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Synthesis and structure–activity relationship of novel diarylpyrazole imide analogues as CB1 cannabinoid receptor ligands

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ABSTRACT

A myriad of research groups have been engaged in searching for novel CB1 receptor antagonists, since SR141716A (rimonabant), a CB1 receptor antagonist, was discovered for an obesity treatment. In this research, extended series, based on the 1,5-diarylpyrazole template of rimonabant, was synthesized and tested for CB1 receptor binding affinity. In the present study, *N*-piperidinylcarboxamide group of rimonabant was replaced with the corresponding sulfonamide, imide, *N*-methyl imide and methylenediamide, respectively. The SAR studies to optimize the CB1 binding affinity led to the potent imide derivatives. The in vivo efficacy test of a derivative (**16f**) gave a promising result for this novel scaffold. In order to explore physicochemical properties (hydrophobic, steric and electronic) of the representative imide derivatives responsible for their CB1 receptor binding affinity, quantitative structure activity relationship (QSAR) studies were performed. Hansch QSAR models, which were moderate in the explanation for SAR, were generated with hydrophobic, steric and electronic properties of substituents. Especially, the Taft Es-based parabolic model was obtained with the best correlation result (r^2 = 0.846).

1. Introduction

It is now widely accepted that obesity is not a cosmetic issue, but is a major health issue. At last, the World Health Organization (WHO) officially declared obesity a disease in 1998.[1,2] Obesity has received considerable attention due to its high prevalence and association with serious health risks, especially metabolic syndrome including insulin resistance, hypertension, and heart disease.[3–5] Most medical experts absolutely insist that the best way to reduce the risk of the metabolic syndrome is to overcome the obesity. It has indeed been demonstrated that a body weight reduction of only 5–10% leads to a significant fall in blood pressure in hypertensive patients and an improved glycemic control in diabetic patients. Accordingly, if a higher safety and efficacy of antiobesity drugs can be achieved, the first choice of the antiobesity drug could replace the treatment of the individual metabolic diseases associated with obesity by the treatment of obesity itself.[6] However, the currently available antiobesity drugs have not satisfied medical experts and obese patients, because of their serious side-effect issues and lower efficacy than expected. Accordingly, there are still unmet medical needs for novel target developments for antiobesity drug.[1,7]

Under these circumstances, the endocannabinoid system offered the good clue for a new approach to the antiobesity drug development. This endogenous signaling system has been known to play a key role in the regulation of food intake, fat accumulation, and energy balance. The overactivation of the endocannabinoid system seems to be strongly linked to the abdominal obesity and the development of the metabolic syndrome.[8] The first particular interest has been focused on the characteristic role of CB1 receptor, which could effectively modulate endocannabinoid system. The down-regulation of the endocannabinoid system by the specific blockage of CB1 receptors could induce weight loss. Accordingly, a lot of research groups have tried to find CB1 receptor antagonists and at last rimonabant, the first CB1 receptor antagonist, was discovered by Sanofi-Aventis. After this discovery, antagonism of CB1 receptor has been pursued as a highly promising strategy for the treatment of obesity and various CB1 receptor antagonists, with the pharmacophores similar to those of rimonabant, have been discovered and developed by many pharmaceutical companies.[9–11] However, rimonabant was withdrawn from the market due to its severe psychiatric adverse effects and all ongoing clinical trials of rimonabant were also discontinued in November 2008. Developments of other CB1 antagonists, such as taranabant and otenabant, have been also stopped recently. In spite of the successive failures of leading CB1 antagonists to launch on the market, the projects still proceed to find out novel peripherally restricted CB1 antago-

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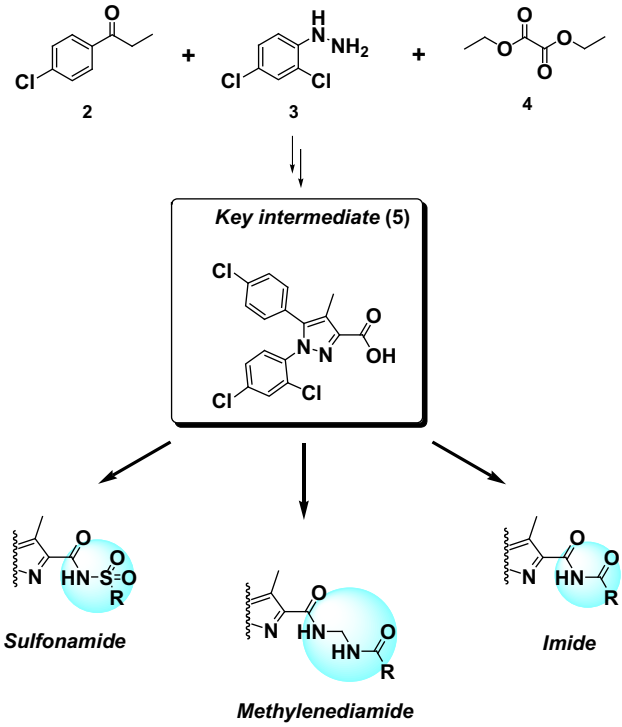
nists which are non-brain penetrants and do not show serious psychiatric disorders.[12]

The main objective of this study was the search for novel CB1 receptor antagonists. A pharmacophore model for the binding of a low energy conformation of rimonabant in the CB1 receptor has well-documented.[13] The key receptor–ligand interaction is known to be a hydrogen bond between the carbonyl group of rimonabant and the Lys192-Asp366 residue of the CB1 receptor, thereby exerting a stabilizing effect on the Lys192-Asp366 salt bridge as shown in Figure 1(A). In the present study, it was suggested that various diketone moieties should be better hydrogen bonding acceptors than the sole carbonyl group of rimonabant. Based on this conception, several derivatives, containing various diketone groups, were prepared to seek for better hydrogen bonding acceptors than a carboxamide group of rimonabant (**1**). The expected hydrogen bonding model of diketone groups is suggested in Figure 1(B).[33] Scheme 1 illustrates the general synthetic idea for the novel scaffolds including sulfonamide, imide and methylenediamide group[34] as hydrogen bonding acceptors. The key intermediate (acid, **5**) could be prepared from 4-chlorophenylpropiophenone (**2**), 2,4-dichlorophenylhydrazine (**3**) and diethyl oxalate (**4**) by a conventional method.[14] Through typical coupling reactions of the key intermediate with the corresponding substrates, the target scaffolds (sulfonamide, imide and methylenediamide in Scheme 1) could be produced.

In the present study, classical QSAR models, based on Hansch's parabolic approach, were obtained by using traditional parameters, describing hydrophobic, steric and electronic properties.[15,16] Two reliable models were applied to predict the biological activity of virtual compounds without the synthesis and biological evaluation.



Scheme 1. The schematic diagram illustrates the general synthetic idea. The key intermediate was prepared from 4-chlorophenylpropiophenone (**2**), 2,4-dichlorophenylhydrazine (**3**) and diethyl oxalate (**4**). Through typical coupling reactions with the key intermediate (**5**), the above target scaffolds (sulfonamide, imide, methylenediamide) could be produced.

2. Results and discussion

2.1. Chemistry

The synthetic pathway of other intermediates from the key intermediate (acid, **5**) is outlined in Scheme 2. The acid intermediate, 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (**5**), was prepared in 18% overall yield by the conventional method.[14] The amide intermediate **7** was then prepared in a high yield (93%) by conventional methods such as conversion to the carbonyl chloride **6** followed by amination with ammonium hydroxide.[17] The *N*-methyl amide intermediate **8**, was also prepared in high yield (99%) by a reaction of the carbonyl chloride **6** with methylamine under Schotten–Baumann reaction conditions.[18] The coupling of the above acid **5** with glycinamide hydrochloride, using conditions such as EDCI (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride)/HOBt (1-hydroxybenzotriazole)/NMM (*N*-methyl morpholine), provides the *N*-carbamoylmethyl amide **9**. Rearrangement of **9** using PIFA [phenyliodine(III) bis(trifluoroacetate)] in a solvent such as a mixture of ACN and water, followed by treatment with 1 M hydrochloric acid affords the *N*-aminomethyl amide as the corresponding salt **10**.[19] The resulting intermediate, *N*-aminomethyl amide hydrochloride salt **10**, was not effectively recovered during the purification step by a diethyl ether extraction, since the intermediate salt **10** was unexpectedly soluble in organic solvent. Accordingly, the yield (28%) was relatively lower than those found in the literature.[19]

The synthetic methods for various target derivatives are illustrated in Schemes 3–6. As shown in Scheme 3, *N*-acyl sulfonamide derivatives **12** were synthesized in good yields (>50%) by the coupling reaction between the acid intermediate **5** and the corresponding sulfonamides **11** in the presence of EDCI and DMAP (4-dimethylaminopyridine) at room temperature.[20] Three sulfona-

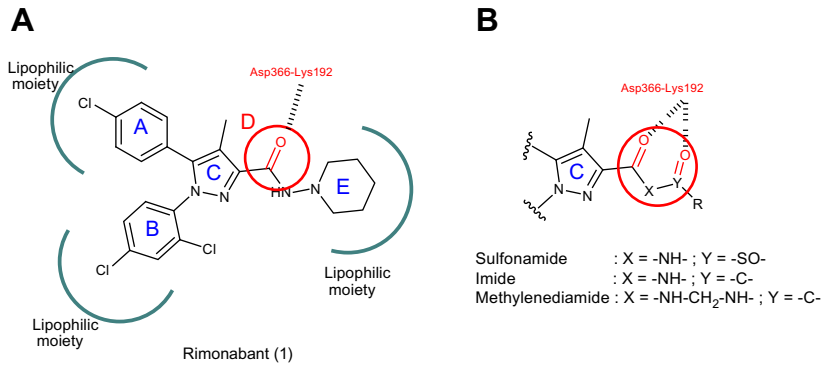
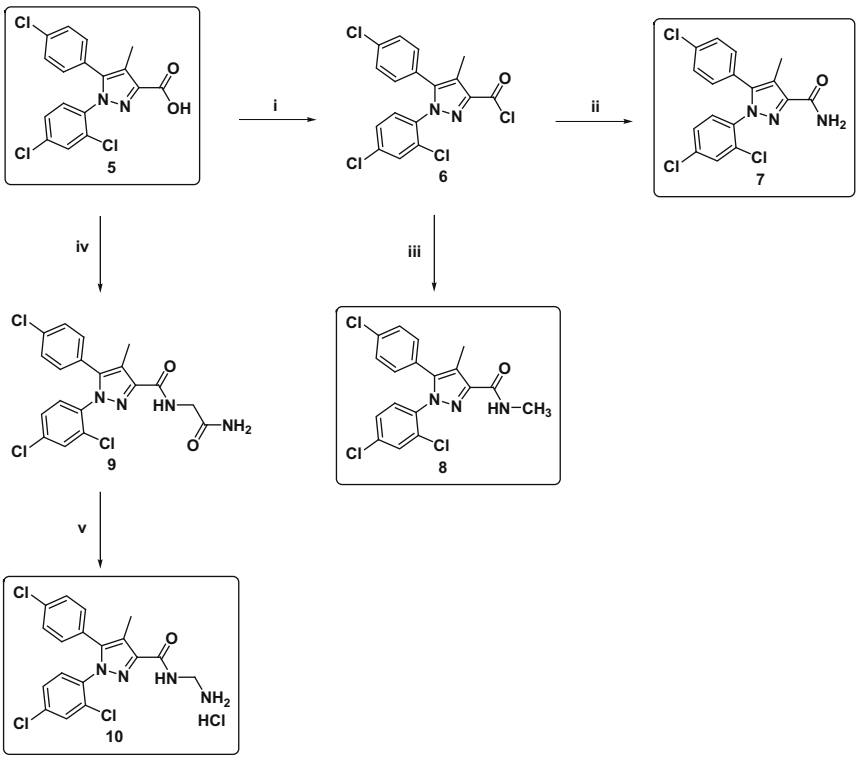
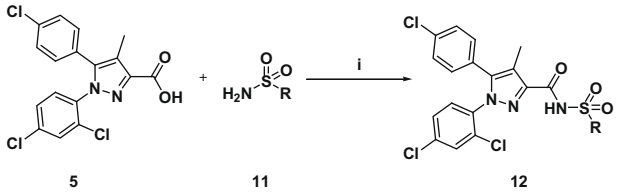


Figure 1. (A) Pharmacophore model in CB1 receptor and rimonabant (**1**). Both aromatic ring A and B attached to a central core C. A carbonyl moiety D serves as a key interaction between rimonabant and the Lys 192 residue of the CB1 receptor. (B) Expected binding model of sulfonamide, imide and methylenediamide derivatives.



Scheme 2. The major intermediates are marked with rectangles. Reagents and conditions: (i) $SOCl_2$, toluene, reflux; (ii) NH_4OH, DCM, 0 °C→rt; (iii) CH_3NH_2, NaOH, THF/water, rt; (iv) glycinamide, EDCI, HOBt, NMM, DMF, rt; (v) PIFA, ACN/water, rt.

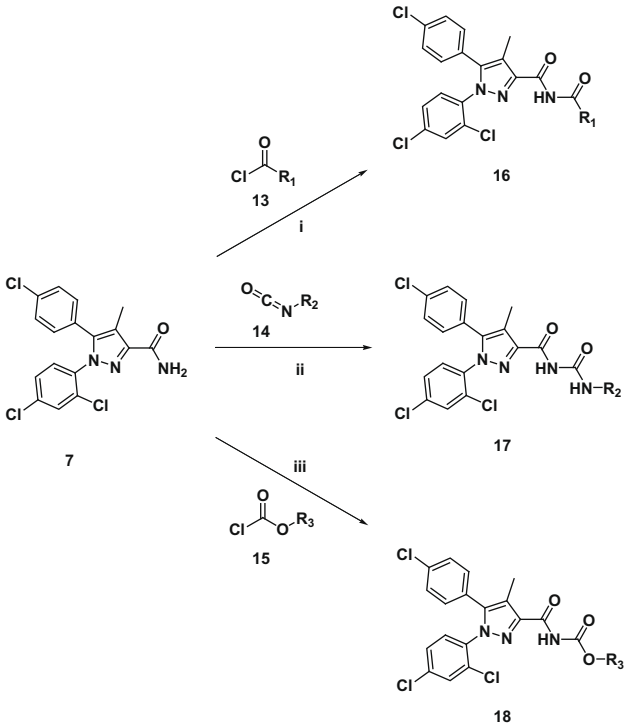


Scheme 3. Reagents and conditions: (i) EDCI, DMAP, DCM, rt.

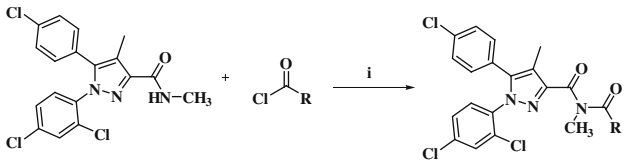
mides (ethyl-, isopropyl- and butyl-) were not commercially available at the moment. Accordingly, they were prepared from the corresponding sulfonyl chlorides by treatment of ammonium hydroxide.[21] The structures of *N*-acyl sulfonamide derivatives are illustrated in Table 1. Synthetic pathways for three different imide derivatives—amide **16**, urea **17** and carbamate **18**—were summarized in Scheme 4. The amide intermediate **7** was employed to prepare imide derivatives **16**, **17**, and **18** by coupling reaction with acyl chloride **13**, isocyanate **14** and chloroformate **15** in the presence of NaHMDS (sodium salt of hexamethyldisilazane) as a base at low temperature (−78 °C to rt).[22] The yields for all the imide derivatives **16**–**18** proved to be moderate to good (>50%). Unfortunately, IC_{50} values of some urea form derivatives **17** could not be obtained due to their low solubility during the biological assay. As shown in Scheme 5, the *N*-methyl imide derivatives **19** were also successfully synthesized in more than 70% yield by coupling reactions of the *N*-methyl amide intermediate **8** and corresponding acyl chlorides **13** in the presence of TEA as a base at refluxing conditions.[18] Synthesis of methylenediamide derivatives **21** is represented in Scheme 6. The methylenediamide derivatives **21** were prepared in more than 45% yield by coupling of *N*-aminomethyl amide hydrochloric acid salt **10** with the corresponding acids **20** in the presence of DMAP and EDCI.[20]

2.2. Structure–activity relationship studies

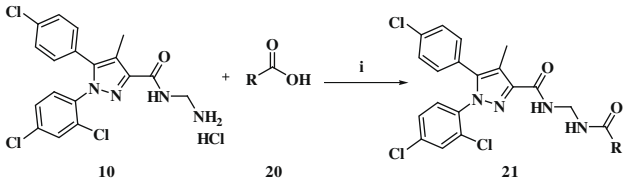
All the derivatives synthesized were first evaluated in vitro at a rat CB1 receptor binding assay,[23,31] and the results are summa-



Scheme 4. Reagents and conditions: (i) R_1-COCl, NaHMDS, THF, −78 °C→rt; (ii) R_2-NCO, NaHMDS, THF, −78 °C→rt; (iii) R_3-OCOCl, NaHMDS, THF, −78 °C→rt.

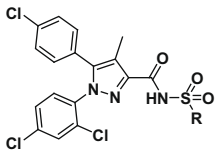


Scheme 5. Reagents and conditions: (i) TEA, DCM, reflux.



Scheme 6. Reagents and conditions: (i) DMAP (2 equiv), EDCI, DCM, rt.

Table 1
Structures and rat CB1 receptor binding affinities of sulfonamide derivatives

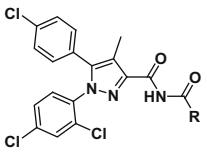


Code	R	IC_{50} for rCB1R[a] (nM)
12a	-Phenyl	3640
12b	-4-Chlorophenyl	>10,000
12c	-3-Chlorophenyl	1200
12d	-4-Fluorophenyl	1200
12e	-3-Fluorophenyl	>10,000
12f	-4-Methylphenyl	>10,000
12g	-Benzyl	>10,000
12h	-Propyl	>10,000
12i	-Isopropyl	>10,000
12j	-Butyl	>10,000

[a] These data were obtained by single determinations.

rized in Tables 1–4. As shown in Table 1, most of the *N*-acyl sulfonamide derivatives **12** had over 1 μM of IC_{50} value at CB1 receptor. This is consistent with the previously reported result of *N*-acyl sulfonamide derivatives in the diarylpyrazoline series.[24] Meanwhile, an introduction of acyl group, instead of sulfonamide group of *N*-acyl sulfonamide derivatives **12**, resulted in a large increase in binding affinity at CB1 receptor. The results of imide derivatives (**16**–**18**) are summarized in Table 2. All the imide form derivatives **16** gave the potent CB1 binding activities (IC_{50} = 21.2–383 nM). The most potent compound in this imide series was compound **16d** (IC_{50} = 21.2 nM), with *tert*-pentyl moiety as a lipophilic pharmacophore. Eight derivatives in total 14 samples demonstrated CB1R binding affinity of IC_{50} < 50 nM. For the further SAR studies, urea **17** and carbamate form **18** were also introduced instead of imide derivatives **16**, respectively. Replacement of imide to urea **17** led to a 10-fold reduction in CB1 binding activity. The carbamate form **18** of imide derivatives showed slightly decreased binding activity for the CB1 receptor as exemplified by both a neopentyl derivative **16e** (IC_{50} = 53.4 nM) versus an *O*-*tert*-butyl derivative **18c** (IC_{50} = 82.5 nM) and a pentyl derivative **16h** (IC_{50} = 33.5 nM) versus an *O*-butyl derivative **18b** (IC_{50} = 99.7 nM). An interesting exception to this observation is an O-benzyl derivative **18a** (IC_{50} = 17.8 nM). Introduction of a benzyl unit at a carbamate functionality resulted in an improvement in binding affinity,

Table 2
Structures and rCB1 receptor binding affinities of imide derivatives (amide, urea and carbamate form)

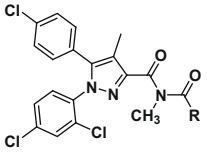


Code	R	IC_{50} for rCB1R[a] (nM)
16a	-3-Pentyl	24.8
16b	-4-Heptyl	26.9
16c	-3-Heptyl	43.1
16d	2-Methyl-2-butyl	21.2
16e	-Neopentyl	53.4
16f	-*tert*-Butyl	32.7
16g	-Isopropyl	161
16h	-Pentyl	33.5
16i	-Propyl	43.7
16j	-Methyl	383
16k	-Cyclohexyl	30.4
16l	-Cyclopentyl	67.2
16m	-Cyclobutyl	127
16n	-Cyclopropyl	124
17a	-NH-Hexyl	138
17b	-NH-Cyclohexyl	390
17c	-NH-Cycloheptyl	750
17d	-NH-Cyclohexylmethyl	Insoluble
17e	-NH-Isopropyl	253
17f	-NH-*tert*-Butyl	Insoluble
17g	-NH-Phenyl	>10,000
17h	-NH-Benzyl	146
18a	-O-Benzyl	17.8
18b	-O-Butyl	99.7
18c	-O-*tert*-Butyl	82.5
18d	-O-Neopentyl	363
1	Rimonabant	5.0 ± 1.0[b]

[a] These data were obtained by single determinations.
[b] **1** is estimated to have rCB1 binding affinity via in-house assay.

suggesting the importance of a nonpolar moiety in order to optimally bind to a hydrophobic area of the CB1 receptor. *N*-Methyl imide derivatives **19** were prepared by introduction of methyl group into imide derivatives. The introduction of *N*-methyl group resulted in a large loss of CB1 binding affinity (Table 3). The binding affinity for CB1R of *N*-methyl imide derivatives decreased in 5- to 10-fold. For example, an imide derivative **16d** (IC_{50} = 21.2 nM), the most potent imide derivative, is compared with the corresponding *N*-methyl imide derivative **19b**

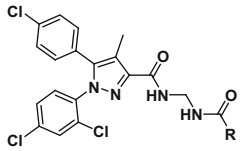
Table 3
Structures and rCB1 receptor binding affinities of *N*-methyl imide derivatives



Code	R	IC_{50} for rCB1R[a] (nM)
19a	-*tert*-Butyl	220
19b	-2-Methyl-2-butyl	227
19c	-3-Pentyl	265
19d	-4-Heptyl	247
19e	-Cyclohexyl	167

[a] These data were obtained by single determinations.

Table 4
Structures and rCB1 receptor binding affinities of methylenediamide derivatives



Code	R	IC_{50} for rCB1R[a] (nM)
21a	-Heptyl	1180
21b	-Propyl	905
21c	-Isopropyl	951
21d	-Cyclobutyl	150
21e	-Cyclohexyl	49.2
21f	-Cycloheptyl	75.6
21g	-3-Methylcyclohexyl	102
21h	-Cyclopenylmethyl	138
21i	-Cyclohexylmethyl	46.5

[a] These data were obtained by single determinations.

(IC_{50} = 227 nM). According to this SAR study, it is confirmed that the *N*-acyl amide group and the hydrogen of imide derivatives are essentially required of CB1 binding affinity. The binding affinity results for methylenediamide derivatives **21** are listed in Table 4. Even though most of methylenediamide derivatives **21** had more than 100 nM of IC_{50} value, two derivatives **21e** (IC_{50} = 49.2 nM) and **21i** (IC_{50} = 46.5 nM) gave decent CB1 binding affinities.

An imide derivative **16f**, prepared at an early stage, was tested on animal model in order to confirm in vivo efficacy of the imide scaffold. Figure 2 shows chronic effects of the reference (rimonabant) and the imide derivative **16f** in DIO mice. Rimonabant reduced body weight by 11.5%, while the imide derivative **16f** by 2.2%. The test compound **16f** was substantially less efficacious than rimonabant. However, considering that body weight of control mice increased to 6.2% and binding affinity of the test compound (IC_{50} = 32.7 nM for **16f**) for CB1 receptor was significantly lower than that of rimonabant (IC_{50} = 5.0 ± 1.0 nM, in-house test), it must be stated that **16f** showed moderate in vivo efficacy for body weight reduction. Since the mice tested maintained their active movements without any apparent abnormalities, it was believed that the weight loss was derived from CB1 antagonism rather than toxicity. However, functional assay for this series of compounds would be very useful, and it will be reported in due course.

2.3. Classical QSAR model developments

QSAR studies were performed to identify the detailed relationship between molecular bioactivity and the changes in substituents. Some statistically significant models, obtained from these QSAR studies, were applied in order to search for compounds with optimized CB1 binding affinity without practical synthesis.

Traditional parameters (hydrophobic, steric, electric parameters) were employed to formulate classical QSAR equations. In order to develop the QSAR models, the 14 representative imide derivatives (**16a**–**n**) were chosen for training set molecules and six different fragment descriptors (π, STERIMOL L, STERIMOL B5, Taft Es, Hammett σ^*, Hammett σ_I) were collected.[15,25] The negative logarithmic values of CB1 binding activities and the values of selected descriptors for training set molecules used in nonlinear regression analysis were summarized in Table S1 (in Supplementary data).

Hydrophobic substituent parameter (π) is related to the difference between the Log*P* of an unsubstituted molecule and that of a substituted molecule. STERIMOL L, STERIMOL B5 and Taft's Es parameters were employed for the quantitation of steric characteristics.[15,25] STERIMOL parameters, developed by Verloop, reflect on the three-dimensional shape of molecule. STERIMOL L represents the length of a substituent along the axis of a bond between the parent molecule and the substituent. STERIMOL B5 is used to describe the maximum distance orthogonal to STERIMOL L. Taft's Es measures the steric effect of the substituent.[15,25] The STERIMOL parameters were calculated MMPplus software (ChemSW, Fairfield, CA) and Taft's Es parameters were collected from the literature.[26,27] Hammett constants (σ^*, σ_I), used in aliphatic systems, were selected to evaluate electronic properties of training set molecules. In this research, σ^* and σ_I were calculated by MMPPLUS software and applied to QSAR model developments.

The molecules were analyzed by nonlinear Hansch approach[15,25] using different fragment descriptors as independent variables and pIC_{50} values as dependent values. In the following QSAR studies, XLSTAT (version 2008, Addinsoft, New York, NY) was employed to give rise to nonlinear regression analysis. Initially, one hydrophobic parameter (π) and one of three different steric parameters (Es, STERIMOL L and B5) was chosen to find out the appropriate parameters for QSAR developments. As a result, the following equations, Eqs. 1–3 were derived. All the parabolic models represented relatively high determination coefficients (r^2 = 0.810–0.833). In particular, as shown in Eq. 3, the best statistical result (r^2 = 0.833) was obtained for the model employing π and Es as hydrophobic and steric parameters, respectively.

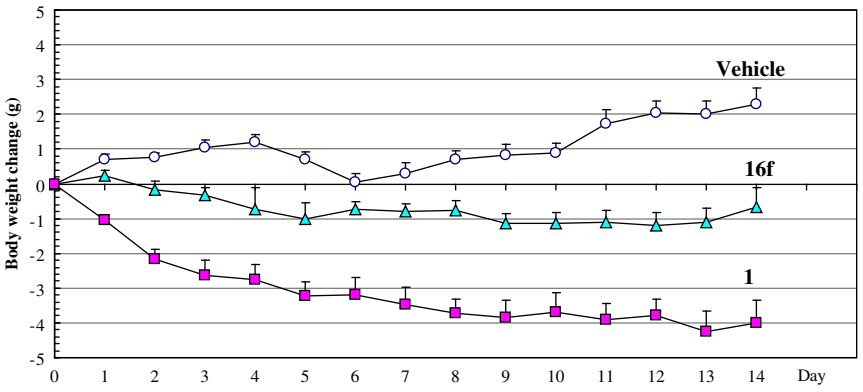


Figure 2. Graphical representation of in vivo efficacy of the control (vehicle, ○), the test compound (**16f**, ▲) and the reference compound (**1**, ■). Body weights at the start were 35.3 g for control, 35.7 g for **16f**, and 34.8 for **1**.

$$pIC_{50} = 3.2016 + 0.9208 \times \pi - 0.1307 \times \pi^2 - 0.04219 \times L$$
$$n = 14,\ r^2 = 0.810,\ MSE = 0.035,\ SSE = 0.346 \quad (1)$$

$$pIC_{50} = 3.3056 + 0.9319 \times \pi - 0.1265 \times \pi^2 - 0.09794 \times B5$$
$$n = 14,\ r^2 = 0.820,\ MSE = 0.033,\ SSE = 0.331 \quad (2)$$

$$pIC_{50} = 2.9831 + 0.9483 \times \pi - 0.1651 \times \pi^2 - 0.1070 \times Es$$
$$n = 14,\ r^2 = 0.833,\ MSE = 0.030,\ SSE = 0.297 \quad (3)$$

The stepwise developments of nonlinear regression models were documented in Eqs. 4–9. The hydrophobic parameter (π) and the steric parameters (L, B5, Es) were retained for exploration of further QSAR and the Hammett constants (σ^* or σ_I) of the substituents were combined as electronic parameters to derive better QSAR models. As shown in Eqs. 4–9, addition of electronic parameters slightly improved the correlation coefficients (r^2 = 0.814–0.846), accounting for better results of the models. Generally, improved equations were obtained by adoption of σ_I (Eqs. 5, 7, and 9) rather than σ^* (Eqs. 4, 6, and 8). The observed and predicted activities (pIC_{50}) for the imide derivatives using representative models, Eqs. 7 and 9, were displayed with graphical representations in Figures S1 and S2, respectively (in Supplementary data).

$$pIC_{50} = 3.1418 + 0.9775 \times \pi - 0.1476 \times \pi^2 - 0.03899 \times L - 0.1073 \times \sigma^*$$
$$n = 14,\ r^2 = 0.814,\ MSE = 0.037,\ SSE = 0.335 \quad (4)$$

$$pIC_{50} = 3.1772 + 0.9632 \times \pi - 0.1452 \times \pi^2 - 0.04324 \times L - 0.6065 \times \sigma_I$$
$$n = 14,\ r^2 = 0.814,\ MSE = 0.037,\ SSE = 0.336 \quad (5)$$

$$pIC_{50} = 3.1962 + 1.0157 \times \pi - 0.1503 \times \pi^2 - 0.08560 \times B5 - 0.07249 \times \sigma^*$$
$$n = 14,\ r^2 = 0.824,\ MSE = 0.035,\ SSE = 0.316 \quad (6)$$

$$pIC_{50} = 3.1972 + 1.2043 \times \pi - 0.1808 \times \pi^2 - 0.1491 \times B5 - 0.1854 \times \sigma_I$$
$$n = 14,\ r^2 = 0.832,\ MSE = 0.033,\ SSE = 0.296 \quad (7)$$

$$pIC_{50} = 2.9678 + 0.9828 \times \pi - 0.1835 \times \pi^2 - 0.1144 \times Es - 0.3303 \times \sigma^*$$
$$n = 14,\ r^2 = 0.840,\ MSE = 0.032,\ SSE = 0.284 \quad (8)$$

$$pIC_{50} = 2.9232 + 1.0415 \times \pi - 0.2047 \times \pi^2 - 0.1275 \times Es - 1.7784 \times \sigma_I$$
$$n = 14,\ r^2 = 0.846,\ MSE = 0.030,\ SSE = 0.272 \quad (9)$$

Based on the actual CB1 receptor binding activities of real imide derivatives (Table 2), several virtual imide derivatives, which are expected to have good activities, were selected and listed in Table S2 (in Supplementary data). Their descriptors were collected from the literature (Es) and the calculation (π, L, B5, σ^*, σ_I) by a software (MMPPLUS) and summarized in Table S2, as well (in Supplementary data). The two equations, Eqs. 7 and 9, were selected for their good correlation properties in order to estimate the pIC_{50} values. The collected values of descriptors were substituted into the above different QSAR model equations to predict pIC_{50} values for virtual derivatives. An exponential function, the reversed function of a logarithmic function, was used to calculate the corresponding IC_{50} value from each pIC_{50} value. The resulting IC_{50} values for CB1 receptor were listed in Table S3 (in Supplementary data). Two virtual derivatives (IC_{50} = 17.7 nM and 14.9 nM for **V2** and **V4**) represented less than 20 nM of IC_{50} value, calculated from Eq. 9. Both of the two models used π, Es and σ_I as independent variables. Meanwhile, another equation Eq. 7) used B5 instead of Es as a steric parameter, due to the lack of Es data from literatures. This equation proposed **V3** (IC_{50} = 24.4 nM) as the most potent derivative.

The above QSAR models made good suggestions about active derivatives based on their own characteristic equations. According to estimations from QSAR equations, the virtual derivative **V4** was predicted as the most active compound (IC_{50} = 14.9 nM) among the practical compounds.[32] It appears that the imide derivative **16d** (IC_{50} = 21.2 nM) is so close to the optimization of CB1 binding affinity and any derivatives derived from this imide scaffold are not likely to show superior CB1 binding affinity to that of **1** (IC_{50} = 5.0 ± 1.0 nM).

3. Conclusion

Novel diarylpyrazole amide derivatives, including diketone moiety, as CB1 receptor antagonists were designed, synthesized and evaluated via in vitro rat cannabinoid CB1 receptor binding assays. In general, imide derivatives linked to diarylpyrazole showed the significant CB1 binding affinities. Especially, compound **16a** or **16d** appeared to be the most potent among the compounds tested (IC_{50} = 24.8 nM and 21.2 nM, respectively) in terms of CB1R binding affinity. Meanwhile, compound **16f** (IC_{50} = 32.7 nM) showed the moderate in vivo efficacy from the DIO mice test. Since the derivatives in this study showed moderate in vivo efficacy, further studies should undergo to optimize the CB1 binding affinity and to confirm their antagonistic properties using a functional assay. A CB2 binding assay should be also performed to evaluate their selectivities for CB1 over CB2 receptor.

The QSAR models, based on Hansch nonlinear approach, were developed with traditional descriptors of the substituents. These models gave rise to equations with relatively good relationships between descriptors and biological activities. In particular, the Taft's Es based equations showed the best correlation (r^2 = 0.846). The results of these studies indicate that hydrophobic (π) and steric (Taft's Es) properties of substituents appear to be the governing factors for the CB1 binding affinity. Unfortunately, the QSAR models do not suggest any imide derivatives with better CB1 binding affinity than rimonabant. A virtual compound, **V4** (IC_{50} = 14.9 nM), is estimated to have the optimized CB1 binding affinity by the significant QSAR equation Eq. 9).

4. Experimental

4.1. General methods

^{1}H NMR spectra were recorded on either a Jeol ECX-400, or a Jeol JNM-LA300 spectrometer for solution in $CDCl_3$. Chemical shifts were expressed in parts per million (ppm, δ units) downfield from the signal for TMS. Coupling constants are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), br (broad). Mass spectra were obtained with either Micromass, Quattro LC Triple Quadruple Tandem Mass Spectometer or Agilent, 1100LC/MSD system equipped with XTerra®MS C18 3.5 μm 2.1 × 50 mm column with a 9 min gradient from 30% CH_3CN to 90% CH_3CN in H_2O.

For preparative HPLC, ca. 100 mg of a product was injected in 1 mL of DMSO or MeOH onto a SunFire™ Prep C18 OBD 5 μm 19 × 100 mm column with a 10 min gradient from 10% CH_3CN to 90% CH_3CN in H_2O. Flash chromatography was carried using Merck Silica Gel 60 (230–400 mesh). Most of the reactions were monitored by thin-layer chromatography on 0.25 mm E. Merck silica gel plates (60F-254), visualized with UV light.

4.2. Chemistry

4.2.1. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (5)

To a solution of LHMDS (62.5 mL, 1 M in THF solution, 62.5 mmol) in diethyl ether (250 mL) was added a solution of 4′-chloropropiophenone (10.5 g, 62.3 mmol) in diethyl ether (50 mL) at −78 °C. After stirring at −78 °C for additional 1 h, diethyl oxalate (9.6 mL, 70.8 mmol) was added to the mixture. The reaction mixture was allowed to warm to room temperature and stirred for

16 h. The precipitate was filtered, washed with diethyl ether and dried under vacuum to provide 5.44 g (19.8 mmol, 32% yield) of the lithium salt (**3**).

To a solution of the above lithium salt (5.44 g, 19.8 mmol) in EtOH (30.2 mL) was added 2,4-dichlorophenylhydrazine hydrochloride (5.07 g, 23.7 mmol) at room temperature. The resulting mixture was stirred at room temperature for 16 h. The precipitate was filtered, washed with EtOH and diethyl ether, and then dried under vacuum to provide a yellow solid of the hydrazone (4.95 g). This solid (4.95 g) was dissolved in acetic acid (39.3 mL) and refluxed for 24 h. The reaction mixture was poured into cold water (196.5 mL) and extracted with EtOAc (330 mL). The EtOAc extract was washed successively with water, dried over $MgSO_4$ and evaporated under vacuum to provide 5.00 g (12.2 mmol) of a pale yellow solid of the ester. To a solution of the above ester (5.00 g) in MeOH (36.5 mL) was added a solution of KOH (1.72 g) in water (36.5 mL). The mixture was refluxed for 3 h. After refluxing, the mixture was poured into cold water (104 mL) and acidified to pH 1 with a 10% HCl solution. The precipitate was filtered, washed with water and dried under vacuum to provide 4.31 g of the title compound (11.3 mmol, 57% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 1.8 Hz, 1H), 7.33–7.27 (m, 4H), 7.09 (d, J = 8.6 Hz, 2H), 2.35 (s, 3H). MS, m/z = 381 ([M+H]$^+$).

4.2.2. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (7)

To a suspension of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (***5***, 3.82 g, 10 mmol) in toluene (75 mL) was added thionyl chloride (3.64 mL, 50 mmol) and the mixture was refluxed for 3 h and then cooled to the room temperature. The solvent was evaporated off under the reduced pressure. The residue was redissolved in toluene (30 mL) and the solvent was evaporated off again (procedure repeated twice) to yield the carboxyl chloride (3.94 g, 98% yield). Concentrated ammonium hydroxide solution (30 mL) was added dropwise to a solution of the carboxyl chloride obtained above in DCM (40 mL) at 0 °C. The mixture was subsequently stirred at room temperature for 16 h and then extracted with DCM (2 × 40 mL). The combined DCM was washed successively with water, dried over $MgSO_4$ and evaporated under vacuum to provide 3.56 g (9.3 mmol, 93% yield) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 2.0 Hz, 1H), 7.33–7.25 (m, 4H), 7.07 (d, J = 8.4 Hz, 2H), 6.82 (br s, 1H, –NH–), 5.43 (br s, 1H, –NH–), 2.37 (s, 3H). MS, m/z = 380 ([M+H]$^+$).

4.2.3. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*,4-dimethyl-1*H*-pyrazole-3-carboxamide (8)

To a suspension of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (***5***, 1.15 g, 3 mmol) in toluene (25 mL) was added thionyl chloride (1.09 mL, 15 mmol) and the mixture was refluxed at 120 °C for 3 h and then cooled to the room temperature. The solvent was evaporated off under the reduced pressure. The residue was redissolved in toluene (30 mL) and the solvent was evaporated off again (procedure repeated twice) to yield the carboxyl chloride. Methylamine hydrochloride (0.243 g, 3.6 mmol) was added to a solution of the carboxyl chloride obtained above in THF (15 mL) at room temperature. An aqueous NaOH solution (2 M, 3.6 mL) was slowly added to the mixture. The reaction mixture was subsequently stirred at room temperature for 16 h and then extracted with ethylacetate (2 × 30 mL). The combined ethylacetate was washed successively with water, dried over $MgSO_4$ and evaporated under vacuum to provide 1.17 g (2.96 mmol, 99% yield) of the title compound as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 2.0 Hz, 1H), 7.32–7.24 (m, 4H), 7.07 (d, J = 8.1 Hz, 2H), 6.93 (br s, 1H, –NH–), 2.98 (d, J = 4.8 Hz, 3H), 2.38 (s, 3H). MS, m/z = 380 ([M+H]$^+$).

4.2.4. *N*-(Aminomethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (10)

To a mixture of glycinamide hydrochloric acid (166 mg, 1.5 mmol), HOBt (243 mg, 1.8 mmol), NMM (1.82 g, 18 mmol) and 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (572 mg, 1.5 mmol) in DMF (15 mL) was added EDCI (345 mg, 1.8 mmol). The reaction mixture was subsequently stirred at room temperature overnight and then the solvent was evaporated off under the reduced pressure. The residue was redissolved in DCM (30 mL) and then washed successively with water, dried over $MgSO_4$ and evaporated under vacuum to provide 550 mg (1.26 mmol, 84% yield) of *N*-carbamoylmethyl amide (**9**) as an intermediate. PIFA (320 mg, 0.74 mmol) was dissolved in ACN (1.9 mL). To this solution water (1.9 mL) was added. Finally, 310 mg (0.71 mmol) of *N*-carbamoylmethyl amide (**9**) was added, and the mixture was stirred at room temperature overnight. The mixture was diluted with 1 M HCl (20 mL) and then washed with ether (2 × 20 mL). The aqueous layer was concentrated under vacuum to provide 108 mg (0.24 mmol, 33% yield) of title compound. The resulting residue was used in the next step without further purification.

4.2.5. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(phenylsulfonyl)-1*H*-pyrazole-3-carboxamide (12a)

To a solution of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic acid (228 mg, 0.6 mmol), DMAP (80.6 mg, 0.66 mmol) and EDCI (126.5 mg, 0.66 mmol) in DCM (5 mL) was added benzenesulfonamide (103.8 mg, 0.66 mmol). After stirring at room temperature for 16 h, the reaction mixture was quenched by addition of 1 M HCl (10 mL). The aqueous layer was separated and extracted with DCM (2 × 10 mL). The combined organic extracts were washed successively with water, dried over $MgSO_4$ and evaporated under vacuum. The residue was further purified by prep HPLC to provide the title compound (280 mg, 0.54 mmol, 90%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (s, 1H), 8.17 (d, J = 9.2 Hz, 2H), 7.65 (t, J = 7.2 Hz, 1H), 7.57 (t, J = 8.0 Hz, 2H), 7.44 (d, J = 2.0 Hz, 1H), 7.34–7.24 (m, 4H), 7.01 (d, J = 8.4 Hz, 2H), 2.28 (s, 3H). MS, m/z = 522 ([M+H]$^+$).

4.2.6. 5-(4-Chlorophenyl)-*N*-(4-chlorophenylsulfonyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (12b)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and 4-chlorobenzenesulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.4 Hz, 2H), 7.44 (s, 1H), 7.34–7.23 (m, 4H), 7.01 (d, J = 8.0 Hz, 2H), 2.28 (s, 3H). MS, m/z = 556 ([M+H]$^+$).

4.2.7. 5-(4-Chlorophenyl)-*N*-(3-chlorophenylsulfonyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12c)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and 3-chlorobenzenesulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.60 (d, J = 9.2 Hz, 1H), 7.50 (t, J = 7.8 Hz, 1H), 7.43 (d, J = 2.3 Hz, 1H), 7.34–7.24 (m, 4H), 7.01 (d, J = 8.7 Hz, 2H), 2.28 (s, 3H). MS, m/z = 556 ([M+H]$^+$).

4.2.8. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(4-fluorophenylsulfonyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12d)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and 4-fluorobenzenesulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 8.22–

8.17 (m, 2H), 7.44 (d, J = 2.4 Hz, 1H), 7.44–7.21 (m, 6H), 7.01 (d, J = 9.2 Hz, 2H), 2.28 (s, 3H). MS, m/z = 538 ($[M+H]^+$).

4.2.9. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(3-fluorophenylsulfonyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12e)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and 3-fluorobenzenesulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.58–7.52 (m, 1H), 7.44 (s, 1H), 7.36–7.24 (m, 5H), 7.01 (d, J = 8.4 Hz, 2H), 2.29 (s, 3H). MS, m/z = 538 ($[M+H]^+$).

4.2.10. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-tosyl-1*H*-pyrazole-3-carboxamide (12f)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and tolylsulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (s, 1H), 8.05 (d, J = 8.0 Hz, 2H), 7.44 (s, 1H), 7.37–7.33 (m, 2H), 7.32–7.23 (m, 4H), 7.01 (d, J = 8.4 Hz, 2H), 2.44 (s, 3H), 2.28 (s, 3H). MS, m/z = 536 ($[M+H]^+$).

4.2.11. *N*-(Benzylsulfonyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12g)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and phenylmethanesulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73–7.57 (m, 2H), 7.44–7.39 (m, 3H), 7.34–7.23 (m, 5H), 7.05 (d, J = 8.7 Hz, 2H), 4.75 (s, 2H), 2.38 (s, 3H). MS, m/z = 536 ($[M+H]^+$).

4.2.12. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(ethylsulfonyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12h)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and ethylsulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 6.4 Hz, 1H), 7.33–7.22 (m, 4H), 7.06 (m, 2H), 3.57 (q, J = 7.4 Hz, 2H), 2.36 (s, 3H), 1.45 (t, J = 7.3 Hz, 3H). MS, m/z = 474 ($[M+H]^+$).

4.2.13. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(isopropylsulfonyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12i)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and isopropylsulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.41 (m, 1H), 7.32–7.23 (m, 4H), 7.06 (d, J = 8.7 Hz, 2H), 3.96–3.87 (m, 1H), 2.34 (s, 3H), 1.48 (d, J = 6.9 Hz, 6H). MS, m/z = 488 ($[M+H]^+$).

4.2.14. *N*-(Butylsulfonyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12j)

The procedure described for the synthesis of **12a** was applied to the acid intermediate (**5**) and butylsulfonamide providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 1.8 Hz, 1H), 7.33–7.24 (m, 4H), 7.04 (d, J = 8.3 Hz, 2H), 3.57–3.51 (m, 2H), 2.34 (s, 3H), 1.92–1.84 (m, 2H), 1.54–1.45 (m, 2H), 0.95 (t, J = 7.4 Hz, 6H). MS, m/z = 500 ($[M+H]^+$).

4.2.15. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(2-ethylbutanoyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16a)

To a solution of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (228 mg, 0.6 mmol) in THF (5 mL) was added 1 M NaHMDS (0.9 mL, 0.9 mmol) at −78 °C under a nitrogen atmosphere. After stirring for 20 min, 2-ethylbutyryl chloride (80.8 mg, 0.6 mmol) dissolved in THF (1 mL) was added dropwise thereto, and the mixture was reacted for 30 min. Then, the mixture was returned to room temperature and further reacted for 16 h. After completion of the reaction, the reaction mixture was pour into saturated $NaHCO_3$ solution (30 mL) and extracted with EtOAc (50 mL). The organic layer was washed successively with water, dried over $MgSO_4$ and evaporated under vacuum. The residue was further purified by prep HPLC to provide the title compound (111 mg, 0.23 mmol, 38%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (br s, 1H, –NH–), 7.45 (d, J = 1.8 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.2 Hz, 2H), 3.33–3. 26 (m, 1H), 2.38 (s, 3H), 1.84–1.73 (m, 2H), 1.66–1.55 (m, 2H), 0.97 (t, J = 7.8 Hz, 6H). MS, m/z = 478 ($[M+H]^+$).

4.2.16. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(2-propylpentanoyl)-1*H*-pyrazole-3-carboxamide (16b)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and 2-propylpentanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (br s, 1H, –NH–), 7.45 (d, J = 2.0 Hz, 1H), 7.35–7.24 (m, 4H), 7.06 (d, J = 8.4 Hz, 2H), 3.45 (m, 1H), 2.38 (s, 3H), 1.81–1.68 (m, 2H), 1.60–1.30 (m, 6H), 0.92 (t, J = 7.1 Hz, 6H). MS, m/z = 506 ($[M+H]^+$).

4.2.17. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(2-ethylhexanoyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16c)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and 2-ethylhexanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.38 (br s, 1H, –NH–), 7.45 (d, J = 2.2 Hz, 1H), 7.36–7.25 (m, 4H), 7.06 (d, J = 8.3 Hz, 2H), 3.35 (m, 1H), 2.38 (s, 3H), 1.82–1.69 (m, 2H), 1.66–1.47 (m, 2H), 1.42–1.26 (m, 4H), 0.97 (t, J = 7.3 Hz, 3H), 0.89 (t, J = 6.8 Hz, 3H). MS, m/z = 506 ($[M+H]^+$).

4.2.18. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(2,2-dimethylbutanoyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16d)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and 2,2-dimethylbutanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.79 (br s, 1H, –NH–), 7.47 (d, J = 2.2 Hz, 1H), 7.34–7.22 (m, 4H), 7.07 (d, J = 9.0 Hz, 2H), 2.38 (s, 3H), 1.64 (q, J = 7.5 Hz, 2H), 1.25 (s, 6H), 0.92 (t, J = 7.5 Hz, 3H). MS, m/z = 478 ($[M+H]^+$).

4.2.19. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(3,3-dimethylbutanoyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16e)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and 3,3-dimethylbutanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.32 (br s, 1H, –NH–), 7.45 (d, J = 2.3 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.7 Hz, 2H), 2.86 (s, 2H), 2.37 (s, 3H), 1.12 (s, 9H). MS, m/z = 478 ($[M+H]^+$).

4.2.20. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-pivaloyl-1*H*-pyrazole-3-carboxamide (16f)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and pivaloyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (br s, 1H, –NH–), 7.46 (d, J = 2.2 Hz, 1H), 7.34–7.22 (m, 4H), 7.06 (d, J = 8.6 Hz, 2H), 2.38 (s, 3H), 1.29 (s, 9H). MS, m/z = 464 ($[M+H]^+$).

4.2.21. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-isobutyryl-4-methyl-1*H*-pyrazole-3-carboxamide (16g)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and isobutyryl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (br s, 1H, –NH–), 7.45 (d, J = 2.2 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.4 Hz, 2H), 3.58–3.48 (m, 1H), 2.38 (s, 3H), 1.26 (d, J = 6.8 Hz, 6H). MS, m/z = 450 ($[M+H]^+$).

4.2.22. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-hexanoyl-4-methyl-1*H*-pyrazole-3-carboxamide (16h)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and hexanoyl chloride providing the ti-

tle product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.35 (br s, 1H, –NH–), 7.44 (d, J = 1.8 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.7 Hz, 2H), 2.96 (t, J = 7.3 Hz, 2H), 2.37 (s, 3H), 1.77–1.69 (m, 2H), 1.42–1.33 (m, 4H), 0.91 (t, J = 6.9 Hz, 3H). MS, m/z = 478 ([M+H]$^+$).

4.2.23. *N*-Butyryl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16i)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and butanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (br s, 1H, –NH–), 7.45 (d, J = 2.3 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.3 Hz, 2H), 2.94 (t, J = 7.3 Hz, 2H), 2.37 (s, 3H), 1.81–1.71 (m, 2H), 1.03 (t, J = 7.3 Hz, 3H). MS, m/z = 450 ([M+H]$^+$).

4.2.24. *N*-Acetyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16j)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and acetyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (br s, 1H, –NH–), 7.45 (d, J = 2.2 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.6 Hz, 2H), 2.60 (s, 3H), 2.38 (s, 3H). MS, m/z = 422 ([M+H]$^+$).

4.2.25. 5-(4-Chlorophenyl)-*N*-(cyclohexanecarbonyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16k)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and cyclohexanecarbonyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (br s, 1H, –NH–), 7.44 (d, J = 1.8 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.2 Hz, 2H), 3.29–3.21 (m, 1H), 2.38 (s, 3H), 2.03–1.96 (m, 2H), 1.86–1.78 (m, 2H), 1.75–1.68 (m, 1H), 1.56–1.33 (m, 4H), 1.31–1.20 (m, 1H). MS, m/z = 490 ([M+H]$^+$).

4.2.26. 5-(4-Chlorophenyl)-*N*-(cyclopentanecarbonyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16l)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and cyclopentanecarbonyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.33 (br s, 1H, –NH–), 7.44 (d, J = 1.8 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.7 Hz, 2H), 3.74–3.66 (m, 1H), 2.38 (s, 3H), 2.06–1.97 (m, 2H), 1.95–1.86 (m, 2H), 1.80–1.71 (m, 2H), 1.69–1.60 (m, 2H). MS, m/z = 476 ([M+H]$^+$).

4.2.27. 5-(4-Chlorophenyl)-*N*-(cyclobutanecarbonyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16m)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and cyclobutanecarbonyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (br s, 1H, –NH–), 7.44 (d, J = 1.8 Hz, 1H), 7.34–7.24 (m, 4H), 7.06 (d, J = 8.2 Hz, 2H), 4.06–3.97 (m, 1H), 2.44–2.28 (m, 7H), 2.09–1.99 (m, 1H), 1.97–1.86 (m, 1H). MS, m/z = 462 ([M+H]$^+$).

4.2.28. 5-(4-Chlorophenyl)-*N*-(cyclopropanecarbonyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (16n)

The procedure described for the synthesis of **16a** was applied to the amide intermediate (**7**) and cyclopropanecarbonyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (br s, 1H, –NH–), 7.44 (d, J = 1.8 Hz, 1H), 7.34–7.24 (m, 4H), 7.07 (d, J = 8.2 Hz, 2H), 3.04–2.97 (m, 1H), 2.39 (s, 3H), 1.23–1.19 (m, 2H), 1.05–1.00 (m, 2H). MS, m/z = 448 ([M+H]$^+$).

4.2.29. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(hexylcarbamoyl)-4-methyl-1*H*-pyrazole-3-carboxamide (17a)

To a solution of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (228 mg, 0.6 mmol) in THF (5 mL) was added 1 M NaHMDS (0.9 mL, 0.9 mmol) at −78 °C under a nitrogen atmosphere. After stirring for 20 min, hexyl isocyanate (76.3 mg, 0.6 mmol) dissolved in THF (1 mL) was added dropwise thereto, and the mixture was reacted for 30 min. Then, the mixture was returned to room temperature and further reacted for 16 h. After completion of the reaction, the reaction mixture was pour into saturated $NaHCO_3$ solution (30 mL) and extracted with EtOAc (50 mL). The organic layer was washed successively with water, dried over $MgSO_4$ and evaporated under vacuum. The residue was further purified by prep HPLC to provide the title compound (90.0 mg, 0.18 mmol, 30%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (br s, 1H, –NH–, imide), 8.36 (t, J = 5.5 Hz, 1H, –NH–, amide), 7.44 (d, J = 2.3 Hz, 1H), 7.34–7.23 (m, 4H), 7.06 (d, J = 8.2 Hz, 2H), 3.35 (q, J = 6.0 Hz, 2H), 2.36 (s, 3H), 1.64–1.56 (m, 2H), 1.42–1.29 (m, 6H), 0.90 (t, J = 6.44 Hz, 3H). MS, m/z = 507 ([M+H]$^+$).

4.2.30. 5-(4-Chlorophenyl)-*N*-(cyclohexylcarbamoyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (17b)

The procedure described for the synthesis of **17a** was applied to the amide intermediate (**7**) and cyclohexyl isocyanate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (br s, 1H, –NH–, imide), 8.32 (d, J = 8.2 Hz, 1H, –NH–, amide), 7.44 (d, J = 2.3 Hz, 1H), 7.33–7.23 (m, 4H), 7.06 (d, J = 8.2 Hz, 2H), 3.84–3.74 (m, 1H), 2.36 (s, 3H), 2.03–1.96 (m, 2H), 1.78–1.71 (m, 2H), 1.65–1.57 (m, 1H), 1.46–1.21 (m, 5H). MS, m/z = 505 ([M+H]$^+$).

4.2.31. 5-(4-Chlorophenyl)-*N*-(cycloheptylcarbamoyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (17c)

The procedure described for the synthesis of **17a** was applied to the amide intermediate (**7**) and cycloheptyl isocyanate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (br s, 1H, –NH–, imide), 8.38 (d, J = 7.8 Hz, 1H, –NH–, amide), 7.44 (d, J = 2.3 Hz, 1H), 7.33–7.23 (m, 4H), 7.06 (d, J = 8.7 Hz, 2H), 4.03–3.94 (m, 1H), 2.36 (s, 3H), 2.04–1.96 (m, 2H), 1.71–1.49 (m, 10H). MS, m/z = 519 ([M+H]$^+$).

4.2.32. 5-(4-Chlorophenyl)-*N*-(cyclohexylmethylcarbamoyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (17d)

The procedure described for the synthesis of **17a** was applied to the amide intermediate (**7**) and cyclohexanemethyl isocyanate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (br s, 1H, –NH–, imide), 8.42 (t, J = 5.9 Hz, 1H, –NH–, amide), 7.44 (d, J = 1.8 Hz, 1H), 7.33–7.23 (m, 4H), 7.06 (d, J = 8.2 Hz, 2H), 3.21 (t, J = 6.4 Hz, 2H), 2.36 (s, 3H), 1.84–1.64 (m, 5H), 1.62–1.52 (m, 1H), 1.32–1.12 (m, 3H), 1.04–0.94 (m, 2H). MS, m/z = 519 ([M+H]$^+$).

4.2.33. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(isopropylcarbamoyl)-4-methyl-1*H*-pyrazole-3-carboxamide (17e)

The procedure described for the synthesis of **17a** was applied to the amide intermediate (**7**) and isopropyl isocyanate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (br s, 1H, –NH–, imide), 8.24 (d, J = 7.8 Hz, 1H, –NH–, amide), 7.44 (d, J = 2.3 Hz, 1H), 7.33–7.23 (m, 4H), 7.06 (d, J = 8.7 Hz, 2H), 4.14–4.04 (m, 1H), 2.36 (s, 3H), 1.26 (d, J = 6.4 Hz, 6H). MS, m/z = 465 ([M+H]$^+$).

4.2.34. *N*-(*tert*-Butylcarbamoyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (17f)

The procedure described for the synthesis of **17a** was applied to the amide intermediate (**7**) and *tert*-butyl isocyanate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (br s, 1H, –NH–, imide), 8.36 (br s, 1H, –NH–, amide), 7.44 (d, J = 2.3 Hz, 1H), 7.33–7.23 (m, 4H), 7.06 (d, J = 8.7 Hz, 2H), 2.35 (s, 3H), 1.44 (m, 9H). MS, m/z = 479 ([M+H]$^+$).

4.2.35. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(phenylcarbamoyl)-1*H*-pyrazole-3-carboxamide (17g)

The procedure described for the synthesis of **17a** was applied to the amide intermediate (**7**) and phenyl isocyanate providing the ti-

tle product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.53 (br s, 1H, –NH–, amide), 9.00 (br s, 1H, –NH–, imide), 7.59 (d, J = 7.4 Hz, 2H), 7.46 (d, J = 1.8 Hz, 1H), 7.37–7.28 (m, 6H), 7.14–7.06 (m, 3H), 2.40 (s, 3H). MS, m/z = 499 ([M+H]$^+$).

4.2.36. *N*-(Benzylcarbamoyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (17h)

The procedure described for the synthesis of **17a** was applied to the amide intermediate (**7**) and benzyl isocyanate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (br s, 1H, –NH–, imide), 8.75 (t, J = 5.5 Hz, 1H, –NH–, amide), 7.48–7.44 (m, 2H), 7.37–7.25 (m, 6H), 7.23–7.14 (m, 2H), 7.07–7.02 (m, 2H), 4.59–4.48 (m, 2H), 2.38 (s, 3H). MS, m/z = 513 ([M+H]$^+$).

4.2.37. Benzyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carbonyl carbamate (18a)

To a solution of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (228 mg, 0.6 mmol) in THF (5 mL) was added 1 M NaHMDS (0.9 mL, 0.9 mmol) at -78 °C under a nitrogen atmosphere. After stirring for 20 min, benzyl chloroformate (102 mg, 0.6 mmol) dissolved in THF (1 mL) was added dropwise thereto, and the mixture was reacted for 30 min. Then, the mixture was returned to room temperature and further reacted for 16 h. After completion of the reaction, the reaction mixture was pour into saturated $NaHCO_3$ solution (30 mL) and extracted with EtOAc (50 mL). The organic layer was washed successively with water, dried over $MgSO_4$ and evaporated under vacuum. The residue was further purified by prep HPLC to provide the title compound (164 mg, 0.32 mmol, 53%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (br s, 1H, –NH–, imide), 7.45–7.24 (m, 10H), 7.06 (d, J = 8.6 Hz, 2H), 5.26 (s, 2H), 2.37 (s, 3H). MS, m/z = 514 ([M+H]$^+$).

4.2.38. Butyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carbonyl carbamate (18b)

The procedure described for the synthesis of **18a** was applied to the amide intermediate (**7**) and butyl chloroformate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.99 (br s, 1H, –NH–, imide), 7.45 (d, J = 2.2 Hz, 1H), 7.35–7.26 (m, 4H), 7.07 (d, J = 8.4 Hz, 2H), 4.24 (t, J = 6.8 Hz, 2H), 2.38 (s, 3H), 1.73–1.59 (m, 2H), 1.48–1.35 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H). MS, m/z = 480 ([M+H]$^+$).

4.2.39. *tert*-Butyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carbonylcarbamate (18c)

The procedure described for the synthesis of **18a** was applied to the amide intermediate (**7**) and *tert*-butyl chloroformate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (br s, 1H, –NH–, imide), 7.45 (d, J = 2.0 Hz, 1H), 7.34–7.24 (m, 4H), 7.07 (d, J = 8.8 Hz, 2H), 2.37 (s, 3H), 1.53 (s, 9H). MS, m/z = 480 ([M+H]$^+$).

4.2.40. Neopentyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carbonylcarbamate (18d)

The procedure described for the synthesis of **18a** was applied to the amide intermediate (**7**) and neopentyl chloroformate providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (br s, 1H, –NH–, imide), 7.45 (-d, J = 1.8 Hz, 1H), 7.36–7.27 (m, 4H), 7.07 (d, J = 8.4 Hz, 2H), 3.94 (s, 2H), 2.38 (s, 3H), 0.98 (s, 9H). MS, m/z = 494 ([M+H]$^+$).

4.2.41. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*,4-dimethyl-*N*-pivaloyl-1*H*-pyrazole-3-carboxamide (19a)

To a mixture of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-*N*,4-dimethyl-1*H*-pyrazole-3-carboxamide (165 mg, 0.42 mmol) and TEA (0.12 mL, 0.84 mmol) in DCM (4 mL) was slowly added pivaloyl chloride (51 mg, 0.42 mmol). The reaction mixture was refluxed at 70 °C for 16 h. Then, the mixture was diluted with DCM (40 mL) and successively washed with saturated NH_4Cl, dried over $MgSO_4$ and evaporated under vacuum. The residue was further purified by prep HPLC to provide the title compound (159 mg, 0.32 mmol, 79%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 2.0 Hz, 1H), 7.32–7.23 (m, 4H), 7.07 (d, J = 8.4 Hz, 2H), 3.32 (s, 3H), 2.30 (s, 3H), 1.27 (s, 9H). MS, m/z = 478 ([M+H]$^+$).

4.2.42. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(2,2-dimethylbutanoyl)-*N*,4-dimethyl-1*H*-pyrazole-3-carboxamide (19b)

The procedure described for the synthesis of **18a** was applied to the *N*-methyl amide intermediate (**8**) and 2,2-dimethylbutanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (d, J = 2.0 Hz, 1H), 7.34–7.23 (m, 4H), 7.07 (d, J = 6.4 Hz, 2H), 3.32 (s, 3H), 2.29 (s, 3H), 1.70 (q, J = 7.6 Hz, 2H), 1.23 (s, 6H), 0.86 (t, J = 7.6 Hz, 3H). MS, m/z = 492 ([M+H]$^+$).

4.2.43. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(2-ethylbutanoyl)-*N*,4-dimethyl-1*H*-pyrazole-3-carboxamide (19c)

The procedure described for the synthesis of **18a** was applied to the *N*-methyl amide intermediate (**8**) and 2-ethylbutanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 2.4 Hz, 1H), 7.33–7.24 (m, 4H), 7.08 (d, J = 8.4 Hz, 2H), 3.36 (s, 3H), 2.96–2.90 (m, 1H), 2.28 (s, 3H), 1.78–1.68 (m, 2H), 1.61–1.50 (m, 2H), 0.89 (t, J = 7.6 Hz, 6H). MS, m/z = 492 ([M+H]$^+$).

4.2.44. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*,4-dimethyl-*N*-(2-propylpentanoyl)-1*H*-pyrazole-3-carboxamide (19d)

The procedure described for the synthesis of **18a** was applied to the *N*-methyl amide intermediate (**8**) and 2-propylpentanoyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (d, J = 3.6 Hz, 1H), 7.34–7.21 (m, 4H), 7.08 (d, J = 8.4 Hz, 2H), 3.35 (s, 3H), 3.07–3.03 (m, 1H), 2.28 (s, 3H), 1.72–1.63 (m, 2H), 1.50–1.41 (m, 2H), 1.35–1.26 (m, 4H), 0.86 (t, J = 7.2 Hz, 6H). MS, m/z = 520 ([M+H]$^+$).

4.2.45. 5-(4-Chlorophenyl)-*N*-(cyclohexanecarbonyl)-1-(2,4-dichlorophenyl)-*N*,4-dimethyl-1*H*-pyrazole-3-carboxamide (19e)

The procedure described for the synthesis of **18a** was applied to the *N*-methyl amide intermediate (**8**) and cyclohexanecarbonyl chloride providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44 (d, J = 2.4 Hz, 1H), 7.33–7.23 (m, 4H), 7.08 (d, J = 8.4 Hz, 2H), 3.35 (s, 3H), 2.89–2.82 (m, 1H), 2.29 (s, 3H), 1.92–1.89 (m, 2H), 1.77–1.73 (m, 2H), 1.68–1.58 (m, 1H), 1.53–1.43 (m, 2H), 1.25–1.17 (m, 3H). MS, m/z = 504 ([M+H]$^+$).

4.2.46. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(octanamidomethyl)-1*H*-pyrazole-3-carboxamide (21a)

To a mixture of octanoic acid (32.8 mg, 0.23 mmol), DMAP (55.5 mg, 0.46 mmol) and *N*-(aminomethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloric acid salt (92.2 mg, 0.21 mmol) in DCM (5 mL) was added EDCI (43.6 mg, 0.23 mmol). After stirring at room temperature overnight, the reaction mixture was pour into 1 M HCl solution (10 mL) and extracted with DCM (2 × 20 mL). The combined organic layer was washed successively with water, dried over $MgSO_4$ and evaporated under vacuum. The residue was further purified by prep HPLC to provide the title compound (60 mg, 0.11 mmol, 53%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (br t, J = 6.4 Hz, 1H, –NH–, amide), 7.41 (d, J = 2.3 Hz, 1H), 7.31–7.27 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.56 (br t, J = 6.0 Hz, 1H, –NH–, amide), 4.80 (t, J = 6.4 Hz, 2H), 2.35 (s, 3H), 2.17 (t, J = 7.8 Hz, 2H), 1.64–1.57 (m, 2H), 1.31–1.20 (m, 8H), 0.85 (t, J = 6.4 Hz, 3H). MS, m/z = 535 ([M+H]$^+$).

4.2.47. *N*-(Butyramidomethyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21b)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and butanoic acid providing the title product. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.80 (br t, J = 6.4 Hz, 1H, –NH–, amide), 7.42 (d, J = 1.8 Hz, 1H), 7.32–7.24 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.55 (br t, J = 6.0 Hz, 1H, –NH–, amide), 4.81 (t, J = 6.4 Hz, 2H), 2.36 (s, 3H), 2.16 (t, J = 7.3 Hz, 2H), 1.70–1.62 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). MS, m/z = 479 ($[M+H]^+$).

4.2.48. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(isobutyramidomethyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21c)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and isobutyric acid providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (br t, J = 6.0 Hz, 1H, –NH–, amide), 7.42 (d, J = 1.8 Hz, 1H), 7.32–7.24 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.58 (br t, J = 5.9 Hz, 1H, –NH–, amide), 4.82 (t, J = 6.4 Hz, 2H), 2.39–2.32 (m, 1H), 2.36 (s, 3H), 1.15 (d, J = 6.9 Hz, 6H). MS, m/z = 479 ($[M+H]^+$).

4.2.49. 5-(4-Chlorophenyl)-*N*-(cyclobutanecarboxamidomethyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21d)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and cyclobutanecarboxylic acid providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (br t, J = 5.9 Hz, 1H, –NH–, amide), 7.42 (d, J = 2.3 Hz, 1H), 7.32–7.25 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.45 (br t, J = 6.4 Hz, 1H, –NH–, amide), 4.81 (t, J = 6.4 Hz, 2H), 3.03–2.95 (m, 1H), 2.36 (s, 3H), 2.32–2.21 (m, 2H), 2.18–2.09 (m, 2H), 2.01–1.81 (m, 2H). MS, m/z = 491 ($[M+H]^+$).

4.2.50. 5-(4-Chlorophenyl)-*N*-(cyclohexanecarboxamidomethyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21e)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and cyclohexanecarboxylic acid providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (br t, J = 6.4 Hz, 1H, –NH–, amide), 7.42 (d, J = 2.3 Hz, 1H), 7.32–7.25 (m, 4H), 7.05 (d, J = 8.3 Hz, 2H), 6.58 (br t, J = 6.4 Hz, 1H, –NH–, amide), 4.81 (t, J = 6.4 Hz, 2H), 2.36 (s, 3H), 2.11–2.03 (m, 1H), 1.88–1.62 (m, 6H), 1.47–1.36 (m, 2H), 1.30–1.16 (m, 2H). MS, m/z = 519 ($[M+H]^+$).

4.2.51. 5-(4-Chlorophenyl)-*N*-(cycloheptanecarboxamidomethyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21f)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and cycloheptanecarboxylic acid providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (br t, J = 6.4 Hz, 1H, –NH–, amide), 7.42 (d, J = 1.8 Hz, 1H), 7.32–7.25 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.52 (br t, J = 6.4 Hz, 1H, –NH–, amide), 4.80 (t, J = 6.9 Hz, 2H), 2.36 (s, 3H), 2.25–2.16 (m, 1H), 1.90–1.83 (m, 2H), 1.78–1.69 (m, 2H), 1.68–1.38 (m, 8H). MS, m/z = 533 ($[M+H]^+$).

4.2.52. 5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-((3-methylcyclohexane carboxamido)methyl)-1*H*-pyrazole-3-carboxamide (21g)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and 3-methylcyclohexanecarboxylic acid providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (br t, J = 6.4 Hz, 1H, –NH–, amide), 7.42 (d, J = 1.8 Hz, 1H), 7.32–7.24 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.56 (br t, J = 6.0 Hz, 1H, –NH–, amide), 4.84–4.78 (m, 2H), 2.36 (s, 3H), 2.13–2.06 (m, 1H), 1.88–1.75 (m, 4H), 1.66–1.63 (m, 1H), 1.43–1.16 (m, 2H), 1.11–1.02 (m, 1H), 0.90 (d, J = 6.4 Hz, 3H), 0.89–0.81 (m, 1H). MS, m/z = 533 ($[M+H]^+$).

4.2.53. 5-(4-Chlorophenyl)-*N*-((2-cyclopentylacetamido)methyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21h)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and cyclopentylacetic acid providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (br t, J = 6.4 Hz, 1H, –NH–, amide), 7.42 (d, J = 2.3 Hz, 1H), 7.32–7.24 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.62 (br t, J = 6.4 Hz, 1H, –NH–, amide), 4.81 (t, J = 6.4 Hz, 2H), 2.36 (s, 3H), 2.23–2.17 (m, 3H), 1.86–1.77 (m, 2H), 1.65–1.48 (m, 4H), 1.19–1.08 (m, 2H). MS, m/z = 519 ($[M+H]^+$).

4.2.54. 5-(4-Chlorophenyl)-*N*-((2-cyclohexylacetamido)methyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (21i)

The procedure described for the synthesis of **21a** was applied to the *N*-aminomethyl amide hydrochloric acid salt intermediate (**10**) and cyclohexylacetic acid providing the title product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (br t, J = 6.4 Hz, 1H, –NH–, amide), 7.42 (d, J = 2.3 Hz, 1H), 7.32–7.24 (m, 4H), 7.05 (d, J = 8.7 Hz, 2H), 6.55 (br t, J = 5.9 Hz, 1H, –NH–, amide), 4.80 (t, J = 6.4 Hz, 2H), 2.36 (s, 3H), 2.04 (d, J = 6.8 Hz, 2H), 1.82–1.61 (m, 5H), 1.31–1.19 (m, 2H), 1.17–1.06 (m, 2H), 0.98–0.87 (m, 2H). MS, m/z = 533 ($[M+H]^+$).

4.3. CB1 receptor binding assay

For CB1 receptor binding studies, rat cerebellar membranes were prepared by the previously described methods.[23] Male Sprague Dawley rats (200–300 g) were sacrificed by decapitation and the cerebella rapidly removed. The tissue was homogenized in 30 volumes of TME buffer (50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, pH 7.4) using a Dounce homogenizer. The crude homogenates were immediately centrifuged (48,000 *g*) for 30 min at 4 °C. The resultant pellets were resuspended in 30 volumes of TME buffer and protein concentration was determined by the method of Bradford and stored at −70 °C until use.[28]

Competitive binding assays were performed as described.[29] Briefly, approximately 10 μg of rat cerebella membranes containing CB1 receptor were incubated in 96-well plate with TME buffer containing 0.5% essentially fatty acid free bovine serum albumin (BSA), 3 nM [^{3}H]CP55,940 (for CB1 receptor, NEN; specific activity 120–190 Ci/mmol) and various concentrations of the synthesized cannabinoid ligands in a final volume of 200 μL. The assays were incubated for 1 h at 30 °C and then immediately filtered over GF/B glass fiber filter (PerkinElmer Life and Analytical Sciences, Boston, MA) that had been soaked in 0.1% PEI for 1 h by a cell harvester (PerkinElmer Life and Analytical Sciences, Boston, MA). Filters were washed five times with ice-cold TBE buffer containing 0.1% essentially fatty acid free BSA, followed by oven-dried for 60 min and then placed in 5 mL of scintillation fluid (Ultima Gold XR; PerkinElmer Life and Analytical Sciences, Boston, MA), and radioactivity was quantitated by liquid scintillation spectrometry. In CB1 receptor competitive binding assay, nonspecific binding was assessed using 1 μM SR141716A. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1 μM concentrations of SR 141716A and was 70 to 80% of the total binding. IC_{50} was determined by nonlinear regression analysis using GRAPHPAD PRISM. All data were collected in triplicate and IC_{50} was determined from three independent experiments.

4.4. Measurements of in vivo activity analysis

Male C57BL/6 J mice weighing over 35 g were housed 1 per cage on a 12-/12-h light/dark cycle, had free access to food (rodent sterilizable diet) and water, and were experimentally native before testing. Mice were allowed at least 7 days to habituate to the experimental room prior to testing, and testing was conducted during the light period. Mice were maintained and experiments were conducted in accordance with the Institutional Animal Care. The reference (rimonabant) and the test compound **16f** was prepared daily by dissolving it in deionized water containing 10% DMSO. By oral administration, animals received at a volume of

Table 5
Hydrophobic, steric and electronic parameters for substituents of representative imide derivatives in the present QSAR study. This series was used for QSAR development

Code	pIC_{50}	Hydrophobic parameter	Steric parameters			Electonic parameters	
		π	Es[a]	L[b]	B5[b]	σ^{*}[c]	σ_I[c]
16a	4.606	2.58	−2.00	5.154	3.631	−0.152	−0.027
16b	4.570	3.42	−2.03	6.208	4.906	−0.308	−0.113
16c	4.366	3.42	−2.03	7.313	4.928	−0.176	−0.050
16d	4.674	2.48	−2.28	5.146	3.726	−0.118	−0.033
16e	4.272	2.48	−1.63	5.221	4.362	−0.186	−0.021
16f	4.485	1.98	−1.43	4.152	3.327	−0.043	−0.027
16g	3.793	1.30	−0.48	4.284	3.341	−0.002	−0.015
16h	4.475	2.50	−0.31	7.085	4.937	−0.060	−0.032
16i	4.360	1.50	−0.31	5.091	3.467	−0.036	−0.009
16j	3.417	0.50	0.00	3.049	2.218	−0.080	0.009
16k	4.517	2.51	−0.69	6.291	3.774	−0.216	−0.047
16l	4.173	2.14	−0.41	5.409	3.748	−0.108	−0.007
16m	3.896	1.51	−0.03	5.110	3.404	0.060	−0.027
16n	3.907	1.14	−1.09	4.461	3.411	0.228	0.061

[a] Taft's steric parameter.
[b] STERIMOL parameters.
[c] Hammett's constants.

10 mL/kg for 14 days. All control animals received 10% DMSO dissolved in deionized water. The vehicle (10% DMSO) treated group was comprised of 5 mice in oral test. There were six mice in each of the other experimental groups ($n = 6$ in each group). By oral administration, the losing weight was checked everyday for the drug treated group and the control group.[30]

4.5. QSAR analysis

All the 14 molecules (as shown in Table 5) for the data set were drawn in 2D structures, and subsequently converted into 3D structures with ACD/CHEMSKETCH (version 11.0, Toronto, ON, Canada). After an energy minimization, the conformation was used in descriptor calculation for each molecule. Most of the descriptors, such as Log*P* and steric parameters (STERIMOL L and STERIMOL B5), were calculated with ACD/CHEMSKETCH (version 11.0, Toronto, ON, Canada), CHEMDRAW ULTRA (version 9.0.1, CambridgeSoft, Cambridge, MA), and Molecular Modeling Pro Plus (ChemSW, Fairfield, CA). Hydrophobic and steric parameters (Taft parameter, Es) of substituents were taken from literature.[26,27]

XLSTAT (version 2008.5.01, Addinsoft, New York, NY), statistical software for EXCEL, was used for the statistical data analysis and development of QSAR equations. A relationship between independent (physicochemical parameters) and dependent (biological activity) variables was determined by using multiple linear regression analysis.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.03.006.

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31. In this article, CB1R data were obtained by single determinations. Whenever we use rimonabant as our reference in our in-house assay, the CB1R binding affinity for rimonabant has showed a certain number in the close range ($IC_{50} = 5.0 \pm 1.0$ nM) in each different assay (>1500 compounds tested). Therefore, we believe that all SAR discussions in the manuscript are scientifically meaningful.
32. The requisite acyl chlorides or acids are not commercially available to date and the predicted IC_{50} values were not convincingly impressive or attractive enough to make the real compounds.
33. In the reported homology modeling study of rimonabant, the carboxamide oxygen forms a hydrogen bond with Lys192, which should be responsible for

the high affinity for the receptor. According to our model in Ref.[30b], the 1,3,4-oxadiazole ring, a bioisostere of amide, forms a bidentate H-bond with Lys192, and this explains boosted affinity of the 1,3,4-oxadiazole series of compounds for CB1 receptor. Here we hoped to mimic 1,3,4-oxadiazole ring moiety with an acyclic imide functionality, since they present similarity in several aspects.

34. The methylenediamide moiety has been reported in the structure of pantocine B and related natural product[19b] as well as other antibiotic.[19c] They did not observe any instability caused by its chemical structure. Neither did us. The synthesis of methylenediamide compounds proved to be uneventful.