.

4.1.22. *N*-piperidinyl-1-(cyclohexyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (21). Yield 151 mg (32.4%) as a white crystalline solid. $R_f = 0.42$ (ethyl acetate/petroleum ether 1:3), mp: 150 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.15 (m, 2H), 1.38 (m, 2H), 1.59 (m, 2H), 1.69 (p, $J = 5.5$ Hz, 4H), 1.76 (m, 4H), 1.86 (m, 2H), 2.11 (s, 3H), 2.84 (brs, 4H), 3.79 (tt, $J = 3.5$, 11.5 Hz, 1H), 7.11 (d, $J = 8.5$ Hz, 2H), 7.40 (d, $J = 8.5$ Hz, 2H), 7.61 (s, 1H); MS: m/z 423.9 (M + 23) Anal. (calcd for $C_{22}H_{29}ClN_4O$) C, H, N (Theoretical: C-65.90%, H-7.29%, N-13.97% Found: C-65.78%, H-7.35%, N-13.55).

4.1.23. *N*-piperidinyl-1- (cycloheptyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (22). Yield 614 mg (30.8%) as a white crystalline solid. $R_f = 0.42$ (ethyl acetate–petroleum ether 15:85), mp: 154–155 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.42 (m, 2H), 1.51 (m, 2H), 1.63 (m, 4H), 1.82 (m, 6H), 1.92 (m, 2H), 2.14 (m, 2H), 2.24 (s, 3H), 2.98 (t, $J = 4.5$ Hz, 4H), 4.14 (h, $J = 4.5$ Hz, 1H), 7.24 (d, $J = 8.5$ Hz, 2H), 7.52 (d, $J = 8.5$ Hz, 2H), 7.75 (s, 1H); MS: m/z 415.5 (M + 1) Anal. (calcd for $C_{23}H_{31}ClN_4O$) C, H, N (Theoretical: C-66.57%, H-7.53%, N-13.5% Found: C-66.53%, H-7.47%, N-13.52).

4.1.24. *N*-piperidinyl-1- (3-methyl-cyclohexyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (23). Yield 473 mg (42.4%) as a white crystalline solid. $R_f = 0.4$ (ethyl acetate–hexane 1:3), mp: 151 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.28 (m, 4H), 1.50 (m, 2H), 1.77 (m, 7H), 2.00 (m, 2H), 2.12 (s, 3H), 2.85 (t, $J = 5.4$ Hz, 4H), 4.13 ($J = 4$, 10.5 Hz, 1H), 7.12 (d, $J = 8.4$ Hz, 2H), 7.39 (d, $J = 8.7$ Hz, 2H), 7.61 (s, 1H); MS: m/z 415.4 (M + 1) Anal. (calcd for $C_{23}H_{31}ClN_4O.0.5H_2O$) C, H, N (Theoretical: C-65.16%, H-7.61%, N-13.21% Found: C-64.97%, H-7.61%, N-13.16).

4.1.25. *N*-piperidinyl-1-(4-methyl-cyclohexyl)-5-(4-chlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (24). Yield 645 mg (47.2%) as a white crystalline solid. $R_f = 0.3$ (ethyl acetate–hexane 1:4), mp: 171 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.55 (m, 4H), 1.76 (m, 9H), 2.17 (m, 2H), 2.18 (s, 3H), 2.92 (t, $J = 5.4$ Hz, 4H), 3.88 (tt, $J = 3.0$, 11.0 Hz, 1H), 7.18 (d, $J = 8.4$ Hz, 2H), 7.46 (d, $J = 8.4$ Hz, 2H), 7.71 (s, 1H); MS: m/z 415.5 (M + 1) Anal. (calcd for $C_{23}H_{31}ClN_4O$) C, H, N (Theoretical: C-66.57%, H-7.53%, N-13.5% Found: C-66.4%, H-7.53%, N-13.30).

4.1.26. N-piperidinyl-1- (2,4-dichlorophenyl)-5-(4-methylphenyl)-4-methyl-1H-pyrazole-3-carboxamide (27). Yield 613 mg (30.8%) as a white crystalline solid. $R_f = 0.32$ (ethyl acetate–hexane 6:94), mp: 154 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.49 (m, 2H), 1.81 (p, $J = 6$ Hz, 4H), 2.39 (s, 3H), 2.42 (s, 3H), 2.92 (brs, 4H), 7.05 (d, $J = 8$ Hz, 2H), 7.17 (d, $J = 8$ Hz, 2H), 7.32 (m, 2H), 7.48 (dd, $J = 1$, 2 Hz, 1H), 7.69 (s, 1H); MS: m/z 415.5 (M + 1) Anal. (calcd for $C_{23}H_{31}ClN_4O$) C, H, N (Theoretical: C-62.31%, H-5.46%, N-12.64% Found: C-62.08%, H-5.51%, N-12.48).

4.1.27. *N*-piperidinyl-1- (4-chlorophenyl)-5-(cyclohexyl)-4-methyl-1H-pyrazole-3-carboxamide (36). Yield 104 mg (20%) as a white crystalline solid. $R_f = 0.45$ (ethyl acetate/petroleum ether 1:3), mp: 165 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.19 (m, 3H), 1.41 (m, 2H), 1.73 (m, 11H), 2.46 (s, 3H), 2.57 (tt, $J = 4.5$, 11.5 Hz, 1H), 2.82 (brs, 4H), 7.28 (d, $J = 8.4$ Hz, 2H), 7.48 (d, $J = 8.5$ Hz, 2H), 7.59 (s, 1H); MS: m/z 401.5 (M + 1) Anal. (calcd for $C_{22}H_{29}ClN_4O$) C, H, N (Theoretical: C-65.9%, H-7.29%, N-13.97% Found: C-65.63%, H-7.18%, N-13.81).

4.1.28. *N*-piperidinyl-1-(4-chlorophenyl)-5-(cycloheptyl)-4-methyl-1H-pyrazole-3-carboxamide (37). Yield 84 mg (25%) as a white crystalline solid. $R_f = 0.3$ (ethyl acetate/petroleum ether 1:4), mp: 175–176 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.32 (m, 4H), 1.46 (m, 4H), 1.68 (m, 8H), 1.79 (m, 2H), 2.35 (s, 3H), 2.65 (tt, $J = 3.5$, 11 Hz, 1H), 2.76 (brs, 4H), 7.22 (d, $J = 8.5$ Hz, 2H), 7.41 (d, $J = 8.5$ Hz, 2H), 7.52 (s, 1H); MS: m/z 415.4 (M + 1) Anal. (calcd for $C_{23}H_{31}ClN_4O.\ 0.1H_2O$) C, H, N (Theoretical: C-66.28%, H-7.55%, N-13.44% Found: C-65.9%, H-7.55%, N-13.19).

4.1.29. *N*-piperidinyl-1,5-dicyclohexyl-4-methyl-1H-pyrazole-3-carboxamide (39). Yield 55 mg (25%) as a white crystalline solid. $R_f = 0.54$ (ethyl acetate–petroleum ether 1:3), mp: 197–198 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.34 (m, 8H), 1.74 (m, 10H), 1.89 (m, 8H), 2.36 (s, 3H), 2.68 (p, $J = 7.5$ Hz, 1H), 2.87 (brs, 4H), 4.02 (tt, $J = 3.5$, 11 Hz, 1H), 7.63 (s, 1H); MS: m/z 373.5 (M + 1) Anal. (calcd for $C_{22}H_{36}N_4O.\ 0.5H_2O$) C, H, N (Theoretical: C-69.25%, H-9.77%, N-14.68% Found: C-69.12%, H-9.53%, N-14.58).

4.2. NMR studies and molecular modeling

All spectra were acquired at 23 °C and 500 MHz on a Varian Inova-500 spectrometer using a 5-mm HCN triple resonance probe. Both proton and carbon chemical shifts were referenced to the residual solvent peak of DMSO (2.49 ppm for proton and 40 ppm for carbon). For two-dimensional NOESY measurements, a total of 1024 fids were recorded for the indirect dimension, with a 2 second recycle delay, with a 500 ms mixing time. The TRIAD NMR package within the Sybyl software was used for data processing and analysis.[34] Peaks in the NOESY spectra were assigned and integrated using TRIAD standard functions. MARDIGRAS was then used to generate distance constraints using these peak integrals. The resulting constraints were then examined to ensure that the error in distances conformed to established errors for NOE constraints wherein; $x < 2.5$ Å was ±0.1 Å; $x \leq 3.0$ Å was ±0.2 Å; $x \leq 3.5$ Å was ±0.3 Å; and $x \geq 3.5$ was ±0.4 Å.

Ten cycles of simulated annealing using the constraints generated by MARDIGRAS was performed on compounds **21**, **24**, **27**, **36**, **39**, and AM-251 by heating to 1000 K for 1ps followed by exponential cooling to 200 K then equilibrating for 5 ps. The experimentally obtained NOE distance constraints were applied during all steps of the simulated annealing runs. These averaged conformations of unique rotomers were then minimized

with a gradient tolerance of 0.005 Kcal·mol^{-1}·Å^{-1} without experimental NOE distance constraints to obtain the final average conformations. Systematic searches were performed around τ_1 and τ_2 from 0 to 350° using 10 degree increments. Additional parameters included the Tripos force field with MMFF94 charges, an 8 Å nonbonding cutoff, and distance dependent dielectric constant function.

4.3. Receptor binding assays

Cell membranes from HEK293 cells transfected with the human CB1 receptor (Lot #1929, B_{max}: 1.7 pmol/mg protein, K_d for [^{1}H]CP 55,940 binding: 186 pM) and membranes from CHO-K1 cells transfected with the human CB2 receptor (Lot #1930, B_{max}: 3.3 pmol/mg protein, K_d for [^{3}H]CP 55,940 binding: 0.12 nM) were purchased from Perkin–Elmer Life Sciences, Inc. [^{3}H]CP 55,940 having a specific activity of 120 Ci/mmol was obtained from Perkin–Elmer Life Sciences, Inc. All other chemicals and reagents were obtained from Sigma-Aldrich. The assays were carried out in 96-well plates obtained from Millipore, Inc. fitted with glass fiber filters (hydrophilic, GFC filters) having a pore size of 1.2 μ. The filters were soaked with 0.05% polyethyleneimine solution and washed 5x with deionized water prior to carrying out the assays. The filtrations were carried out on a 96-well vacuum manifold (Millipore Inc.), the filters punched out with a pipette tip directly into scintillation vials at the end of the experiment and vials filled with 5 mL scintillation cocktail Ecolite (+) (Fisher Scientific). Counting was carried out on a Beckmann Scintillation Counter model LS6500. Drug solutions were prepared in DMSO and the radioligand was dissolved in ethanol.

Incubation buffer: 50 mM Tris–HCl, 5mM $MgCl_2$, 2.5 mM EDTA, 0.5 mg/mL fatty acid free bovine serum albumin, pH 7.4.

Binding protocol for the CB1 receptor: 8 μg of membranes (20 μL of a 1:8 dilution in incubation buffer) was incubated with 5 μL of drug solution (10^{-4}–10^{-12} M) and 5 μL of 5.4 nM [^{3}H]CP 55,940 in a total volume of 200 μL for 90 min at 30 °C. Non-specific binding was determined using 10 μM WIN55,212-2 (K_i = 4.4 nM). The membranes were filtered and the filters washed 7× with 0.2 mL ice-cold incubation buffer and allowed to air dry under vacuum.

Binding protocol for the CB2 receptor: 15.3 μg of membranes (20 μL of a 1:20 dilution in incubation buffer) was incubated with 5 μL of drug solution (10^{-4} M to 10^{-12} M) and 5 μL of 10 nM [^{3}H]CP 55,940 in a total volume of 200 μL for 90 min at 30 °C. Non-specific binding was determined using 10 μM WIN55,212-2 (K_i = 4.4 nM). The membranes were filtered and the filters washed 7× with 0.2 mL ice-cold incubation buffer and allowed to air dry under vacuum.

Data accumulation and statistical analysis: Varying concentrations of drug ranging from 10^{-4} M to 10^{-12} M were added in triplicate for each experiment and the individual molar IC_{50} values were determined using GraphPad Prism. The corresponding K_i values for each drug were determined utilizing the Cheng and Prusoff equation[37] and final data are presented as $K_i \pm$ S.E.M. of $n \geq 2$ experiments.

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