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Solid Phase Synthesis of 1,5-Diarylpyrazole-4-carboxamides: Discovery of Antagonists of the CB-1 Receptor

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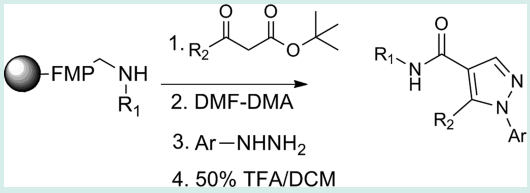
Supporting Information

ABSTRACT: We have developed a solid phase synthesis route to 1,5-substituted pyrazole-4-carboxamides with three diversity points aimed at the discovery of new compounds as potential G-Protein coupled receptor (GPCR) ligands. The new chemistry involves acylation of a resin bound secondary amine with a β-ketoester via transamidation, conversion of the resulting β-ketoamide to the corresponding vinylogous amide, pyrazole formation upon reaction with a aryl hydrzine, and cleavage of the product from the resin. Using the reported methodology, we describe the syntheses of multiple arrays of pyrazoles that were used collectively to construct a library of more than 1000 analogues. Several members of this library displayed submicromolar antagonist activities at the cannabinoid subtype 1 (CB-1) receptor.



KEYWORDS: *solid phase synthesis, pyrazole-4- carboxamide, GPCR ligands, privileged scaffolds*

INTRODUCTION

The solid-phase synthesis of compound libraries for general screening has been shown to be effective for lead identification, and can facilitate subsequent lead optimization when the solid phase route has sufficient generality to allow access to novel analogues of interest. However, a challenge in these exercises is selecting a structural motif to serve as the library scaffold, given the diversity of structures that can now be prepared on solid-phase and the related range of available chemistries.[1,2]

Our efforts in this area have been guided by the concept of "privileged scaffolds", which are defined as structural templates known to yield biologically active molecules for a range of therapeutically interesting targets.[3,4] In the current work, we identified the pyrazole heterocycle as one such privileged scaffold based on its wide-ranging biological activities.[5] As shown in Figure 1, bioactive pyrazoles include the following: G-Protein coupled receptor (GPCR) targeted drugs such as the serotonin $5HT_{2A}$ inverse agonist Nelotanserin (**1a**), used for the treatment of insomnia;[6] Celecoxib (**1b**), a selective cyclooxygenase-2 (COX-2) inhibitor that is marketed for the treatment of osteoarthritis, rheumatoid arthritis, and acute pain;[7] and the N-substituted pyrazole Doramapimod (**1c**), an inhibitor of p38 MAP kinase used for the treatment of cancer.[8]

In this current study, we report a solid phase synthesis of 1,5-diarylpyrazole-4-carboxamides, as typified by **2** in Figure 2, and comment on the generality of the reported chemistry. From a library synthesis perspective, compounds of this type are attractive because they contain three vectors (denoted R_1, R_2, and Ar) that allow the introduction of significant structural diversity. The pendant functionality is also disposed in a less common 1,4,5-pattern, which we felt would explore a chemical space that had not been adequately addressed in currently disclosed structural classes.[9−27]

Using the reported methodology, we describe the syntheses of multiple arrays of pyrazoles that were used collectively to construct a library of more than 1000 analogues. This compound collection was subsequently screened against a range of GPCR targets, with hits being identified against multiple GPCR receptors. Of particular interest to us was the finding that several members of this library displayed submicromolar antagonist activities at the cannabinoid subtype 1 (CB-1) receptor, vide infra.

CHEMISTRY

The following chemistry was conducted using IRORI Micro-Kan technology as a synthesis platform.[28] The chemical protocol developed (Scheme 1) involves the introduction of the first diversity element (R_1) by loading a primary amine reagent chemset **3** (Figure 3) onto a 4-formyl-3-methoxyphenoxy (FMP) resin[29] via standard reductive amination[30] conditions to provide resin-bound amine chemset **4**. The subsequent transacylacetylation[31] of **4** with an aromatic *tert*-butyl-β-ketoester (R_2) reagent chemset **5** (Figure 4) requires heating in a resin-compatible solvent such as NMP in the presence of catalytic DMAP, and provides corresponding resin-bound β-ketoamide chemset **6**, containing the nascent R_2 substituent. The success of this step is highly dependent on temperature, with 80 °C found to be optimal. Conversion of the resulting β-ketoamide chemset **6** to the corresponding vinylogous amide chemset **7** is accomplished by reaction with dimethylformamide-dimethylacetal in DMF at 95 °C for 48 h. Cyclization of chemset **7** to resin-bound chemset **9** can be

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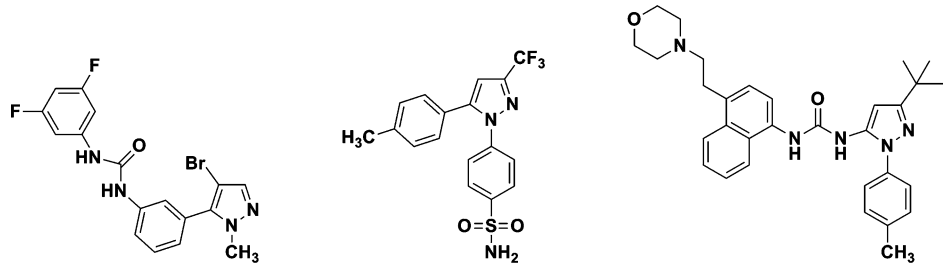
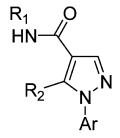


Figure 1. Approved trisubstituted pyrazole based drugs.



2

Figure 2. 1,5-Diaryl-4-carboxamide substituted pyrazoles.

achieved via reaction with an aryl hydrazine (Ar) reagent chemset **8** (Figure 5), this step resulting in the introduction of the Ar moiety. The desired 1,5-diarylpyrazole-4-carboxamide chemset **2** are then liberated from the solid support by treatment with 50% TFA in DCE. Interestingly, in all cases examined, only a single regioisomer is obtained.[32]

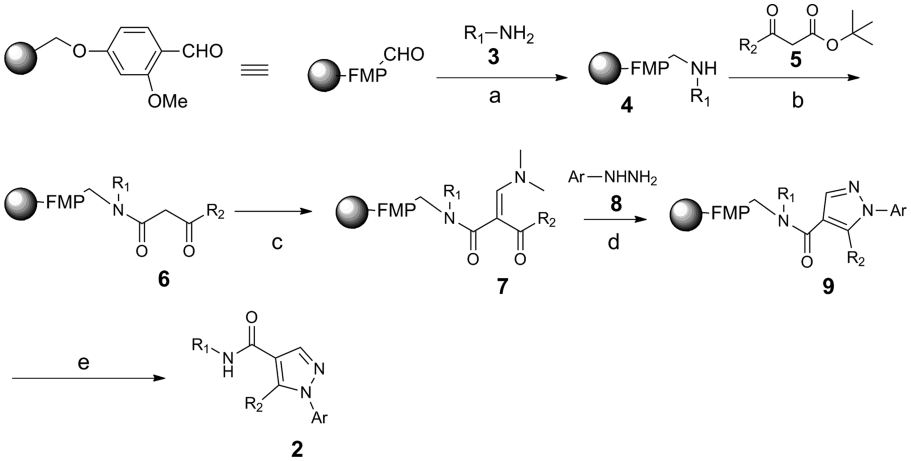
Using this methodology we synthesized multiple compound arrays that resulted in the generation of thousands of analogues. The protocol was found to be general in scope, as can be appreciated by the product yields and purities obtained with the representative chemset analogues presented in Table 1. These crude yields were determined gravimetrically, and the overall yields were based on initial FMP resin loading of 36 μmol for the five step sequence and ranged between 10 and 30 μmol. LCMS analyses of these products showed the desired products to be present in >80% purity, as determined by UV (220 nm) and light scattering detection.

The data presented in Table 1 also demonstrates that all three points of diversity (i.e., R_1, R_2, and Ar) tolerate a wide range of substituents. With respect to the amide component, it is apparent that primary amines of varying basicity (cf. (R_1{*1*–*3*})) react in an essentially similar fashion, and that branching alpha to the amine group is also tolerated without a significant impact on product yields, as is seen with analogue **2**{*2,1,1*}. In relation to the aryl moiety (R_2{*1*–*5*}) at C5, both electron donating and electon withdrawing groups can be accommodated, without an undue impact on product yields, as noted with analogues **2**{*1,2,1*}, **2**{*1,3,1*}, and **2**{*1,4,1*}. In relation to the hydrazine component (R_3{*1*–*4*}) used in the above protocol, electron rich and deficient hydrazines are well tolerated, as noted with analogues **2**{*4,5,2*} and **2**{*4,5,4*}, and ortho-substitution in the hydrazine moiety is accommodated as reflected in the similar yields obtained with pyrazole **2**{*4,5,3*}.

DISCUSSION

As part of an effort to identify novel GPCR ligands,[33,34] analogues from the above compound arrays were screened in a high throughput screening (HTS) format against a library of GPCR targets, and hits were observed against several GPCRs of interest. Specifically, several examples exhibited binding affinities against the cannabinoid subtype 1 (CB-1) GPCR.[35−37] CB-1 is expressed in the central nervous system on presynaptic terminals, and modulates neurotransmitter release. Endogenous (endocannabinoids) and many exogenous

Scheme 1[a]



[a]Reagents and conditions: (a) **3**, 2% HOAc in DMF/$(CH_3O)_3CH$ (7:3), 0.25 M $NaBH(OAc)_3$, 25 °C, 72 h; (b) **5**, NMP, DMAP, 80 °C, 48 h; (c) $(CH_3O)_2CHNMe_2$/DMF, 95 °C, 48 h; (d) **8**, NMP, DMAP, 85 °C, 72 h; (e) 50% TFA/DCM, RT, 1 h.

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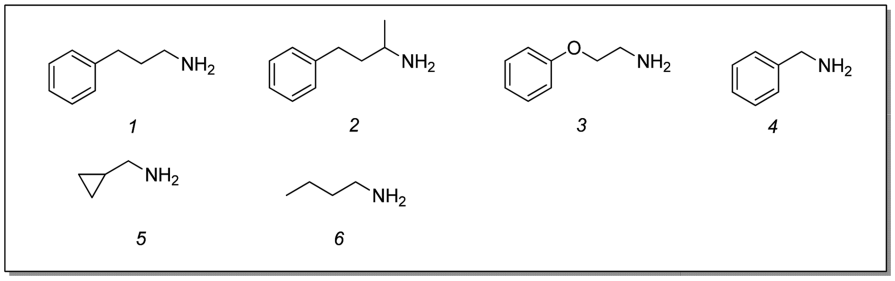

Figure 3. R_1 Diversity reagents **3**{*1*–*6*}.

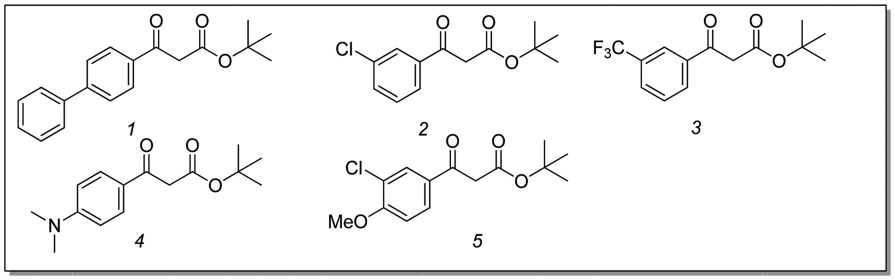

Figure 4. R_2 Diversity reagents **5**{*1*–*5*}.

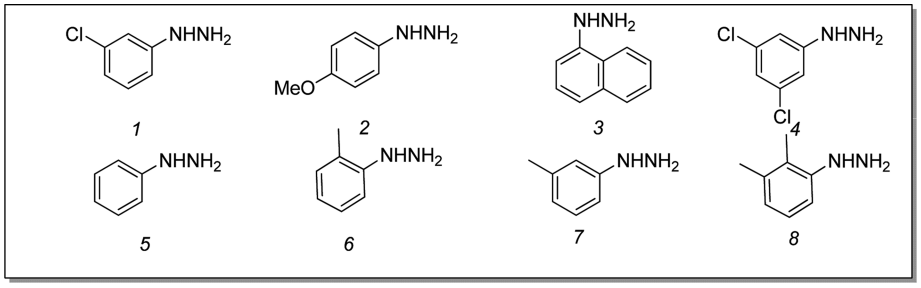

Figure 5. Ar Diversity reagents **8**{*1*–*8*}.

ligands of CB-1 have been identified, and these have been demonstrated to modulate a number of neurological processes, including several related to metabolic function. Correspondingly, the CB-1 receptor has been identified as a target for the treatment of obesity as well as psychiatric and neurodegenerative disorders,[38−43] and the development of suitable ligands for CB-1 receptors is of obvious interest. CB-1 binding assay is used to measure the binding affinity of the library samples toward the CB-1 receptor.[44]

The initial CB-1 binding results of a number of 1,5-diarylpyrazole-4-carboxamide **2** derivatives synthesized in the above array format were encouraging. The CB-1 IC_{50} values for an active set of analogues are presented in Table 2.

Significant structure activity relationships (SARs) can be elucidated from the activity data presented in Table 2. In the case of cyclopropylmethyl amides R_1{*5*}, a range of activities from >10 uM to approximately 300 nM is observed, with the most active derivative being the meta-chloro Ar{*1*} analogue **2**{*5,1,1*}. In the related n-butyl amide series R_1{*6*}, similar trends are observed, although these compounds tend to be less active than their cyclopropylmethyl congeners. Within this class, the meta-chloro Ar{*1*} analogue **2**{*6,1,1*} is again the most active derivative (hCB-1 IC_{50} = 500 nM). Interestingly, in the case of 3-phenylpropyl amides R_1{*1*}, the SAR at Ar (Ar{*1*–*4*}) tends to be flat, with all compounds generally showing enhanced levels of activity relative to other series, but not displaying any significant dependence on substitution pattern. Finally, the naphthyl derivative **2**{*1,1,3*} represented the most active compound (hCB-1 IC_{50} = 90 nM) identified from the compound library.

Several active samples from the library were subsequently resynthesized and purified by preparative HPLC, and the CB-1 IC_{50} values were determined, and are shown in Table 3. The close correlation observed for the IC_{50} values of crude and purified samples suggests that reliable biological data can be obtained from crude library samples of established purity and quantity. Compound **2**{*6,1,1*} with an IC_{50} value of 0.5 μM (Entry 2) also demonstrated full functional CB-1 antagonism (IC_{50} 0.3 μM) in a GTP-γS assay.[45]

In conclusion, a novel series of CB-1 antagonists were identified from a library of 1,5-diarylpyrazole-4-carboxamides synthesized on solid support using IRORI MicroKan technology. This solid phase synthesis route facilitated the rapid synthesis of compounds for SAR exploration by providing diversity at three points on the pyrazole scaffold. Analysis of the CB-1 binding data from the library indicated that biphenyl substitution at the 5-position of the pyrazole was essential for activity. Even though the original library comprised crude samples (>80% pure by HPLC), the activity of crude and resynthesized samples showed excellent correlation. The results of the subsequent lead optimization of the 5-biphenylpyrazole-

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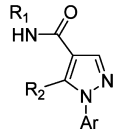
Table 1. Product Examples Illustrative of the Generality of the Chemistry Depicted in Scheme 1



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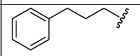
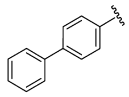
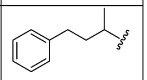
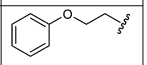
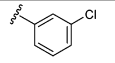
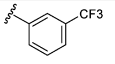
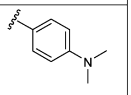
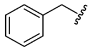
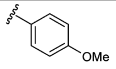
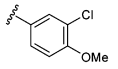
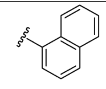
Compound	R_1	R_2	Ar	Yield (%)	Purity (%)
2 {*1,1,1*}				51	92
2 {*2,1,1*}				58	92
2 {*3,1,1*}				50	96
2 {*1,2,1*}				42	100
2 {*1,3,1*}				31	87
2 {*1,4,1*}				43	95
2 {*4,5,2*}				39	100
2 {*4,5,3*}				58	89
2 {*4,5,4*}				53	87

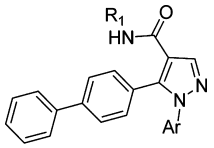
4-carboxamide series for CB-1 antagonist activity will be reported in a future manuscript.

EXPERIMENTAL SECTION

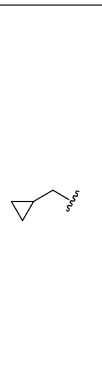
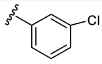
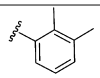
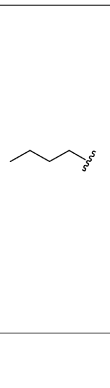
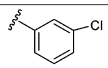
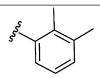
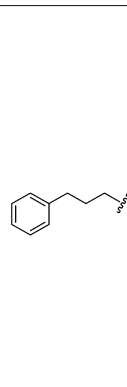
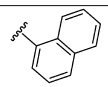
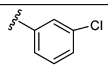
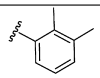
All compounds synthesized were analyzed by LC-MS with UV (λ = 220 nm) to determine their purity: Conditions: Phenomenex-prime S5 C-18, 4.6 × 30 mm. eluted with 0% to 100% B, 2 min gradient. (A = 10% MeOH/H_2O containing 0.1% TFA and B = 90% MeOH/H_2O containing 0.1% TFA); Flow rate at 5 mL/min. UV detection at 220 nm. The molecular mass of the compounds was determined by MS (ES) by the formula m/z.

General Procedure for Chemset 4. To a reaction vessel with Microkans containing 4-formyl-3-methoxyphenoxy (FMP) resin, (Polymer laboratories, 1.2 mmol/g, 75−150 uM mesh, 30 mg) and an Rf tag was added 2.5 mL per Microkan of 7:3 DMF/trimethyl orthoformate (TMOF), the appropriate R_1 amine (15.0 equiv), acetic acid (50 μL per Microkan), and $NaBH(OAc)_3$ (15.0 equiv). The reaction vessel was shaken at room temperature for 2 days, then the Microkans were filtered and washed with DMF (4 × 3 mL/Microkan), 2:1 DMF/MeOH (3 × 3 mL/Microkan), THF (2 × 3 mL/Microkan), DCM (2 × 3 mL/Microkan), and dried under vacuum for 12 h in a vacuum oven at 30 °C to afford resin-bound amine chemset **4**.

Table 2. Compounds with hCB1 Activity Identified from Initial Screening of Compound Library



2

Compound	R_1	Ar	hCB1 IC_{50} (nM)	Purity (%)
2 {*5,1,5*}			55%Inh @10uM	92
2 {*5,1,1*}			270	92
2 {*5,1,6*}			390	96
2 {*5,1,7*}			620	100
2 {*5,1,8*}			61%Inh @10uM	87
2 {*6,1,5*}			57%Inh @10uM	95
2 {*6,1,1*}			500	100
2 {*6,1,6*}			2800	89
2 {*6,1,7*}			2700	87
2 {*6,1,8*}			2500	96
2 {*1,1,3*}			90	95
2 {*1,1,1*}			410	92
2 {*1,1,6*}			200	96
2 {*1,1,7*}			200	94
2 {*1,1,8*}			200	86

General Procedure for Chemset 6. To a reaction vessel with Microkans containing the above resin-bound amine chemset **4** was added 2 mL per Microkan of *N*-methylpyrrolidine (NMP), the appropriate *t*-butyl-β-keto ester R_2 reagent chemset **5** (5.0 equiv) and 4-dimethylaminopyridine (DMAP, 0.1%.). The reaction vessel was agitated for 48 h at 80 °C using a turbocoil shaker, then the Microkans were filtered and washed

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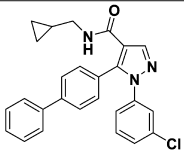
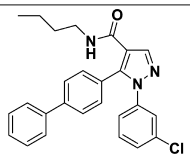
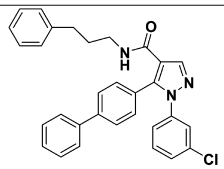
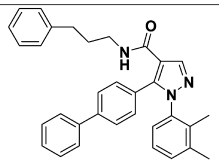
Table 3. CB-1 IC_{50} Values for Crude and Resynthesized Library Samples

Entry	Sample Number	Sample	CB-1 IC_{50} crude sample	IC_{50} resynthesized sample
1	**2** {*5,1,1*}		**0.23**	**0.19**
2	**2** {*6,1,1*}		**0.50**	**0.35**
3	**2** {*1,1,1*}		**0.41**	**0.20**
4	**2** {*1,1,8*}		**0.20**	**0.04**

with DMF (2 × 3 mL/Microkan), 1:1 DMF-MeOH (2 × 3 mL/Microkan), DCM (3 × 3 mL/Microkan) and dried under vacuum for 12 h in a vacuum oven at 30 °C to afford resin-bound amide chemset **6**.

General Procedure for Chemset 7. To a reaction vessel with Microkans containing the above resin-bound amide chemset **6** was added 1 mL per Microkan of DMF and 1 mL per Microkan of dimethylformamide-dimethylacetal (DMF-DMA). The reaction vessel was agitated for 24 h at 80 °C using a turbocoil shaker; then the Microkans were filtered and washed with DMF (2 × 3 mL/Microkan), 1:1 DMF-MeOH (2 × 3 mL/Microkan), DCM (3 × 3 mL Microkan) and dried under vacuum for 12 h in a vacuum oven at 30 °C to afford resin-bound enamine chemset **7**.

General Procedure for Chemset 9. To a reaction vessel with Microkans containing the above resin-bound enamine chemset **7** was added 2 mL per Microkan of *N*-methylpyrrolidine (NMP) and the appropriate arylhydrazine reagent chemset **8** as Ar reagent (15.0 equiv). When the arylhydrazine is an hydrochloride an equivalent amount of diisopropylethyl amine is used to free base the hydrochloride The reaction vessel was agitated for 72 h at 90 °C using a turbocoil shaker; then the Microkans were filtered and washed with DMF (2 × 3 mL/Microkan), 1:1 DMF-MeOH (2 × 3 mL/Microkan), DCM (3 × 3 mL/Microkan) and dried under vacuum for 12 h in a vacuum oven at 30 °C to afford resin-bound pyrazoleamide chemset **9**.

General Procedure for Product 2 Cleavage. The Microkan containing resin-bound pyrazole amide chemset **9** was treated with 1.5 mL of 50% trifluoroacetic acid (TFA) in DCM at room temperature for 1 h, then filtered. The filtrate was concentrated in speed vacuum to afford the crude product chemset **2.**

Using the above procedures, the following compounds were prepared and characterized.

5-(biphenyl-4-yl)-1-(3-chlorophenyl)-N-(3-phenylpropyl)-1H-pyrazole-4-carboxamide (**2**{*1,1,1*}). LCMS [M+H] 492.4, RT 2.14 min, HPLC purity 92%, (18.4 μmol, 51% yield).

5-(biphenyl-4-yl)-1-(3-chlorophenyl)-N-(4-phenylbutan-2-yl)-1H-pyrazole-4-carboxamide (**2**{*2,1,1*}). LCMS [M+H] 506.1, RT 1.86 min, HPLC purity 92%, (21 μmol, 58% yield).

5-(biphenyl-4-yl)-1-(3-chlorophenyl)-N-(2-phenoxyethyl)-1H-pyrazole-4-carboxamide (**2**{*3,1,1*}). LCMS [M+H] 494.2, RT 1.74 min, HPLC purity 96%, (18 μmol, 50% yield).

1-(4-chloro-2-methylphenyl)-5-(3-chlorophenyl)-N-(3-phenylpropyl)-1H-pyrazole-4-carboxamide (**2**{*1,2,1*}). LCMS [M+H] 464.4, RT 1.93 min, .HPLC purity 100% (15 μmol, 42% yield).

1-(4-chloro-2-methylphenyl)-N-(3-phenylpropyl)-5-(3-(trifluoromethyl)phenyl)-1H-pyrazole-4-carboxamide (**2**{*1,3,1*}). LCMS [M+H] 499.17, RT 2.06 min, HPLC purity 87%, (11 μmol, 31% yield).

1-(4-chloro-2-methylphenyl)-5-(4-(dimethylamino)phenyl)-N-(3-phenylpropyl)-1H-pyrazole-4-carboxamide (**2**{*1,4,1*}). LCMS [M+H] 474.3, RT 1.90 min, HPLC purity 100%, (15 μmol, 39% yield).

N-benzyl-5-(3-chloro-4-methoxyphenyl)-1-(4-methoxyphenyl)-1H-pyrazole-4-carboxamide (**2**{*4,4,2*}). LCMS [M+H] 448.17, RT 1.8 min, HPLC purity 100%, (14 μmol, 39% yield).

N-benzyl-5-(3-chloro-4-methoxyphenyl)-1-(naphthalen-1-yl)-1H-pyrazole-4-carboxamide (**2**{*4,4,3*}). LCMS [M+H] 469.5, RT 1.74 min, HPLC purity 89%, (21 μmol, 58% yield).

N-benzyl-5-(3-chloro-4-methoxyphenyl)-1-(3,5-dichlorophenyl)-1H-pyrazole-4-carboxamide (**2**{*4,4,4*}). LCMS [M+H] 486.8, RT 2.12 min, HPLC purity 87%, (19 μmol, 53% yield).

5-(biphenyl-4-yl)-N-(cyclopropylmethyl)-1-phenyl-1H-pyrazole-4-carboxamide (**2**{*5,1,5*}). LCMS [M+H] 394.2, RT 1.93 min, HPLC purity 100%, (19 μmol, 53% yield).

5-(biphenyl-4-yl)-1-(3-chlorophenyl)-N-(cyclopropylmethyl)-1H-pyrazole-4-carboxamide (**2**{*5,1,1*}). LCMS [M+H] 428.4, RT 1.95 min, HPLC purity 88% (23 μmol, 64% yield).

5-(biphenyl-4-yl)-N-(cyclopropylmethyl)-1-o-tolyl-1H-pyrazole-4-carboxamide (**2**{*5,1,6*}). LCMS [M+H] 408.3, RT 1.96 min, HPLC purity 98%, (19 μmol, 55% yield).

5-(biphenyl-4-yl)-N-(cyclopropylmethyl)-1-o-tolyl-1H-pyrazole-4-carboxamide (**2**{*5,1,7*}). LCMS [M+H] 408.6, RT 1.98 min, HPLC purity 96%, (22 μmol, 61% yield).

5-(biphenyl-4-yl)-N-(cyclopropylmethyl)-1-(2,3-dimethylphenyl)-1H-pyrazole-4-carboxamide (**2**{*5,1,8*}). LCMS [M+H] 422.5, RT 1.88 min, HPLC purity 100%, (28 μmol, 78% yield).

5-(biphenyl-4-yl)-N-butyl-1-phenyl-1H-pyrazole-4-carboxamide (**2**{*6,1,5*}). LCMS [M+H] 396.22, RT 2.2 min, HPLC purity 91%, (13 μmol, 36% yield).

5-(biphenyl-4-yl)-N-butyl-1-(3-chlorophenyl)-1H-pyrazole-4-carboxamide (**2**{*6,1,1*}). LCMS [M+H] 430.9, RT 2.14 min, HPLC purity 85%, (23 μmol, 64% yield).

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5-(biphenyl-4-yl)-N-butyl-1-o-tolyl-1H-pyrazole-4-carboxamide (**2**{*6,1,6*}). LCMS [M+H] 410.25, RT 2.27 min., HPLC purity 81%, (17 μmol 47% yield).

5-(biphenyl-4-yl)-N-butyl-1-m-tolyl-1H-pyrazole-4-carboxamide (**2**{*6,1,7*}). LCMS [M+H] 410.5, RT 2.23 min, HPLC purity 84% (14 μmol 55% yield).

5-(biphenyl-4-yl)-N-butyl-1-(2,3-dimethylphenyl)-1H-pyrazole-4-carboxamide (**2**{*6,1,8*}). LCMS [M+H] 424.6, RT 1.98 min, HPLC Purity 92%, (21 μmol, 58% yield).

5-(biphenyl-4-yl)-1-(naphthalen-1-yl)-N-(3-phenylpropyl)-1H-pyrazole-4-carboxamide (**2**{*1,1,3*}). LCMS [M+H] 508.4, RT 2.2 min, HPLC purity 90%, (15 μmol, 42% yield).

5-(biphenyl-4-yl)-N-(3-phenylpropyl)-1-o-tolyl-1H-pyrazole-4-carboxamide (**2**{*1,1,6*}). LCMS [M+H] 472.5, RT 2.13 min, HPLC purity 96%, (12 μmol, 33% yield).

5-(biphenyl-4-yl)-N-(3-phenylpropyl)-1-m-tolyl-1H-pyrazole-4-carboxamide (**2**{*1,1,7*}). LCMS [M+H] 472.6, RT 2.12 min, HPLC purity 94%, (12 μmol, 33% yield).

5-(biphenyl-4-yl)-1-(2,3-dimethylphenyl)-N-(3-phenylpropyl)-1H-pyrazole-4-carboxamide (**2**{*1,1,8*}). LCMS [M+H] 486.6, RT 1.99 min, HPLC purity 98%, (17 μmol 47% yield).

General Procedure for the Purification of Resynthesized Compounds. Four samples from Table 3 compounds **2**{*5,1,1*}, **2**{*6,1,1*}, **2**{*1,1,1*}, and **2**{*1,1,8*} were resynthesized on solid support using loose resin using the protocol described above, and the reagents specified in the following below. The resulting product was purified by chromatography (YMC combiprep ODS-A, 30 mm × 50 mm, MeOH/H_2O/0.1% TFA) to yield the title compounds.

5-([1,1′-Biphenyl]-4-yl)-1-(3-chlorophenyl)-*N*-(3-phenylpropyl)-1*H*-pyrazole-4-carboxamide (2{5,1,1}). (26 mg, 55% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (1 H, s), 7.68 (3 H, d, *J* = 8.5 Hz), 7.55−7.61 (3 H, m), 7.42−7.48 (3 H, m), 7.35−7.42 (4 H, m), 7.24 (2 H, br. s.), 7.11−7.21 (4 H, m), 6.98−7.05 (3 H, m), 3.25−3.33 (2 H, m), 2.43−2.51 (2 H, m), 1.65−1.74 (2 H, m). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.7, 142.7, 141.7, 140.3, 140.3, 140.0, 139.1, 133.1, 131.1, 130.6, 129.0, 128.3, 128.3, 127.9, 127.9, 127.9, 126.1, 126.1, 125.7, 125.3, 124.1, 118.1, 38.3, 32.6, 30.9. HRMS (ESI) calcd for $C_{31}H_{27}ClN_3O$ $[M+H]^+$ 492.1837, found 492.1823.

5-([1,1′-Biphenyl]-4-yl)-1-(3-chlorophenyl)-*N*-(cyclopropylmethyl)-1*H*-pyrazole-4-carboxamide (2{6,1,1}). (24 mg, 58% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.20 (1 H, s), 7.63−7.74 (2 H, m), 7.58−7.64 (2 H, m), 7.47 (2 H, t), 7.36−7.44 (4 H, m), 7.24 (1 H, s), 7.18 (1 H, t), 7.00−7.08 (1 H, m), 3.12 (2 H, s), 0.77 (1 H, s), 0.30−0.37 (2 H, m), 0.02 (2 H, s). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.1, 142.4, 139.9, 139.8, 139.5, 138.7, 132.6, 130.7, 130.1, 128.6, 127.5, 127.4, 127.4,126.17, 125.6, 124.9, 123.6, 117.5, 42.4, 33.9, 10.5, 2.7. HRMS (ESI) calcd for $C_{26}H_{23}ClN_3O$ $[M+H]^+$ 428.1524, found 428.1511.

5-([1,1′-Biphenyl]-4-yl)-*N*-butyl-1-(3-chlorophenyl)-1*H*-pyrazole-4-carboxamide (2{1,1,1}). (33 mg, 79% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.19 (1 H, s), 7.70 (2 H, d, *J* = 13.4 Hz), 7.61 (2 H, s), 7.47 (2 H, s), 7.40 (4 H, s), 7.18 (1 H, s), 7.02 (1 H, s), 3.26 (2 H, s), 2.69 (2 H, s), 1.30 (4 H, s), 1.28−1.35 (2 H, m), 1.11 (2 H, s), 0.80 (3 H, t). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.1, 142.3, 139.9, 139.8, 139.5, 138.7, 132.6, 130.6, 130.1, 128.6, 127.4, 127.4, 126.2, 125.6, 124.8, 123.6, 117.6, 37.8, 30.8, 19.1, 13.2.HRMS (ESI) calcd for $C_{26}H_{25}ClN_3O$ $[M+H]^+$ 430.1681, found 430.1669.

5-([1,1′-Biphenyl]-4-yl)-1-(2,3-dimethylphenyl)-*N*-(3-phenylpropyl)-1*H*-pyrazole-4-carboxamide (2{1,1,8}). (24 mg, 51% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (1 H, s), 7.53−7.58 (2 H, m), 7.49−7.53 (2 H, m), 7.41 (2 H, t, *J* = 7.5 Hz), 7.34−7.37 (1 H, m), 7.30−7.34 (2 H, m), 7.10−7.21 (4 H, m), 7.00−7.08 (4 H, m), 3.27−3.34 (2 H, m), 2.45−2.52 (2 H, m), 2.22 (3 H, s), 1.86 (3 H, s), 1.71 (2 H, d). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.6, 143.3, 141.2, 138.8, 138.6, 139.4, 137.9, 137.3, 133.6, 130.2, 130.0, 128.0, 127.7, 127.7, 127.3, 126.1, 125.5, 125.3(s, 2C), 125.2, 116.1, 37.8, 32.1, 30.5, 19.3, 13.4. HRMS (ESI) calcd for $C_{33}H_{32}N_3O$ $[M+H]^+$ 486.2540, found 486.2526.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H, ^{13}C NMR, HRMS and LCMS data on purified compounds are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(44) CB-1 binding assay The CHO-CB-1-C3 cell line was licensed from Euroscreen (cell line EC-110-C) with a B_{max} value of 0.55 pmol/mg protein (using [^{3}H]-CP-55940). Test compounds were serially diluted in DMSO and added 1:100 to 384 well microtiter plates containing 5 μg of CHO-CB-1 membrane protein and 2 nM [^{3}H]-CP-55940 in a binding buffer containing 25 mM HEPES, pH 7.4; 150 mM NaCl; 2 mM MgCl2; 1 mM EDTA; 0.25% BSA. After 1 h at room temp., the binding reaction was terminated by transferring the reaction mixtures onto GF/B filter plates using a Vprep. The filter plates were washed with cold buffer containing 1X PBS and 0.025% Tween 20; then the contents of the plates were counted on a Packard TopCount Scintillation Counter. Nonspecific binding was determined by the addition of 30 μM cold CP-55940. Specific binding was calculated by subtracting nonspecific binding from total binding. CPM were

converted into % inhibition based on total and nonspecific binding and K_i values were calculated by the Cheng-Prusoff correction (Cheng, Y.-C.; Prusoff, W. H. *Br. Pharmacol.* **1973**, *22*, 3099−3108) using the parameters of radioligand concentration and the K_d values determined for each radioligand. Reported K_i values are the average of at least 3 independent determinations.

(45) CB-1 functional assay: CB-1 functional assay is used to measure the functionality of molecules to the receptor. The [35S]GTPgammaS binding assay is considered as a functional assay. The [35S]GTPgS assay measures the level of G protein activation following agonist occupation of a GPCR, by determining the binding of the nonhydrolyzable analogue [35S]GTPgS to Ga subunits. Thus, the assay measures a functional consequence of receptor occupancy at one of the earliest receptor-mediated events. Compounds were diluted in 100% DMSO and then added 1:100 into 96 well plates containing 1 μg of CHO-CB-1 membrane protein in assay buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM $MgCl_2$, 10 μg/mL saponin, 1 mM EDTA, 0.25% BSA, WGA-PVT beads 150 μg, cold GTPγ-S 10 μM and 60 μM GDP). After adding [^{35}S]-GTPγ-S to all the wells, the plates were incubated for 1 h at room temp. The binding reaction was terminated by centrifuging at 1000 rpm for 5 min. The plates were then counted on a Packard TopCount Scintillation Counter immediately.

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