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***N*-(4-Cyano-tetrahydro-2*H*-pyran-4-yl) and *N*-(1-Cyanocyclohexyl) Derivatives of 1,5-Diarylpyrazole 3-Carboxamides Showing High Affinity for 18 kDa Translocator Protein and/or Cannabinoid Receptors**

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Abstract

In order to develop improved radioligands for imaging brain CB₁ receptors with positron emission tomography (PET) based on rimonabant (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide; **1**), we synthesized compounds **9a–s** in which the *N*-piperidinyl ring was replaced with a 4-(4-cyano-tetrahydro-2*H*-pyran-4-yl) or 1-cyano-cyclohexyl ring. Such changes were expected to be almost isosteric with **1**, confer greater metabolic resistance and, in the case of the 4-(4-cyano-tetrahydro-2*H*-pyran-4-yl) compounds, substantially reduce lipophilicity. One derivative, 1-(2-bromophenyl)-*N*-(1-cyanocyclohexyl)-5-(4-methoxyphenyl)-4-methyl-pyrazole-3-carboxamide (**9n**), showed high affinity ($K_i = 15.7$ nM) and selectivity for binding to CB₁ receptors. The corresponding 4-(4-cyano-tetrahydro-2*H*-pyran-4-yl) derivative (**9m**) also showed quite high affinity for CB₁ receptors ($K_i = 62$ nM), but was found to have even higher affinity ($K_i = 29$ nM) for the structurally unrelated 18 kDa translocator protein (TSPO). Some other minor structural changes among **9a–s** were also found to switch binding selectivity from CB₁ receptors to TSPO or vice versa. These unexpected findings and their implications for the development of selective ligands or PET radioligands for CB₁ receptors or TSPO are discussed in relation to current pharmacophore models of CB₁ receptor and TSPO binding sites.

Keywords

CB₁ receptor; CB₂ receptor; TSPO receptor; pharmacophore

Introduction

Marijuana (*Cannabis sativa*) is one of the oldest known plant-derived substances used medicinally or as a drug of abuse.¹ Marijuana's principle psychoactive phytocannabinoid, Δ⁹-tetrahydrocannabinol (Δ⁹-THC)², binds with high affinity to two known receptor subtypes, cannabinoid type-1 (CB₁)³ and cannabinoid type-2 (CB₂)⁴. Both the CB₁ and CB₂

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receptor sub-types are G-protein coupled receptors (GPCRs) with 7-transmembrane domains. They have 48% protein sequence homology.⁵ CB₁ receptors have high density in brain and are also located in some peripheral tissues, whereas CB₂ receptors are mostly located in peripheral tissues.⁵ Brain CB₁ receptors are believed to play a role in the regulation of neurotransmitter (e.g., glutamate and γ -aminobutyric acid (GABA)) release through retrograde endocannabinoid-mediated depolarization-induced suppression of inhibition.⁶ Alterations in brain CB₁ receptor densities and function have been implicated in several psychiatric disorders, such as drug dependence,⁷ obesity,⁸ depression,⁹ and schizophrenia¹⁰. Therefore, there is keen interest to study brain CB₁ receptors in relation to such disorders in living human subjects with molecular imaging techniques, such as positron emission tomography (PET), and suitably selective radioligands.¹¹

In 1994 Sanofi Recherche, presently Sanofi-Aventis, introduced the first high-affinity and selective CB₁ receptor ‘antagonist’, rimonabant (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide; SR141716A **1**, Chart 1).¹² This ligand is now considered to be an inverse-agonist. The key 1,5-diarylpyrazole pharmacophore of **1** has been modified extensively to evaluate substituent-effects on receptor affinity, selectivity and physicochemical properties.¹³ Additionally, **1** has been modified to allow labeling with a short-lived imaging radionuclide (e.g., carbon-11 or fluorine-18) in the development of PET radioligands.¹¹ Compound **1** is highly lipophilic with a computed *cLogD* value of 6.32 (Table 1), and hence many candidate PET radioligands derived from **1** retain high lipophilicity.¹¹ This is usually detrimental to the success of a PET radioligand^{14–18} because high lipophilicity generally promotes low and poorly measurable free radioligand fraction in plasma, low radioligand penetration from blood to brain, and high non-specific binding to brain tissue. Therefore, success in the development of PET radioligands from **1** has been quite limited. PET CB₁ receptor radioligands developed from other structural platforms also tend to be quite lipophilic, although some are gaining application in human subjects.¹¹ Examples are [¹¹C]OMAR¹⁹ (**2**), [¹¹C] MEPPEP^{20,21} (**3**), [¹⁸F]FMPEP-*d*₂²² (**4**), and [¹⁸F]MK-9470²³ (**5**) (Chart 1).

The possible efficacious roles of the nitrile group in pharmacophore development have recently been highlighted,²⁴ and include its ability to act as a carbonyl or halogen isostere, to act as a hydroxyl or carboxyl surrogate, and to reduce lipophilicity without adverse steric effect. In our search for leads to improved PET radioligands for CB₁ receptors, we considered that the replacement of the *N*-piperidinyl ring in **1** with a 1-cyanocyclohexyl or 4-(4-cyano-tetrahydro-2*H*-pyranyl) ring (Scheme 1) would be a minor steric perturbation and would confer lower lipophilicity. Moreover, these rings, because of their nitrile substitution, may show greater resistance to metabolism than the replaced piperidinyl ring, which has been shown to be the exclusive region of metabolism of **1** in vitro²⁵ and in vivo^{25,26}. We therefore set out to synthesize a set of compounds with replaced *N*-piperidinyl rings and to evaluate their structure-activity relationships at CB₁ receptors. Selective high-affinity CB₁ ligands were discovered and also very unexpectedly selective high-affinity ligands for a structurally and functionally distinct site, the 18 kDa translocator protein, previously known as the peripheral benzodiazepine receptor (PBR) and now known as TSPO.²⁷ These findings and their implications for ligand and PET radioligand development are discussed and rationalized in the context of an identified level of congruence between previously proposed pharmacophore models of the CB₁ receptor and TSPO.

^aAbbreviations: CB₁, cannabinoid type-1, CB₂, cannabinoid type-2; D, dopamine; DAT, dopamine transporter; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMF, dimethylformamide; DOR, δ opiate receptor; GABA_A-Bz site, γ -aminobutyric acid_A-benzodiazepine site; GPCR, G-protein coupled receptor; H, histamine; 5-HT, 5-hydroxytryptamine; KOR, κ opiate receptor; MeCN, acetonitrile; MOR, μ opiate receptor; NBS, *N*-bromosuccinimide; NCA, no-carrier added; NET noradrenaline transporter; PET, positron emission tomography; PBR, peripheral benzodiazepine receptor; rt, room temperature; SERT, serotonin transporter; THF tetrahydrofuran; Δ^9 -THC, Δ^9 -tetrahydrocannabinol.

Results and Discussion

Chemistry

N-(4-Cyano-tetrahydro-2*H*-pyran-4-yl) and *N*-(1-cyanocyclohexyl) derivatives of 1,5-diarylpyrazole 3-carboxamides constituted the two core scaffolds of all newly synthesized compounds. Substituents in the 2-, 3- and 4-positions of the pyrazole-*N*¹ and -C⁵ aryl rings, and at the pyrazole 3-carboxamide and 4-positions of the core scaffolds were also varied. Because of our strong interest in developing radioligands for imaging with PET, nearly all of the new ligands were designed to be amenable to labeling with cyclotron-produced, carbon-11 ($t_{1/2} = 20.4$ min) by commonly used procedures such as C-¹¹C-methylation (**9b–f**, **9h–o**, **9r** and **9s**), O-¹¹C-methylation (**9l–q**), N-¹¹C-methylation (**9d**), or ¹¹C-cyanation (**9g**, and **9p**).²⁸

Compounds were synthesized as shown in Scheme 2. Substituted anilines were first converted into diazonium salts and then treated with ethyl 2-chloroacetoacetate under basic conditions to give **6a–g** in good yields. A series of aryl ketones (**7a–f**) were converted into enamines with morpholine, TiCl₄ and DIPEA (diisopropylethylamine) in toluene solution using a modified general procedure.²⁹ Compounds **6a–g** were then treated with the morpholine enamines under basic conditions to give the 1,5-diarylpyrazoles **8a–k**, **8m** and **8n** in low but useful yields by a regioselective 1,3-dipolar cycloaddition reaction.³⁰ Treatment of the acyl acetonitrile, **7h** with **6c** gave the diarylpyrazole **8p** in low yield. Diarylpyrazole **8l** was prepared in high yield by bromination of **8k** with NBS (*N*-bromosuccinimide) using acetonitrile as a polar solvent to favor the formation of bromine. Each of the diarylpyrazoles **8a–q** were hydrolyzed to the acids, converted into acyl chlorides and treated with either 4-amino-tetrahydro-2*H*-pyran-4-carbonitrile or 1-amino-cyclohexanecarbonitrile HCl to give the desired carboxamides (**9a–c** and **9e–s**). Treatment of **9c** with NaH and MeI gave the tertiary amide **9d** in high yield.

Computed ligand lipophilicities

The most common structural change made to the scaffold of **1**, namely replacement of the *N*-(piperidin-1-yl)-4*H*-pyrazole-3-carboxamide moiety with an *N*-(4-cyano-tetrahydro-2*H*-pyran-4-yl)-4*H*-pyrazole-3-carboxamide moiety was computed to reduce lipophilicity by about 0.65 *cLogD* units. Consequently, the majority of the prepared ligands have much lower *cLogD* values than **1** (Table 1). These *cLogD* values approach values in the range that is generally preferred for PET radioligands (~ 1.5– 3.5).^{14–18} Replacement of the 4-(4-cyano-tetrahydro-2*H*-pyran-4-yl) group with a 1-cyano-cyclohexyl group increases the computed lipophilicity substantially (Table 1). Nevertheless, the prepared examples still have substantially lower lipophilicity than **1**.

Structure–affinity relationships

The sequence homologies and structures of TSPO, and CB₁ and CB₂ receptors are each highly conserved between species.^{5,27} Therefore, the binding affinities of ligands determined for each of these sites in tissue from one species in vitro are expected to be quite representative of those in other animal species. Prompted by the serendipitous and unexpected discovery of the high affinity of **9m** for TSPO in a broad receptor screen of this compound (see later), each new ligand was assayed at TSPO, CB₁, CB₂ and GABA_A-Bz sites (Table 1). All compounds exhibited low affinity ($K_i > 10$ μM, $n = 4$) for the GABA_A-Bz site. Across the synthesized series of compounds, the K_i values for CB₁ and CB₂ receptors and TSPO ranged widely, namely from 15.7 to > 10,000 nM, 175 to > 10,000 nM and 29.0 to > 10,000 nM, respectively.

The substituent in the N^1 -aryl ring (R^1 , Table 1) was mainly varied with respect to halogen (F, Cl, Br and I) and position. In compounds having a pyrazole- C^5 phenyl substituent (R^2 , Table 1) (**9b–f**, **9h–k**), such variations failed to generate compounds with affinity for CB₁ or CB₂ receptors in the $K_i < 100$ nanomolar range. All these compounds had measurable affinity for TSPO, with the majority of 4-(4-cyano-tetrahydro-2H-pyran-2-yl) compounds showing less than micromolar affinity. One compound, the relatively lipophilic 1-cyanocyclohexyl compound **9f**, showed relatively higher TSPO affinity ($K_i = 75$ nM). Remarkably, this compound was wholly selective for TSPO versus CB₁ or CB₂ receptors. Generally, high-affinity TSPO ligands are tertiary amides and their secondary amide counterparts have lower affinity.^{31,32} However, methylation of the amido nitrogen in the ligand **9c** led to compound **9d** with a marked two-fold reduction in TSPO receptor affinity (to $K_i = 880$ nM), but with retained selectivity versus CB₁ or CB₂ receptors. Introduction of the large phenyl substituent into the 2-position of the N^1 -aryl ring in place of halogen (Cl, Br or I), as in **9k**, switched the selectivity from TSPO to CB₁ receptors. A 4-bromo substituent, as in **9i**, also induced selectivity for CB₁ receptors. In this group of compounds, replacement of the 4-methyl group (R^3 , Table 1) on the pyrazole ring with hydrogen (**9a**) or nitrile (**9g**) conferred CB₁ selectivity.

Introduction of a substituent into the C^5 -aryl ring (R^2 , Table 1) had a major influence on receptor affinity and selectivity. Compounds with a 4-methoxy substituent in the C^5 -aryl ring plus a 2-fluoro or 2-bromo substituent on the N^1 -aryl ring (**9l–n**) showed quite high affinity for both CB₁ receptors and TSPO, with very low affinity for CB₂ receptors. Remarkably, the 4-(4-cyano-tetrahydro-2H-pyran-2-yl) compound (**9m**) with a 2-bromo substituent in the C^5 -aryl ring showed high affinity ($K_i = 29$ nM) for binding to TSPO with two-fold preference over binding to CB₁ receptors. Even more remarkably, replacement of the tetrahydro-2H-pyran ring oxygen atom with a methylene group, as in the 1-cyanocyclohexyl analog (**9n**), caused selectivity to be reversed and affinity for CB₁ receptors to be enhanced ($K_i = 15.7$ nM). In the whole group of pyrazole- C^5 4-methoxyphenyl compounds (**9l–q**), binding site affinity and selectivity were sensitive to position and type of substituent in the pyrazole- C^5 aryl ring. Thus, a compound with a 3-bromo substituent (**9o**) showed quite a high affinity ($K_i = 62$ nM) and selectivity for binding to TSPO versus CB₁ receptors. This compound also showed very low affinity for CB₂ receptors, as did the other N^1 -3-bromophenyl compounds (**9h**, **9q**). A 2-iodo substituent in the N^1 -aryl ring and a 4-nitrile group in the pyrazole ring (**9p**) led to very low affinity at CB₁ and CB₂ receptors and at TSPO. Introduction of a 4-fluoro substituent into the C^5 -aryl ring, as in **9r**, in place of a 4-methoxy substituent enhanced selectivity of binding to CB₁ receptors versus TSPO. However, shift of this substituent to the 3-position, as in **9s**, abolished high-affinity CB₁ receptor binding.

Receptor and binding site screening of **9m** and **9n**

Ligands **9m** and **9n** at 10 μ M concentration showed $< 50\%$ inhibition ($n = 4$) for radioligand binding to the following receptors and binding sites: 5-HT_{1A,E}, 5-HT_{1D} (CycMEP), 5-HT_{2A–C}, 5-HT₃, 5-HT_{5A}, 5-HT₆, 5-HT₇, $\alpha_{1A,B}$, $\alpha_{2A–C}$, $\beta_{1,3}$, D_{1–4}, DAT, DOR, H_{1–4}, M_{1–5}, MOR, NET, SERT, $\sigma_{1,2}$, and V_{1A,1B,2}. The K_i values ($n = 3$) of **9m** at 5-HT_{1A}, 5-HT_{1D}, D₅ and KOR were $6,280 \pm 1,100$, $> 10,000$, $> 10,000$ and 798 ± 84 nM, respectively. The corresponding K_i values for **9n** were $1,130 \pm 96$, $> 10,000$, $> 10,000$ and 597 ± 67 nM. Thus, **9m** is highly selective for TSPO and CB₁ receptors versus other tested binding sites and **9n** is highly selective for CB₁ receptors versus all other tested binding sites and receptors.

Relationship of ligand structures to previously proposed CB₁ receptor and TSPO pharmacophore models

CB₁ receptors and TSPO are structurally distinct proteins. The CB₁ receptor belongs to the family of GPCRs having 7-transmembrane domains and considerable information has been obtained on the likely topography and make-up of the binding site for ligands structurally resembling **1**. This information has been summarized and a pharmacophore model proposed.¹³ The model proposes i) a hydrogen bond interaction between the amide oxygen of the ligand and the Asp366-Lys192 salt bridge in the receptor, ii) a pocket formed by Val 196, Phe 170, Leu 387 and Met 384 that can accommodate a lipophilic alicyclic group, and iii) two distal pockets, one formed by Trp255, Tyr 275 and Phe 278 and the other by Trp279, Trp356 and Phe 200 which can each accept a substituted phenyl ring, as depicted in Panel A, Scheme 3. It may be hypothesized that the highest affinity and most CB₁-selective ligand found in this study, **9n**, fits into the proposed pharmacophore in the same manner as the prototypical selective CB₁ receptor ligand **1** (Panel A, Scheme 3). TSPO protein is not a GPCR, but a tryptophan-rich 18 kDa protein that is a component of a trimeric complex with the 32 kDa voltage-dependent anion channel and the 30 kDa adenine nucleotide translocase to constitute the mitochondrial permeability transition pore.²⁷ Relatively less is known about the amino acids composing the binding site for high-affinity TSPO ligands from a plethora of structural classes. Nevertheless, a pharmacophore model has been proposed which comprises three lipophilic pockets (dubbed L1, L3 and L4) and an H-bond donor group (Panel B, Scheme 3).^{33–35} This pharmacophore model accommodates classical TSPO ligands such as the isoquinoline 3-carboxamide **10** (see Table 1 for structure) and TSPO ligands from some other structural classes, including the recently described *N*¹-methyl-2-phenylindol-3-ylglyoxylamides, of which **11** (Table 1) is a recent example.³⁶ The model also accommodates the highest affinity TSPO ligand found in this study, namely **9m** (Panel B, Scheme 3).

We observe that the previously described pharmacophore models for CB₁ receptors and TSPO are strikingly similar in the arrangement of key domains of binding site-ligand interaction; that is the pharmacophores appear quite congruent at this macro level of description. Ligands with the general structural motif shown in Panel C (Scheme 3) therefore have potential to bind to either or both pharmacophores. This provides a rational basis for our unexpected findings on binding selectivity. Clearly, our CB₁ and TSPO ligand binding data also reveal marked sensitivities to subtle differences in ligand structure, as exemplified by **9m** and **9n** (Table 1). At a deeper level of description the pharmacophores would be expected to be noticeably different and this would account for the severe alterations or even reversals of ligand selectivity with minor structural changes.

Implications for PET radioligand development

As for CB₁ receptors, the development of PET radioligands for imaging brain and peripheral TSPO currently attracts immense effort.³² This effort on TSPO radioligands is driven by their potential to become important biomarkers of inflammation in a variety of neuropsychiatric and peripheral conditions. The majority of radioligands being developed for CB₁ receptors or TSPO share the general structural motif depicted in Panel C (Scheme 3). PET radioligands are required to bind selectively to their target proteins. Therefore, a message from this study is to screen candidate PET radioligands for CB₁ receptors for high affinity binding at TSPO and vice versa in order to confirm adequate target selectivity. Screens against CB₂ receptors are also of course advised.

The ligand **9m** possesses too low affinity and selectivity to be developed as a PET radioligand for TSPO, but could perhaps be a lead to better candidates. [¹¹C]**2** (Chart 1) has been developed as a promising PET radioligand for brain CB₁ receptors and is now in use in

human subjects. This radioligand is selective with moderately high affinity ($K_b = 30$ nM) and a $cLogD$ value of 5.3. By comparison, ligand **9n** also shows excellent selectivity for CB₁ receptors, high affinity ($K_i = 16$ nM) and only somewhat higher $cLogD$ value (5.87; Table 1). The labeling of **9n** with carbon-11, which is feasible in its 4-methoxy group, for evaluation in vivo may therefore be warranted.

Conclusion

Modification of the prototypical CB₁ receptor ligand **1**, primarily by replacing the *N*-piperidinyl ring with a 4-(4-cyano-tetrahydro-2*H*-pyranyl) or 1-cyano-cyclohexyl ring, led respectively to one high-affinity CB₁ selective ligand (**9n**), and also surprisingly to a ligand with a preference to bind with high affinity to TSPO (**9m**). Many of the prepared ligands showed quite high affinity for both CB₁ and TSPO. These unexpected findings may be rationalized on the basis of the observed congruence between previously proposed pharmacophore models for CB₁ receptors and TSPO.

Experimental

Materials

All reagents and solvents (ACS or HPLC grade) were purchased from commercial sources and used as supplied. 1-(2-Chlorophenyl)-*N*-methyl-*N*-sec-butyl-isoquinoline-3-carboxamide (PK 11195, **10**) was purchased from Tocris Biosciences.

Chloro[(phenyl)hydrazono]ethyl acetates **6a–d** were synthesized according to a previously described procedure.^{30,37} The 1,5-diarylpyrazoles **8o** and **8q** were prepared as previously described.^{38,39}

General methods

¹H (400 MHz) and ¹³C (100 MHz) NMR spectra were recorded at r.t. on a Varian-400 spectrometer (Varian, Walnut Creek, CA) or Avance-400 spectrometer (Bruker; Billerica, MA). Chemical shifts are reported in δ units (ppm) downfield relative to the chemical shift of tetramethylsilane. Signals are quoted as s (singlet), d (doublet), dd (double doublet), dt (double triplet), t (triplet), q (quartet) or m (multiplet). High-resolution mass spectra (HRMS) were determined at the University of Illinois at Urbana (Champaign, IL, USA) or Notre Dame Mass Spectrometry Facility (Notre Dame, Indiana). Melting points were determined using Mel-temp II (Laboratory Devices, Holliston, MA, USA) or Mel-temp apparatus (Electrothermal, Fisher Scientific, USA) and are uncorrected. Flash column chromatography was performed on silica gel (230–400 mesh, 60 Å) (Sigma-Aldrich). Elemental analyses were acquired from Midwest Microlab, LLC (Indianapolis, IN, USA).

Chemistry

Chloro[(2-phenylphenyl)hydrazono]ethyl acetate (6e)—A solution of 2-aminobiphenyl (7.6 g, 45 mmol) in concentrated HCl (12 M, 5.4 mL) plus H₂O (200 mL) was stirred in a 1-L round-bottomed flask for 30 min. The reaction mixture was cooled to about 5 °C in an ice-water bath. A solution of sodium nitrite (3.2 g, 46 mmol) in H₂O (10 mL) was slowly added to the reaction mixture so that the reaction temperature remained below 15 °C. The reaction mixture was then stirred for another 30 min. A separate solution of sodium acetate (3.5 g, 43 mmol) and ethyl 2-chloroacetoacetate (6.2 mL, 45 mmol) in EtOH-H₂O (400 mL, 4:1 v/v) was added dropwise. The reaction mixture was allowed to warm to r.t. and stirred overnight (16 h). The precipitate was filtered off, washed with water and dried to give **6e** (11.3 g, 83 %) as a light yellow solid. Mp 82–84 °C; ¹H NMR δ 8.60 (s, 1H), 7.69 (d, $J = 8.4$ Hz, 1H), 7.51–7.36 (m, 6H), 7.26–7.22 (m, 1H), 7.12 (t, $J = 6.8$ Hz, 1H), 4.40 (q, $J = 7.2$ Hz, 2H), 1.41 (t, $J = 6.8$ Hz, 3H).

Chloro[(3-bromophenyl)hydrazono]ethyl acetate (6f)—The procedure described for the synthesis of **5e** was applied to 3-bromo-aniline to give **6f** (52%) as a light brown solid. Mp 84–86 °C; ¹H NMR δ 8.60 (s, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.53 (t, *J* = 6.8, 2H), 7.45–7.36 (m, 4H), 7.26 (t, *J* = 6.8 Hz, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 4.40 (q, *J* = 7.2 Hz, 2H), 1.41 (t, *J* = 6.8 Hz, 3H).

Chloro[(4-bromophenyl)hydrazono]ethyl acetate (6g)—The procedure described for the synthesis of **5e** was applied to 4-bromo-aniline to give **6g** (78%) as a pale white solid. Mp 84–86 °C; ¹H NMR δ 8.30 (s, 1H), 7.44 (t, *J* = 1.6, 1H), 7.21–7.11 (m, 3H), 4.43 (q, *J* = 7.2 Hz, 2H), 1.43 (t, *J* = 6.8 Hz, 3H).

Ethyl 1-(2-fluorophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxylate (8a)—Morpholine (33 mL, 358 mmol) was added to a suspension of MgSO₄ (4 g) in toluene 60 mL) and was stirred at r.t. TiCl₄ (47 mL, 1 M in toluene) was slowly added to give a dark green suspension. Propiophenone (**7a**, 8.0 g, 60 mmol) and DIPEA (52 mL, 298 mmol) were dissolved separately in toluene (20 mL). This mixture was slowly added to the solution containing TiCl₄ and morpholine. The reaction was heated to 60 °C for 16 h, cooled to r.t. and then filtered. The cake was washed with toluene, which was then added to the filtrate. The combined filtrate was concentrated *in vacuo* to give crude the morpholine enamine (8.2 g, 68%) as a pale yellow oil.

DIPEA (7.2 mL, 41.4 mmol) was added to a stirred solution of **6a** (3.3 g, 13.7 mmol) and the morpholine enamine (2.8 g, 13.7 mmol) in EtOH (100 mL). The mixture was stirred at r.t. for 16 h and then concentrated *in vacuo*. The resulting crude product was purified by column chromatography on silica gel (20% EtOAc in hexanes) to give **8a** (1.1 g, 25%) as a white solid. Mp 66–68 °C; ¹H NMR δ 7.47 (td, *J* = 1.6 and 7.6 Hz, 1H), 7.32–7.29 (m, 4H), 7.18–7.15 (m, 3H), 7.02 (td, *J* = 1.6 and 7.6 Hz), 4.49 (q, *J* = 7.2 Hz, 2H), 2.35 (s, 3H), 1.43 (t, *J* = 6.8 Hz, 3H).

Ethyl 1-(2-chlorophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxylate (8b)—The procedure described for the synthesis of **8a** was applied to **6b** and **7a** to yield **8b** (21%) as a solid. Mp 116–118 °C; ¹H NMR δ 7.42 (dd, *J* = 1.6 and 7.2 Hz, 1H), 7.36–7.26 (m, 6H), 7.17–7.14 (m, 2H), 4.49 (q, *J* = 7.2 Hz, 2H), 2.36 (s, 3H), 1.45 (t, 6.8 Hz, 3H).

Ethyl 1-(2-bromophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxylate (8c)—The procedure described for the synthesis of **8a** was applied to **6c** and **7a** to yield **8c** (23%) as a solid. Mp 116–118 °C; ¹H NMR δ 7.54 (dd, *J* = 1.6 and 7.2 Hz, 1H), 7.39 (dd, *J* = 1.6 and 7.2 Hz, 1H), 7.33–7.16 (m, 7H), 4.49 (q, *J* = 7.2 Hz, 2H), 2.37 (s, 3H), 1.45 (t, *J* = 6.8, 3H).

Ethyl 1-(3-bromophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxylate (8d)—The procedure described for the synthesis of **8a** was applied to **6f** and **7a** to yield **8d** (15%) as a semi-solid; ¹H NMR δ 7.56 (t, *J* = 2.0 Hz, 1H), 7.45–7.39 (m, 4H), 7.17–7.00 (m, 4H), 4.49 (q, *J* = 7.2 Hz, 2H), 2.39 (s, 3H), 1.43 (t, *J* = 6.8, 3H).

Ethyl 1-(4-bromophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxylate (8e)—The procedure described for the synthesis of **8a** was applied to **6g** and **7a** to yield **8e** (25%) as a solid. Mp 76–78 °C; ¹H NMR δ 7.42–7.37 (m, 5H), 7.16–7.17 (m, 4H), 4.49 (q, *J* = 7.2 Hz, 2H), 2.39 (s, 3H), 1.43 (t, *J* = 6.8, 3H).

Ethyl 1-(2-iodophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxylate (8f)—The procedure described for the synthesis of **8a** was applied to **6d** and **7a** to yield **8f** (22%) as a

solid. Mp 122–124 °C; ^1H NMR δ 7.80 (d, J = 8.0 Hz, 1H), 7.34–7.27 (m, 5H), 7.20–7.18 (m, 2H), 7.08–7.04 (m, 1H), 4.49 (q, J = 7.2 Hz, 2H), 2.37 (s, 3H), 1.45 (t, J = 6.8 Hz, 3H).

Ethyl 1-(2-phenylphenyl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxylate (8g)—

The procedure described for the synthesis of **8a** was applied to **6e** and **7a** to yield **8g** (17%) as a semi-solid. ^1H NMR δ 7.73 (d, J = 7.2 Hz, 1H), 7.21 (dd, J = 1.6 and 8.0 Hz, 1H), 7.17–7.12 (m, 2H), 7.08 (p, J = 8.4 Hz, 4H), 6.51 (d, J = 7.6 Hz, 2H), 6.42 (d, J = 7.6 Hz, 2H), 4.45 (q, J = 7.2 Hz, 2H), 2.17 (s, 3H), 1.48 (t, J = 7.2 Hz).

Ethyl 1-(2-bromophenyl)-5-phenyl-1H-pyrazole-3-carboxylate (8h)—

The procedure described for the synthesis of **8a** was applied to **6c** and **7b** to yield **8h** (24%) as a solid. Mp 90–92 °C; ^1H NMR δ 7.62 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.49 (t, J = 7.6 Hz, 1H), 7.34–7.20 (m, 6H), 7.10 (s, 1H), 4.49 (q, J = 7.2 Hz, 2H), 1.45 (t, J = 6.8 Hz).

Ethyl 1-(2-fluorophenyl)-5-(4-methoxyphenyl)-4-methyl-1H-pyrazole-3-

carboxylate (8i)—The procedure described for the synthesis of **8a** was applied to **6a** and **7c** to yield **8i** (19%) as a white solid. Mp 86–88 °C; ^1H NMR δ 7.46 (td, J = 1.6 and 7.6 Hz, 1H), 7.35–7.29 (m, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.09 (d, J = 11.2 Hz, 2H), 7.04 (t, J = 9.2 Hz), 6.85 (d, J = 11.2 Hz, 2H), 4.48 (q, J = 7.2 Hz, 2H), 3.79 (s, 3H), 2.33 (s, 3H), 1.45 (t, J = 6.8 Hz, 3H).

Ethyl 1-(3-bromophenyl)-5-(4-methoxyphenyl)-4-methyl-pyrazole-3-

carboxylate (8j)—The procedure described for the synthesis of **8a** was applied to **6f** and **7g** to yield **8j** (12%) as a semi-solid. ^1H NMR δ 7.57 (t, J = 1.9 Hz, 1H), 7.46–7.44 (m, 1H), 7.22–7.11 (m, 3H), 7.06–7.04 (m, 2H), 6.93 (d, J = 8.8), 4.47 (q, J = 7.2 Hz, 2H), 3.84 (s, 3H), 2.29 (s, 3H), 1.45 (t, J = 6.8 Hz, 3H).

Ethyl 1-(3-bromophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate (8k)

—The procedure described for the synthesis of **8a** was applied to **6f** and **7c** to yield **8k** (26%) as a solid. Mp 64–68 °C; ^1H NMR δ 7.61 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.42 (t, J = 7.6 Hz, 1H), 7.33 (t, J = 7.6 Hz, 1H), 7.14 (d, J = 8.0 Hz, 2H), 7.03 (s, 1H), 6.80 (d, J = 7.2 Hz, 2H), 4.49 (q, J = 7.2 Hz, 2H), 3.78 (s, 3H), 1.45 (t, J = 6.8 Hz, 3H).

Ethyl 4-bromo-1-(3-bromophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-

carboxylate (8l)—NBS (334 mg, 1.9 mmol) was added to a stirred solution of **8k** (500 mg, 1.2 mmol) in MeCN (25 mL). The reaction mixture was heated to reflux for 8 h and then concentrated *in vacuo*. The crude was purified with flash chromatography (hexanes–EtOAc, 8:2 v/v) to give **8l** (543 mg, 91%). Mp 118–120 °C; ^1H NMR δ 7.61 (t, J = 2.0 Hz, 1H), 7.46 (dd, J = 6.8 Hz, 1H), 7.21 (d, J = 9.3 Hz, 2H), 7.16 (t, J = 8.4 Hz, 1H), 7.06 (d, J = 7.6 Hz, 1H), 6.93 (d, J = 8.4 Hz, 2H), 4.50 (q, J = 7.2 Hz, 2H), 3.84 (s, 3H), 1.47 (t, J = 6.8 Hz, 3H).

Ethyl 1-(2-bromophenyl)-5-(4-fluorophenyl)-4-methyl-1H-pyrazole-3-

carboxylate (8m)—The procedure described for the synthesis of **8a** was applied to **6c** and **7d** to yield **8m** (20%) as a white solid. Mp 109–110 °C; ^1H NMR δ 7.56 (d, J = 7.6 Hz, 1H), 7.39–7.32 (m, 2H), 7.25–7.23 (m, 1H), 7.17 (dd, J = 5.6 and 3.2 Hz, 2H), 7.01 (t, J = 8.4 Hz, 2H), 4.49 (q, J = 7.2 Hz, 2H), 2.34 (s, 3H), 1.45 (t, J = 6.8 Hz, 3H).

Ethyl 1-(2-bromophenyl)-5-(3-fluorophenyl)-4-methyl-1H-pyrazole-3-

carboxylate (8n)—The procedure described for the synthesis of **8a** was applied to **6c** and **7e** to yield **8n** (19%) as a white solid. Mp 106–108 °C; ^1H NMR δ 7.57 (d, J = 7.6 Hz, 1H),

7.40–7.32 (m, 2H), 7.29–7.24 (m, 2H), 7.02 (t, $J = 6.4$ Hz, 1H), 6.96 (d, $J = 8.0$ Hz, 1H), 6.90 (d, $J = 9.6$ Hz, 1H), 4.49 (q, $J = 7.2$ Hz, 2H), 2.34 (s, 3H), 1.45 (t, $J = 6.8$ Hz, 3H).

Ethyl 1-(2-bromophenyl)-4-cyano-5-phenyl-1H-pyrazole-3-carboxylate (8p)—A solution of **6c** (10.5 g, 34.4 mmol), benzoylacetonitrile (**7h**, 5.0 g, 34.4 mmol) and DIPEA (13.3 g, 103.2 mmol) was refluxed in MeCN (100 mL) for 16 h. The reaction mixture was then concentrated *in vacuo* and the crude product extracted with ether (3 × 100 mL). The ethereal extracts were combined and concentrated *in vacuo*. The crude was purified with flash chromatography (hexanes–EtOAc, 7:3 v/v) to give **8p** (1.1 g, 8%) as a pale white solid. Mp 106–108 °C; ^1H NMR δ 7.63 (d, $J = 8.0$ Hz, 1H), 7.46–7.35 (m, 8H), 4.56 (q, $J = 7.2$ Hz, 2H), 1.49 (t, $J = 6.8$ Hz, 3H).

1-(2-Bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-5-phenyl-1H-pyrazole-3-carboxamide (9a)—An aqueous solution (6 mL) of LiOH (819 mg, 34.2 mmol) was added to a stirred solution of **8c** (500 mg, 966 μmol) in THF (60 mL) and heated to reflux for 6 h. The reaction was cooled to r.t. and volatile compounds were removed *in vacuo*. The residue was dissolved in water (50 mL) and neutralized with *aq.* HCl. The resulting precipitate was filtered-off and dried *in vacuo* yielding crude acid (242 mg, 52%). DMF (1 drop) and oxalyl chloride (66 μL , 777 μmol) were added to a stirred solution of the acid (200 mg, 518 μmol) in dry dichloromethane (DCM; 10 mL). After bubbling had ceased, the reaction was concentrated *in vacuo*. A solution of 4-amino-tetrahydro-2H-pyran-4-carbonitrile (101 mg, 673 μmol) and DIPEA (90 μL , 777 μmol) in DCM (10 mL) was added to the acid chloride and stirred at r.t. for 2 h. The volatile compounds were removed *in vacuo* and the crude residue purified with silica gel chromatography (hexanes–EtOAc, 60:40 v/v) to yield **9a** (58% from acid) as a white solid. Mp 226–228 °C; ^1H NMR δ 7.68 (d, $J = 8.0$ Hz, 1H), 7.44–7.39 (m, 2H), 7.39–7.35 (m, 1H), 7.35–7.25 (m, 3H), 7.12 (s, 1H), 7.06 (s, 1H), 4.01 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.88 (td, $J = 2.0$ and 12.4 Hz, 2H), 2.57 (d, $J = 12.8$ Hz, 2H), 2.11 (td, $J = 4.4$ and 10.0 Hz); HRMS (tof): $[\text{M}+\text{Na}]^+$, calc'd for $\text{C}_{22}\text{H}_{19}^{79}\text{BrN}_4\text{NaO}_2$, 473.0584; found, 473.0562, error 4.5 ppm; Anal. calc'd for $\text{C}_{22}\text{H}_{19}\text{BrN}_4\text{O}_2$: C 58.5, H 4.2, N 12.4; found C 58.4, H 4.3, N 12.3.

N-(4-Cyano-tetrahydro-2H-pyran-4-yl)-1-(2-fluorophenyl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxamide (9b)—The procedure described for the synthesis of **9a** was applied to **8a** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9b** (68% from acid) as a white solid. Mp 212–214 °C; ^1H NMR δ 7.40–7.32 (m, 5H), 7.21–7.05 (m, 5H), 4.00 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.87 (td, $J = 2.0$ and 12.4 Hz, 2H), 2.56 (d, $J = 12.8$ Hz, 2H), 2.40 (s, 3H), 2.07–2.04 (m, 2H); HRMS (tof): $[\text{M}+\text{H}]^+$, calc'd for $\text{C}_{23}\text{H}_{22}\text{FN}_4\text{O}_2$, 405.1721; found, 405.1711, error 2.5 ppm; Anal. calc'd for $\text{C}_{23}\text{H}_{21}\text{FN}_4\text{O}_2 \cdot 0.3 \text{H}_2\text{O}$: C 67.4, H 5.3, N 13.7; found C 67.4, H 5.3, N 13.7.

1-(2-Chlorophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxamide (9c)—The procedure described for the synthesis of **9a** was applied to **8b** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9c** (56% from acid) as a white solid. Mp 208–210 °C; ^1H NMR δ 7.43–7.28 (m, 7H), 7.15–7.12 (m, 2H), 7.10 (s, 1H), 4.01 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.86 (td, $J = 2.0$ and 12.4 Hz, 2H), 2.55 (d, $J = 12.8$ Hz, 2H), 2.40 (s, 3H), 2.06–2.04 (m, 2H); HRMS (tof): $[\text{M}+\text{Na}]^+$, calc'd for $\text{C}_{23}\text{H}_{21}\text{ClN}_4\text{NaO}_2$, 443.1245; found, 443.1231, error 3.2 ppm; Anal. calc'd for $\text{C}_{23}\text{H}_{21}\text{ClN}_4\text{O}_2$: C 65.6, H 5.0, N 13.3; found C 65.7, H 5.0, N 13.0.

1-(2-Chlorophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-N,4-dimethyl-5-phenyl-1H-pyrazole-3-carboxamide (9d)—NaH (60% in dispersion) (17 mg, 428 μmol) was added to a stirred solution of **9c** (90 mg, 223 μmol) and methyl iodide (61 mg,

428 μmol) in DMF (5 mL). The reaction was stirred for 1 h and then concentrated *in vacuo*. The crude was purified with flash chromatography (hexanes–EtOAc, 8:2 v/v) to give **9d** (62 mg, 64%) as a pale white solid. Mp 182–184 °C; ^1H NMR δ 7.45 (d, J = 9.2 Hz, 1H), 7.35–7.20 (m, 6H), 7.18–7.14 (m, 2H), 4.09 (dd, J = 2.8 and 12.8 Hz, 2H), 3.45 (s, 3H), 2.58 (d, J = 2.8 Hz, 2H), 2.28 (s, 3H), 2.17 (td, J = 4.0 and 12.0 Hz, 2H); HRMS (tof): $[\text{M}+\text{Na}]^+$, calc'd for $\text{C}_{24}\text{H}_{23}\text{ClN}_4\text{NaO}_2$, 457.1402; found, 457.1386, error 3.4 ppm; Anal. calc'd for $\text{C}_{24}\text{H}_{23}\text{ClN}_4\text{O}_2$: C 66.3, H 5.3, N 12.9; found C 65.9, H 5.3, N 12.6.

1-(2-Bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxamide (9e)—The procedure described for the synthesis of **9a** was applied to **8c** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9e** (58% from acid) as a white solid. Mp 188–190 °C; ^1H NMR δ 7.62 (d, J = 8.0, 1H), 7.36–7.28 (m, 6H), 7.18–7.14 (m, 2H), 7.10 (s, 1H), 4.01 (td, J = 2.0 and 12.4 Hz, 2H), 3.87 (td, J = 2.0 and 12.4 Hz, 2H), 2.56 (d, J = 12.8 Hz, 2H), 2.41 (s, 3H), 2.07–2.04 (m, 2H); HRMS (tof): $[\text{M}+\text{H}]^+$, calc'd for $\text{C}_{23}\text{H}_{22}^{79}\text{BrN}_4\text{O}_2$, 465.0921; found, 465.0908, error 2.8 ppm; Anal. calc'd for $\text{C}_{23}\text{H}_{21}\text{BrN}_4\text{O}_2$: C 59.4, H 4.5, N 12.0; found C 59.5, H 4.6, N 11.7.

1-(2-Bromophenyl)-N-(1-cyanocyclohexyl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxamide (9f)—The procedure described for the synthesis of **9a** was applied to **8c** and 1-aminocyclohexanecarbonitrile HCl to yield **9f** (62% from acid) as a white solid. Mp 212–214 °C; ^1H NMR δ 7.61 (d, J = 8.0 Hz, 7.37–7.25 (m, 6H), 7.16–7.14 (m, 2H), 7.04 (s, 1H), 2.51 (bs, 2H), 2.41 (s, 3H), 1.78–1.69 (m, 7H), 1.32 (bs, 1H). HRMS (tof): $[\text{M}+\text{H}]^+$, calc'd for $\text{C}_{24}\text{H}_{23}^{79}\text{BrN}_4\text{NaO}$, 485.0953; found, 485.0947, error 3.8 ppm; Anal. calc'd for $\text{C}_{24}\text{H}_{23}\text{BrN}_4\text{O}$: C 62.2, H 5.0, N 12.1; found C 62.1, H 5.2, N 12.3.

1-(2-Bromophenyl)-4-cyano-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-5-phenyl-1H-pyrazole-3-carboxamide (9g)—The procedure described for the synthesis of **9a** was applied to **8p** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9g** (71% from acid) as a solid. Mp 224–226 °C; ^1H NMR δ 7.69 (d, J = 8.4 Hz, 1H), 7.49–7.32 (m, 8H), 6.95 (s, 1H), 4.02 (dt, J = 3.2 and 12.8 Hz, 2H), 3.86 (td, J = 2.0 and 12.4 Hz, 2H), 2.58 (d, J = 12.8 Hz, 2H), 2.09–2.04 (m, 2H); HRMS (tof): $[\text{M}+\text{H}]^+$, calc'd for $\text{C}_{23}\text{H}_{19}^{79}\text{BrN}_5\text{O}_2$, 476.0717; found, 476.0700, error 3.6 ppm; Anal. for $\text{C}_{23}\text{H}_{18}\text{BrN}_5\text{O}_2$: C 58.0, H 3.8, N 14.7; found C 57.6, H 3.9, N 14.5.

1-(3-Bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxamide (9h)—The procedure described for the synthesis of **9a** was applied to **8d** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9h** (53% from acid) as a white solid. Mp 138–140 °C; ^1H NMR δ 7.56 (t, J = 2 Hz, 1H), 7.45 (m, 4H), 7.17–7.12 (m, 3H), 7.10 (s, 1H), 7.00 (d, J = 7.2 Hz, 1H), 4.02 (dt, J = 3.2 and 12.8 Hz, 2H), 3.88 (td, J = 2.0 and 12.4 Hz, 2H), 2.59 (d, J = 12.8 Hz, 2H), 2.36 (s, 3H), 2.10–2.04 (m, 2H); HRMS (tof): $[\text{M}+\text{Na}]^+$, calc'd for $\text{C}_{23}\text{H}_{21}^{79}\text{BrN}_4\text{NaO}_2$, 487.0740; found, 487.0726, error 2.8 ppm; Anal. calc'd for $\text{C}_{23}\text{H}_{21}\text{BrN}_4\text{O}_2$: C 59.4, H 4.5, N, 12.0; found C 59.3, H 4.5, N 12.0.

1-(4-Bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-4-methyl-5-phenyl-1H-pyrazole-3-carboxamide (9i)—The procedure described for the synthesis of **9a** was applied to **8e** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9i** (34% from acid) as a solid. Mp 176–178 °C; ^1H NMR δ 7.46 (dt, J = 2.4 and 9.2 Hz, 2H), 7.41–7.38 (m, 3H), 7.16–7.13 (m, 3H), 7.11 (dt, J = 2.4 and 9.2 Hz, 2H), 4.02 (dt, J = 3.2 and 12.8 Hz, 2H), 3.88 (td, J = 2.0 and 12.4 Hz, 2H), 2.57 (d, J = 12.8 Hz, 2H), 2.36 (s, 3H), 2.08–2.04 (m, 2H); HRMS (tof): $[\text{M}+\text{Na}]^+$, calc'd for $\text{C}_{23}\text{H}_{21}^{79}\text{BrN}_4\text{NaO}_2$, 487.0740; found,

487.0721, error 3.9 ppm; Anal. calc'd for $C_{23}H_{21}BrN_4O_2$: C 59.4, H 4.5, N 12.0; found C 59.2, H 4.6, N 11.9.

***N*-(4-Cyano-tetrahydro-2*H*-pyran-4-yl)-1-(2-iodophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxamide (9j)**—The procedure described for the synthesis of **9a** was applied to **8f** and 4-amino-tetrahydro-2*H*-pyran-4-carbonitrile to yield **9j** (61% from acid) as a solid. Mp 200–202 °C; 1H NMR δ 7.88 (dd, J = 1.2 and 7.6 Hz, 1H), 7.39 (td, J = 1.2 and 7.6 Hz, 1H), 7.31–7.29 (m, 3H), 7.27 (dd, J = 1.6 and 7.6, 1H), 7.18–7.16 (m, 2H), 7.14–7.09 (m, 2H), 4.01 (dt, J = 3.2 and 12.8 Hz, 2H), 3.87 (td, J = 2.0 and 12.4 Hz, 2H), 2.56 (d, J = 12.8 Hz, 2H), 2.41 (s, 3H), 2.08–2.04 (m, 2H); HRMS (tof): $[M+H]^+$, calc'd for $C_{23}H_{22}IN_4O_2$, 513.0728; found, 513.0780, error 0.5 ppm; Anal. calc'd for $C_{23}H_{21}IN_4O_2$: C 53.9, H 4.1, N 10.9; found C 54.0, H 4.1, N 10.8.

***N*-(4-Cyano-tetrahydro-2*H*-pyran-4-yl)-1-(2-phenylphenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxamide (9k)**—The procedure described for the synthesis of **9a** was applied to **8g** and 4-amino-tetrahydro-2*H*-pyran-4-carbonitrile to yield **9k** (44% from acid) as a white solid. Mp 98–100 °C; 1H NMR δ 7.62 (d, J = 7.6, 1H), 7.51–7.47 (m, 2H), 7.29–7.02 (m, 8H), 6.51 (d, J = 7.6 Hz, 2H), 6.47 (d, J = 7.6 Hz, 2H), 4.01 (dt, J = 3.2 and 12.8 Hz, 2H), 3.87 (td, J = 2.0 and 12.4 Hz, 2H), 2.56 (d, J = 12.8 Hz, 2H), 2.41 (s, 3H), 2.08–2.04 (m, 2H); HRMS (tof): $[M+Na]^+$, calc'd for $C_{29}H_{26}N_4NaO_2$, 463.2129; found, 463.2107, error 4.7 ppm; Anal. calc'd for $C_{29}H_{26}N_4O_2$: C 75.3, H 5.7, N 12.1; found C 74.9, H 5.8, N 11.7.

***N*-(4-Cyano-tetrahydro-2*H*-pyran-4-yl)-1-(2-fluorophenyl)-5-(4-methoxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (9l)**—The procedure described for the synthesis of **9a** was applied to **8i** and 4-amino-tetrahydro-2*H*-pyran-4-carbonitrile to yield **9l** (59% from acid) as a white solid. Mp 210–212 °C; δ 7.41–7.34 (m, 2H), 7.22 (t, J = 7.2 Hz, 1H), 7.10–7.05 (m, 4H), 6.85 (d, J = 8.4 Hz, 2H), 4.01 (dt, J = 3.2 and 12.8 Hz, 2H), 3.88 (td, J = 2.0 and 12.4 Hz, 2H), 3.79 (s, 3H), 2.57 (d, J = 12.8 Hz, 2H), 2.35 (s, 3H), 2.11–2.04 (m, 2H); HRMS (tof): $[M+Na]^+$ calc'd for $C_{24}H_{23}FN_4NaO_3$, 457.1646; found, 457.1831, error 3.4 ppm; Anal. calc'd for $C_{24}H_{23}FN_4O_3 \cdot 0.3 H_2O$: C 65.5, H 5.4, N 12.7; found C 65.5, H 5.4, N 12.7.

1-(2-Bromophenyl)-*N*-(4-cyano-tetrahydro-2*H*-pyran-4-yl)-5-(4-methoxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (9m)—The procedure described for the synthesis of **9a** was applied to **8o** and 4-amino-tetrahydro-2*H*-pyran-4-carbonitrile to give **9m** (87 mg, 34% from acid) as a white solid. Mp 208–210 °C; 1H NMR δ 7.62 (dd, J = 6.7 and 1.0 Hz, 1H), 7.37–7.23 (m, 3H), 7.09 (s, 1H), 7.08 (dt, J = 8.8 and 1.6 Hz, 2H), 6.82 (dt, J = 8.8 and 1.6 Hz, 2H), 3.99 (d, J = 8.1 Hz, 2H), 3.85 (t, J = 10.6 Hz, 2H), 3.77 (s, 3H), 2.54 (d, J = 13.4 Hz, 2H), 2.38 (s, 3H), 2.06 (t, J = 10.0 Hz, 3H); ^{13}C NMR δ 162.41, 159.70, 144.30, 143.16, 133.60, 131.07, 130.81, 130.02, 128.08, 122.10, 120.88, 119.03, 113.89, 63.81, 55.21, 49.15, 35.65, 9.48; HRMS (m/z) $[M+H]^+$, calc'd for $C_{24}H_{24}^{79}BrN_4O_3$, 495.1032; found 495.1029, error –0.6 ppm; Anal. calc'd for $C_{24}H_{23}BrN_4O_3$: C 58.2, H 4.7, N 11.3; found C 58.2, N 11.3, H 4.8.

1-(2-Bromophenyl)-*N*-(1-cyanocyclohexyl)-5-(4-methoxyphenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (9n)—The procedure described for the synthesis of **9a** was applied to **8o** to afford **9n** (36% from acid) as a white solid. Mp = 220–222 °C; 1H NMR δ 7.61 (dd, J = 6.7 and 1.0 Hz, 1H), 7.35–7.24 (m, 3H), 7.07 (dt, J = 8.8 and 1.6 Hz, 2H), 7.01 (s, 1H), 3.77 (s, 3H), 2.51 (bs, 2H), 2.39 (s, 3H), 1.77–1.71 (m, 8H), 1.15 (bs, 1H); ^{13}C NMR δ 162.22, 158.67, 144.12, 143.49, 138.98, 133.56, 132.84, 131.06, 130.70, 130.06, 128.05, 122.14, 119.90, 117.99, , 114.56, 68.74, 51.39, 35.64, 24.84, 22.15, 9.55; HRMS (m/z)

z) $[M+H]^+$, calc'd for $C_{25}H_{26}^{79}BrN_4O_2$, 493.1239; found 493.1235, error -0.8 ppm; Anal. calc'd for $C_{25}H_{25}BrN_4O_2$: C 60.9, H 5.1, N 11.4; found C 60.8, H 5.4, N 11.2.

1-(3-Bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-5-(4-methoxyphenyl)-4-methyl-1H-pyrazole-3-carboxamide (9o)—The procedure described for the synthesis of **9a** was applied to **8j** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9o** (53% from acid) as a white solid. Mp 160–162 °C; 1H NMR δ 7.58 (bs, 1H), 7.45 (d, $J = 8.4$ Hz, 1H), 7.15–7.11 (m, 2H), 7.09 (d, $J = 8.8$ Hz, 2H), 7.00 (d, $J = 8.4$ Hz, 1H), 6.92 (d, $J = 8.4$ Hz, 2H), 4.01 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.88–3.83 (m, 5H), 3.79 (s, 3H), 2.57 (d, $J = 12.8$ Hz, 2H), 2.35 (s, 3H), 2.10–2.04 (m, 2H); HRMS (tof): $[M+H]^+$, calc'd for $C_{24}H_{24}^{79}BrN_4O_3$, 495.1028; found, 495.1020, error 1.4 ppm; Anal. calc'd for $C_{24}H_{23}BrN_4O_3$: C 58.2, H 4.7, N 11.3; found C 58.2, H 4.8, N 11.2.

4-Cyano-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-1-(2-iodophenyl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxamide (9p)—The procedure described for the synthesis of **9a** was applied to **8q** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9p** (68% from acid) as a white solid. Mp 232–214 °C; 1H NMR δ 7.95 (dd, $J = 1.2$ and 8.0 Hz, 1H), 7.52 (td, $J = 1.2$ and 7.2 Hz, 1H), 7.38 (dd, $J = 1.6$ and 7.6 Hz, 1H), 7.29–7.22 (m, 3H), 6.95 (s, 1H), 6.88 (dt, $J = 2.8$ and 9.2 Hz), 4.02 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.86 (td, $J = 2.0$ and 12.4 Hz, 2H), 2.58 (d, $J = 12.8$ Hz, 2H), 2.09–2.04 (m, 2H); HRMS (tof): $[M+Na]^+$, calc'd for $C_{24}H_{20}IN_5NaO_3$, 576.0503; found, 576.0483, error 3.5 ppm; Anal. calc'd for $C_{24}H_{20}IN_5O_3$: C 52.1, H 3.6, N 12.7; found C 52.0, H 3.7, N 12.7.

4-Bromo-1-(3-bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxamide (9q)—The procedure described for the synthesis of **9a** was applied to **8l** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9q** (56% from acid) as a solid. Mp 182–184 °C; 1H NMR δ 7.58 (t, $J = 1.6$ Hz, 1H), 7.50 (d, $J = 8.0$ Hz, 1H), 7.21–7.15 (m, 3H), 7.07 (s, 1H), 7.02 (d, $J = 8.0$ Hz, 1H), 6.94 (dt, $J = 2.8$ and 8.8 Hz, 2H), 4.01 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.88–3.83 (m, 5H), 3.79 (s, 3H), 2.57 (d, $J = 12.8$ Hz, 2H), 2.35 (s, 3H), 2.10–2.04 (m, 2H); HRMS (tof): $[M+Na]^+$, calc'd for $C_{23}H_{20}^{79}Br_2N_4NaO_3$, 580.9794; found, 580.9774, error 3.5 ppm; Anal. calc'd for $C_{23}H_{20}Br_2N_4O_3$: C 49.3, H 3.6, N 10.0; found C 49.2, H 3.6, N 9.8.

1-(2-Bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-5-(4-fluorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (9r)—The procedure described for the synthesis of **9a** was applied to **8m** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9r** (54%) as a solid. Mp 188–190 °C; 1H NMR δ 7.62 (d, $J = 6.4$ Hz, 1H), 7.40–7.28 (m, 3H), 7.15–7.11 (m, 2H), 7.09 (s, 1H), 7.02 (t, $J = 9.6$ Hz, 2H), 4.01 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.88–3.83 (m, 5H), 3.79 (s, 3H), 2.56 (d, $J = 12.8$ Hz, 2H), 2.38 (s, 3H), 2.07–2.00 (m, 2H); HRMS (tof): $[M+H]^+$, calc'd for $C_{23}H_{21}^{79}BrFN_4O_2$, 483.0826; found, 483.0821, error 1.2 ppm; Anal. calc'd for $C_{23}H_{20}BrFN_4O_2$: C 57.2, H 4.2, N 11.6; found C 57.2, H 4.2, N 11.4.

1-(2-Bromophenyl)-N-(4-cyano-tetrahydro-2H-pyran-4-yl)-5-(3-fluorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (9s)—The procedure described for the synthesis of **9a** was applied to **8n** and 4-amino-tetrahydro-2H-pyran-4-carbonitrile to yield **9s** (62% from acid) as a solid. Mp 204–206 °C; 1H NMR δ 7.64 (d, $J = 8.0$ Hz, 1H), 7.41–7.25 (m, 4H), 7.09 (s, 1H), 7.04 (td, $J = 2.4$ and 8.4 Hz, 1H), 6.95 (d, $J = 8.0$ Hz, 1H), 6.88 (dt, $J = 2.0$ and 9.6 Hz, 1H), 4.01 (dt, $J = 3.2$ and 12.8 Hz, 2H), 3.88–3.83 (m, 5H), 3.79 (s, 3H), 2.56 (d, $J = 12.8$ Hz, 2H), 2.38 (s, 3H), 2.07–2.00 (m, 2H); HRMS (tof): $[M+Na]^+$, calc'd for $C_{23}H_{20}^{79}BrFN_4O_2$, 505.0646; found, 505.0623, error 4.6 ppm; Anal. calc'd for $C_{23}H_{20}BrFN_4O_2$: C 57.2, H 4.2, N 11.6; found C 57.3, H 4.3, N 11.6.

Computation of *cLogD*

The lipophilicity of each synthesized ligand, expressed as the partition of the compound between *n*-octanol and buffer at pH 7.4 (*cLogD*) was computed with Pallas 3.70 software (Compudrug, South San Francisco, CA).

Receptor assays

Ligands **9a–s** were tested for affinity (K_i values) at GABA_A-Bz site, TSPO, and CB₁, and CB₂ receptors by the National Institute of Mental Health Psychoactive Drug Screening Program. Detailed protocols are available on-line for all binding assays at NIMH-PDSP web site (<http://pdsp.med.unc.edu>).

Receptor screening

Ligands **9m** and **9n** were screened for binding to a wide range of receptors and transporters by National Institute of Mental Health Psychoactive Drug Screening Program. Detailed protocols are available on-line for all binding assays at NIMH-PDSP web site (<http://pdsp.med.unc.edu>).

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Scheme 1.

The main structural changes made to **1** in the newly synthesized set of ligands.

Scheme 2.

Syntheses of **9a–s**. Reagents, conditions and yields: a) HCl-*aq*, NaNO₂, AcONa, ethyl 2-chloroacetoacetate, 52–83%; b) for **7a–f**, morpholine, DIPEA, TiCl₄, toluene, MgSO₄; c) DIPEA, MeCN or EtOH, 18–26%; d) ref. 35; e) DIPEA, *t*-BuOH or MeCN, reflux, 16 h, 6–8%, ref. 36; f) NBS, MeCN, 91%; g) 1: LiOH, *aq.*-THF; 2: oxalyl chloride, DMF (cat.), DCM; 3: 4-amino-tetrahydro-2*H*-pyran-4-carbonitrile or 1-aminocyclohexanecarbonitrile HCl, DIPEA, DCM, 34–71% (for **9a–e**, **9g–n** and **9p–s**), 36–68% for **9f** and **9o**; h) NaH (60% dispersion in oil), DMF, MeI, 64%

Scheme 3.

Compatibility of some new ligands with previously proposed pharmacophore models. **A:** Compatibility of **9n** with a proposed¹³ CB₁ receptor pharmacophore model. **B:** Compatibility of **9m** with a proposed^{31–33} TSPO receptor pharmacophore model. **C:** Common general features of proposed CB₁ receptor and TSPO pharmacophore models. Known CB₁-selective ligands such as **1–5** (Chart 1) and known TSPO-selective ligands **10** and **11** (Table 1) are examples of compounds from different structural classes that are compatible with the pharmacophore motif shown in panel C.

Chart 1.
Structures of **1** and some PET CB₁ receptor radioligands in human use.

Table 1

cLogD values and K_i values at CB₁ and CB₂ receptors and at TSPO for known CB₁ receptor ligands **1**, **3**, **5**, new ligands **9a–s**, and known TSPO ligands **10–12**.

Ligand
	R¹	R²	R³	R⁴	R⁵	cLogD^a	CB₁K_i (nM)^b	CB₂K_i (nM)^b	TSPO K_i (nM)^b			
1						6.32	1.4 ± 0.2	927 ± 66	N.A.			
3						4.76	0.472 ^c	> 1,980 ^c	N.A.			
5						4.98	0.70 ^d	44 ^d	N.A.			
9a	2-Br	H	H	O	H	3.61	297 ± 31	> 10,000	3,110 ± 572			
9b	2-F	H	Me	O	H	3.55	> 10,000	2,180 ± 224	394 ± 62.4			
9c	2-Cl	H	Me	O	H	3.80	> 10,000	> 10,000	436 ± 40.5			
9d	2-Cl	H	Me	O	Me	3.77	> 10,000	> 10,000	880 ± 36.2			
9e	2-Br	H	Me	O	H	3.81	> 10,000	> 10,000	576 ± 27.3			
9f	2-Br	H	Me	CH ₂	H	5.85	> 10,000	> 10,000	75.2 ± 2.51			
9g	2-Br	H	CN	O	H	3.22	437 ± 44	> 10,000	3,690 ± 519			
9h	3-Br	H	Me	O	H	3.81	> 10,000	226 ± 9.5	115 ± 4.9			
9i	4-Br	H	Me	O	H	3.81	259 ± 26	655 ± 36.5	420 ± 20.5			
9j	2-I	H	Me	O	H	3.92	> 10,000	> 10,000	762 ± 51.0			
9k	2-Ph	H	Me	O	H	4.63	500 ± 58	> 10,000	2,800 ± 116			
9l	2-F	4-MeO	Me	O	H	3.66	115 ± 11	2,460 ± 214	132 ± 4.91			
9m	2-Br	4-MeO	Me	O	H	3.94	62.2 ± 12.8	1,890 ± 350	29.0 ± 3.0

Ligand	R ¹	R ²	R ³	R ⁴	R ⁵	<i>cLogD</i> ^a	CB ₁ K _i (nM) ^b	CB ₂ K _i (nM) ^b	TSPO K _i (nM) ^b	
9n	2-Br	4-MeO	Me	CH ₂	H	5.87	15.7 ± 2.3	2,750 ± 183	279 ± 52.3	
9o	3-Br	4-MeO	Me	O	H	3.94	680 ± 108	234 ± 13.8	61.8 ± 2.2	
9p	2-I	4-MeO	CN	O	H	3.86	> 10,000	2,370 ± 168	1,920 ± 116	
9q	3-Br	4-MeO	Br	O	H	4.00	356 ± 40	175 ± 10.4	155 ± 6.7	
9r	2-Br	4-F	Me	O	H	4.08	55 ± 6	> 10,000	492 ± 28.2	
9s	2-Br	3-F	Me	O	H	4.08	> 10,000	> 10,000	693 ± 28.1	
10						4.89	N.A.	N.A.	1.0 ± 0.0	
11						4.29	N.A.	N.A.	5.70 ^e	

^a Computed with Pallas 3.70 software (CompuDrug, South San Francisco, CA) for distribution of compound between *n*-octanol and pH7.4 buffer.

^b Values represent mean ± SD of three determinations.

^c K_b values from reference 20.

^d IC₅₀ value from reference 23.

^e From reference 36.

N.A. = not available.