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Discovery of 7-Oxopyrazolo[1,5-*a*]pyrimidine-6-carboxamides as Potent and Selective CB_2 Cannabinoid Receptor Inverse Agonists

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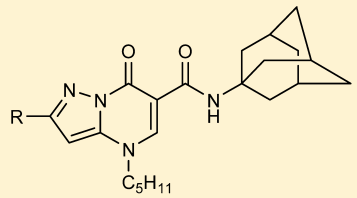
Supporting Information

ABSTRACT: We recently described the medicinal chemistry of a new series of heteroaryl-4-oxopyridine/7-oxopyrimidines as CB_2 receptor partial agonists, showing that the functionality of these ligands is controlled by the nature of the heteroaryl function condensed with the pyridine ring. We describe herein the design and synthesis of the 7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxamides, structural isomers of our previously reported pyrazolo[3,4-*b*]pyridines. All of the new compounds showed high affinity and selectivity for the CB_2 receptor in the nanomolar range. In 3,5-cyclic adenosine monophosphate (cAMP) assays, the novel series shows stimulatory effects on forskolin-induced cAMP production acting as inverse agonists.



R = Ph: hK_i CB_2 = 2.5 nM, hK_i CB_1> 10000 nM, SI > 3906
R = 2-CH_3-Ph: hK_i CB_2 = 2.8 nM, hK_i CB_1> 10000 nM, SI > 3496
R = 4-Cl-Ph: hK_i CB_2 = 3.8 nM, hK_i CB_1> 10000 nM, SI > 2597
R = Furan-2-yl: hK_i CB_2 = 4.9 nM, hK_i CB_1> 10000 nM, SI > 2032

INTRODUCTION

Interest in the potential medicinal use of cannabinoids grew recently with the discovery of two cannabinoid receptors, CB_1 and CB_2.[1,2] The CB_1 receptor is abundantly expressed in the central nervous system (CNS) and is responsible for the psychotropic side effects.[3−5] The CB_2 receptor is mainly found in cells of the immune system, although it may be up-regulated in the CNS under pathological conditions.[6,7] The main signal transduction pathway triggered is through G_i proteins, resulting in an inhibition of adenylate cylase activity and a decrease in cyclic AMP levels.[8] Activation of CB_2 receptors inhibits adenylyl cyclase and activates mitogen-activated protein kinase through binding of the α-subunit of the $G_{i/o}$ protein but does not modulate calcium or potassium conductances.[9,10] Agonist binding to CB_1 receptors, by contrast, suppresses calcium and activates inward-rectifying potassium conductances, effects associated with depression of neuronal excitability and transmitter release.[11,12] Thus, differences in receptor distribution and signal transduction mechanisms are likely to account for the relative absence of the CNS side effects induced by CB_2 agonists. These considerations suggest that novel pharmacotherapies targeting CB_2 receptors may have considerable therapeutic potential. Agonists and partial agonists of the CB_2 receptor have been investigated for their potential utility in the treatment of inflammatory and neuropathic pain,[13−15] while antagonists may have utility in certain inflammatory and allergic conditions.[16] It has been suggested that inverse agonist may be effective for the control of immune cell mobility in arthritis and inflammatory states[17] and for the treatment of osteoporosis via the inhibition of osteoclast differentiation.[18] This therapeutic potential has prompted the development of several CB_2 receptor selective ligands as agonists, partial agonists, or antagonists/inverse agonists (Chart 1).

Among the selective agonists, the best known compound is *R*-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate **1** (*R*-(+)-WIN 55,212-2, Chart 1). This compound displays activity at both CB_1 and CB_2 receptors, although it has been found to possess slightly higher CB_2 than CB_1 affinity.[8] (2-Methoxynaphthalen-1-yl)(1-pentyl-1*H*-indol-3-yl)-methanone (JWH-267) is an *N*-alkylindole derivative with high affinity at the CB_2 receptor that exhibited partial activity in ^{35}S-labeled guanosine 5′-*O*-(3-thio)triphosphate ([^{35}S]GTPγS) binding assays.[19]

Fewer classes of compounds have been reported as selective antagonists or inverse agonists of the CB_2 receptor.[20] The prototypical selective antagonist is SR144528[9] (**2**, Chart 1),

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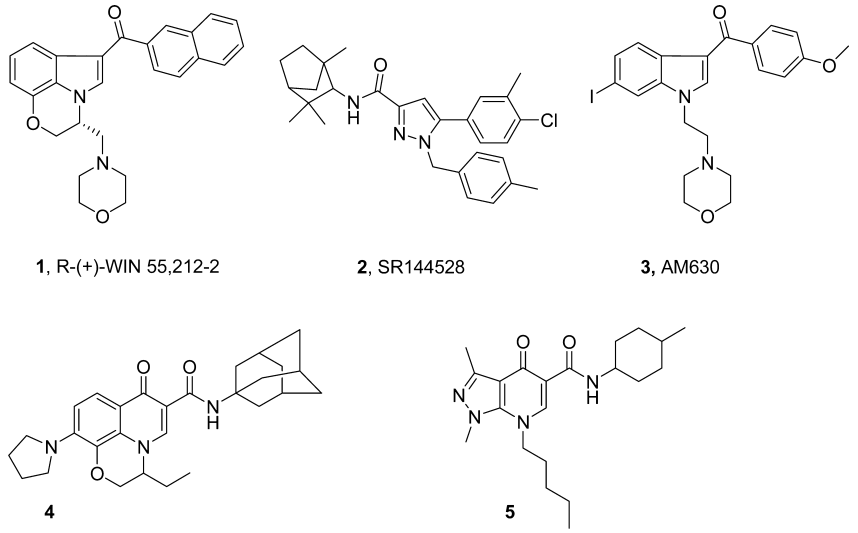
Chart 1. Structures of Representative CB_2 Selective Ligands



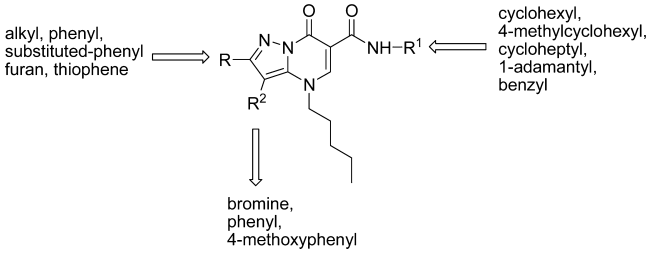
which is based upon a pyrazole scaffold. The 6-iodopravadoline AM630[21] (**3**, Chart 1) is an indole derived cannabinoid ligand that acts as a potent and selective inverse agonist at the CB_2 receptors. These compounds are thought to be CB_2 receptor inverse agonists rather than neutral antagonists because when administered by themselves, they can produce inverse cannabimimetic effects in CB_2 receptor-expressing tissues.[8] *N*-(1,3-Benzodioxol-5-ylmethyl)-1,2-dihydro-7-methoxy-2-oxo-8-(pentyloxy)-3-quinolinecarboxamide (JTE-907)[22] is a potent CB_2 receptor ligand endowed with high selectivity for the CB_2 receptor and inverse-agonistic properties. This compound received attention because of its anti-inflammatory properties, showing antipruritic activity in a model of dermatitis when orally administered.[22,23]

Recently, a new class of CB_2 selective inverse agonists based on a triaryl bis-sulfone scaffold has been described. This class is represented by *N*-[1(*S*)-[4-[[4-methoxy-2-[(4-methoxyphenyl)sulfonyl]phenyl]sulfonyl]phenyl]ethyl]-methanesulfonamide (Sch225336),[24] which was shown to block the recruitment of leucocytes in vivo.[25] Previously, within a research program to identify novel CB_2 agonists, our group designed a hybrid chemical structure that incorporated the structural features of known cannabinoid ligands. The new series of oxazinoquinolone derivatives[26] exemplified by *N*-(adamant-1-yl)-3,7-dihydro-3-ethyl-7-oxo-10-(pyrrolidin-1-yl-2*H*-[1,4]oxazino-[2,3,4-*ij*]quinoline-6-carboxamide (**4**, Chart 1) was synthesized and tested in binding assays, exhibiting high affinity and selectivity for the CB_2 receptor (hCB_2 K_i = 8.12 nM, hCB_1 K_i > 10000, selectivity index (SI) of >1231). The potency of the new oxazinoquinoline-6-carboxamides was measured in functional assays, revealing that the novel series behaved as CB_2 receptor full agonists.

In this context, very recently we have reported the medicinal chemistry of a series of heteroaryl-4-oxopyridine/7-oxopyrimidine derivative[27] as represented by *trans*-4,7-dihydro-1,3-dimethyl-*N*-(4-methylcyclohexyl)-4-oxo-7-pentyl-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxamide **5** (Chart 1), which displayed high affinity at the CB_2 receptor (hCB_2 K_i = 11.4 nM, hCB_1 K_i = 4568, SI = 401). In this study, additional CB_2 ligands were synthesized by replacing the pyrazolo ring with different heterocycles that were found to be potent CB_2 receptor ligands. Moreover, it was shown that the functionality of these ligands is controlled by the nature of the heteroaryl function condensed with the pyridine ring. In 3,5-cyclic adenosine monophosphate (cAMP) assays, they showed a dose-dependent effect in the modulation of forskolin-induced cAMP production, revealing different behaviors as full agonists, partial agonists, and inverse agonists.

Here, we report a novel series of 7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxamides that were found to be potent and selective CB_2 receptor ligands. Our goal was to design and synthesize the structural isomers of our previously reported pyrazolo[3,4-*b*]pyridine that allowed us to conduct a pharmacophore exploration and optimization effort around the heteroaryl central scaffold (Chart 2).

Chart 2. Structure Variations around the 7-Oxopyrazolo[1,5*a*]pyrimidine Scaffold



The newly synthesized pyrazolopyrimidines were tested in competition binding assays toward both rat CB_1 (rCB_1) and rat CB_2 (rCB_2) receptors expressed in native tissues (rat brain or spleen) and human CB_1 (hCB_1) and human CB_2 (hCB_2) receptors expressed in CHO cells. Affinity data (K_i, nM) were used to calculate the selectivity of newly synthesized compounds for the CB_2 receptors. All of these ligands were also examined in cyclic AMP assays on hCB_2 CHO cells, with the aim of evaluating their effect in the modulation of forskolin-induced cAMP production.

Finally a molecular modeling study was performed in order to explain the structure−activity relationships observed experimentally. A series of compounds were docked into CB_1 and CB_2 receptor models, and the observed ligand−receptor interactions were examined while taking into account the site-directed mutagenesis data available for this receptor subtype.

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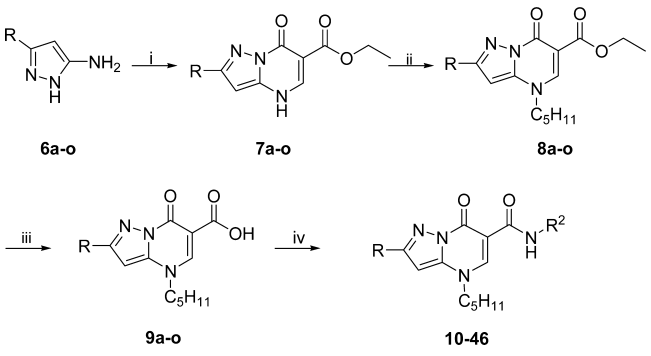
CHEMISTRY

The synthetic routes to obtain the target 4,7-dihydro-7-oxo-4-pentyl-2(3)-substituted[1,5-*a*]pyrimidine-6-carboxamide derivatives **10**–**49** are outlined in Schemes 1 and 2. All substituents are summarized in Table 1.

Scheme 1[a]



[a]Reagents: (i) diethyl ethoxymethylenemalonate, AcOH, 120 °C, 2 h; (ii) K_2CO_3, 1-pentyl bromide, DMF, 100 °C, 16 h; (iii) NaOH 10%, MeOH, rt, 2 h; (iv) EDC, HOBt, amine, DMF, rt, 16 h *or* DIEA, HBTU, adamantan-1-yl-amine, DMF, rt, 16 h. Information on compound designations: **a**, R = Me; **b**, R = Et; **c**, R = *t*-But; **d**, R = phenyl; **e**, R = 2-Me-phenyl; **f**, R = 4-tolyl-Me-phenyl; **g**, R = 2-Cl-phenyl; **h**, R = 4-Cl-phenyl; **i**, R = 2,4-di-Cl-phenyl; **j**, R = 2,6-di-Cl-phenyl; **k**, R = furan-2-yl; **l**, R = furan-3-yl; **m**, R = thiophen-2-yl; **n**, R = 4-Me-thiophen-2-yl; **o**, R = 5-Me-thiophen-2-yl.

The starting materials, 3-(substituted)-5-aminopyrazoles, required for the synthesis of the title compounds were synthesized by the condensation of appropriate β-ketonitriles[28] with hydrazine monohydrate according to the literature procedure.[29] The aminopyrazoles (**6a**–**o**) were reacted smoothly with diethyl ethoxymethylenemalonate (DEEM) in glacial acetic acid to give the cyclized ethyl 2-substituted-4,7-dihydro-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylates (**7a**–**o**) in good yields. The subsequent N-alkylation with 1-pentyl bromide in the presence of K_2CO_3 was performed to yield compounds **8a**–**o**. Hydrolysis in 10% aqueous NaOH yielded the corresponding 4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic acids **9a**–**o**[30] that then underwent coupling reaction with appropriate amines to yield the target compounds **10**–**46**.

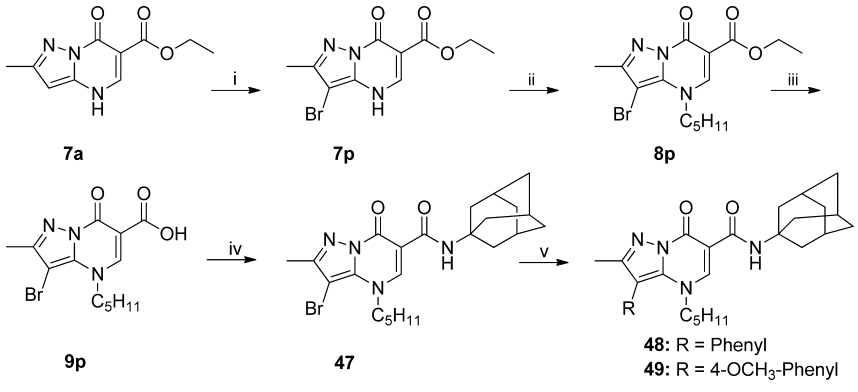
The preparation of the pyrazolopyrimidine derivatives **47**–**49** was accomplished by the route shown in Scheme 2, following synthetic procedures already established for the previous compounds. Bromination of pyrazolo ester **7a** in position 3 led to compound **7p** in high yields. Compound **7p** was alkylated with 1-pentyl bromide to give **8p**. Hydrolysis afforded the carboxylic acid **9p** that was converted to the carboxamide **47** by coupling with 1-adamantylamine. Suzuki coupling with phenylboronic acid and 4-methoxyphenylboronic acid yielded the final compounds **48** and **49**, respectively.

RESULTS AND DISCUSSION

Structure–Activity Relationships (SARs). All of the newly synthesized compounds were examined in [^{3}H]CP-55,940 competition binding experiments for their affinity and selectivity toward the rat and human recombinant CB_1 and CB_2 receptors. The results, in terms of binding affinities for the two receptors (K_i values), are reported in Table 1. While a substantial degree of sequence homology between the human and rat CB receptors is well-known, a significant divergence between the two sequences still exists.[31] In our previous studies, we have observed significant differences in the binding affinities of specific agonists to the receptors from the two species.[26] This may suggest that the ligands were binding to a portion of the receptor where homology was not maintained and could portend that efficacy in a rodent model may not translate into efficacy in humans. As seen in Table 1, the measured affinity values for all of the examined compounds do not differ significantly between rat and human CB_2 receptors.

For the first group of five synthesized 7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxamides (**10**–**14**, Table 1), the structural variations were focused on the moiety borne by the amide at the C-6 position, maintaining the methyl group at the C-2 position and the *n*-pentyl chain at the N-4 constant. As was seen previously, the compounds bearing cyclohexyl, cycloheptyl, or adamantyl moieties on the amide showed high affinity at the CB_2 receptor. The best result in terms of both activity and selectivity profiles was found with the *N*-adamantan-1-ylcarboxamide chain (compound **12** hCB_2 K_i = 12.7 nM, SI = 88). The benzylcarboxamide **13** displayed modest affinity and slight selectivity for the cannabinoid receptors (hCB_2 K_i = 358 nM, hCB_1 K_i = 3290 nM, SI = 9.2). The choice of the 4-methylcyclohexylcarboxamide group in position 6 (compound **14**) was based upon the binding

Scheme 2[a]



[a]Reagents: (i) Br_2, AcOH, rt, 3 h; (ii) K_2CO_3, 1-pentyl bromide, DMF, 100 °C, 16 h; (iii) NaOH, 10%, MeOH, rt, 2 h; (iv) DIEA, HBTU, adamantan-1-yl-amine, DMF, rt, 16 h; (v) (phenyl or 4-methoxyphenyl)boronic acid, $Pd(PPh_3)_4$, K_2CO_3, toluene, 100 °C, 16 h.

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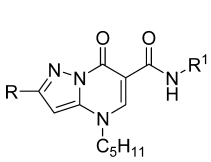
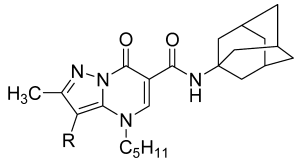
Table 1. Affinity (K_i, nM) and Selectivity Index (SI) on Rat and Human CB_1 and CB_2 Receptors of the Novel CB Compounds 10−49[a]



10-46



47-49

compd	R	R^1	K_i (nM) rCB_1[b]	rCB_2[c]	hCB_1[d]	hCB_2[e]	SI
WIN 55,212-2			15.3 ± 1.3	7.61 ± 0.68	12.2 ± 1.4	4.56 ± 0.45	2.68
10	Me	cyclohexyl	1578 ± 152	26 ± 2	1278 ± 110	22 ± 2	58
11	Me	cycloheptyl	642 ± 65	12.4 ± 1.1	527 ± 48	10.3 ± 1.2	51
12	Me	adamant-1-yl	1460 ± 148	15.3 ± 1.6	1118 ± 96	12.7 ± 1.3	88
13	Me	benzyl	3688 ± 386	428 ± 17	3290 ± 312	358 ± 32	9.2
14	Me	4-Me-cyclohexyl	2600 ± 310	120 ± 11	2432 ± 272	106 ± 9	23
15	Et	cyclohexyl	480 ± 40	11.5 ± 1.2	420 ± 35	10.4 ± 1.2	40
16	Et	cycloheptyl	220 ± 20	5.63 ± 0.57	200 ± 15	5.22 ± 0.45	38
17	Et	adamantyl	150 ± 12	0.96 ± 0.08	120 ± 10	0.84 ± 0.07	143
18	*t*-But	cyclohexyl	720 ± 70	14.2 ± 1.1	675 ± 54	12.3 ± 1.1	55
19	*t*-But	cycloheptyl	650 ± 60	7.52 ± 0.68	605 ± 50	7.22 ± 0.63	84
20	*t*-But	adamant-1-yl	675 ± 65	5.54 ± 0.48	612 ± 57	4.95 ± 0.42	124
21	Ph	cyclohexyl	750 ± 80	50 ± 5	702 ± 68	42 ± 4	17
22	Ph	cycloheptyl	900 ± 100	35 ± 4	825 ± 80	31 ± 3	27
23	Ph	adamant-1-yl	>10000 (40%)	2.74 ± 0.28	>10000 (43%)	2.56 ± 0.22	>3906
24	2-Me-phenyl	cyclohexyl	>10000 (30%)	48 ± 4	>10000 (16%)	40 ± 4	>250
25	2-Me-phenyl	cycloheptyl	>10000 (45%)	9.53 ± 0.92	>10000 (34%)	8.93 ± 0.86	>1119
26	2-Me-phenyl	adamant-1-yl	>10000 (30%)	3.21 ± 0.30	>10000 (22%)	2.86 ± 0.25	>3496
27	4-Me-phenyl	cyclohexyl	>10000 (25%)	62 ± 6	>10000 (20%)	57 ± 5	>175
28	4-Me-phenyl	cycloheptyl	>10000 (49%)	13.2 ± 1.7	>10000 (39%)	10.3 ± 1.1	>970
29	4-Me-phenyl	adamant-1-yl	>10000 (45%)	3.82 ± 0.39	>10000 (38%)	3.41 ± 0.32	>2932
30	2-Cl-phenyl	cyclohexyl	>10000 (40%)	52 ± 5	>10000 (34%)	47 ± 4	>212
31	2-Cl-phenyl	cycloheptyl	>10000 (42%)	12.3 ± 1.6	>10000 (35%)	10.3 ± 1.1	>970
32	2-Cl-phenyl	adamant-1-yl	>10000 (48%)	6.32 ± 0.63	>10000 (41%)	6.21 ± 0.55	>1610
33	4-Cl-phenyl	cyclohexyl	>10000 (8%)	58 ± 6	>10000 (15%)	55 ± 5	>181
34	4-Cl-phenyl	cycloheptyl	>10000 (47%)	6.53 ± 0.58	>10000 (40%)	6.31 ± 0.51	>1584
35	4-Cl-phenyl	adamant-1-yl	>10000 (10%)	4.21 ± 0.42	>10000 (13%)	3.88 ± 0.31	>2577
36	2,4-di-Cl-phenyl	cyclohexyl	>10000 (47%)	60 ± 5	>10000 (38%)	52 ± 5	>192
37	2,4-di-Cl-phenyl	cycloheptyl	>10000 (49%)	40 ± 4	>10000 (39%)	37 ± 4	>270
38	2,4-di-Cl-phenyl	adamant-1-yl	>10000 (40%)	35 ± 3	>10000 (37%)	30 ± 3	>333
39	2,6-di-Cl-phenyl	adamant-1-yl	>10000 (18%)	110 ± 10	>10000 (21%)	98 ± 8	>102
40	furan-2-yl	cyclohexyl	>10000 (30%)	10.4 ± 1.8	>10000 (21%)	9.52 ± 0.92	>1050
41	furan-2-yl	cycloheptyl	>10000 (20%)	7.23 ± 0.81	>10000 (16%)	6.57 ± 0.62	>1522
42	furan-2-yl	adamant-1-yl	>10000 (40%)	5.14 ± 0.42	>10000 (34%)	4.92 ± 0.43	>2032
43	furan-3-yl	adamant-1-yl	>10000 (17%)	12.4 ± 1.5	>10000 (19%)	10.1 ± 1.3	>990
44	thiophen-2-yl	adamant-1-yl	>10000 (22%)	7.52 ± 0.71	>10000 (26%)	6.24 ± 0.61	>1602
45	4-Me-thiophen-2-yl	adamant-1-yl	>10000 (15%)	18 ± 2	>10000 (13%)	15 ± 2	>666
46	5-Me-thiophen-2-yl	adamant-1-yl	>10000 (33%)	12.2 ± 1.8	>10000 (31%)	11.6 ± 1.7	>862
47	Br		>10000 (1%)	272 ± 25	>10000 (1%)	220 ± 18	>45
48	phenyl		>10000 (15%)	214 ± 17	>10000 (15%)	181 ± 16	>55
49	4-OCH_3-Ph		>10000 (5%)	434 ± 41	>10000 (4%)	350 ± 32	>28

[a]The data are espressed as the mean ± SEM of n = 4 independent experiments. The affinity values were calculated by using [^{3}H]CP-55,940 as radioligand on [b]rat brain for CB_1 receptors, [c]rat spleen for CB_2 receptors, [d]human CB_1 CHO membranes, and [e]human CB_2 CHO membranes.

results obtained from our previously reported work on pyrazolo[3,4-*b*]pyridines, where this moiety gave the highest affinity at the CB_2 receptor. In the present series, the 4-methylcyclohexylcarboxamide moiety did not enhance affinity (**14**, hCB_2 K_i = 106 nM, hCB_1 K_i = 2432 nM, SI = 23) relative to the cyclohexylcarboxamide (**10**), possibly because of a different binding mode of the pyrazolopyrimidine derivatives.

Subsequently, the adopted strategy was to prepare analogues by introduction of structural modifications at position 2 of the pyrazolo[1,5-*a*]pyrimidine template while maintaining the cyclohexyl, cycloheptyl, and adamantyl moieties on the carboxamide to investigate their potential to bind to cannabinoid CB_1 and/or CB_2 receptors and to study their structure−affinity relationships.

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Extending the aliphatic chain at the C-2 position of compounds **10**–**12** with ethyl or *tert*-butyl moieties was investigated by preparation of compounds (**15**–**20**). These compounds showed high affinity for the CB2 receptor (**15**–**17**, 0.84–10.4 nM; **18**–**20**, 4.95–12.3 nM) with remarkable selectivity over the CB_1 receptor (**15**–**17**, 38 < SI < 143; **18**–**20**, 55 < SI < 124). Replacing the cyclohexyl moiety with a cycloheptyl or adamantly moiety led to an increase in affinity, as shown by compounds **16**, **17**, **19**, and **20**. In addition the highest selectivity over the CB_1 receptor was observed with the most lipophilic compounds **17** and **20** (SI of 143 and 124, respectively). Compound **17** was found to have the highest CB_2 receptor affinity among the series of 2-alkyl derivatives, with a K_i value of 0.84 nM (Figure 1A).

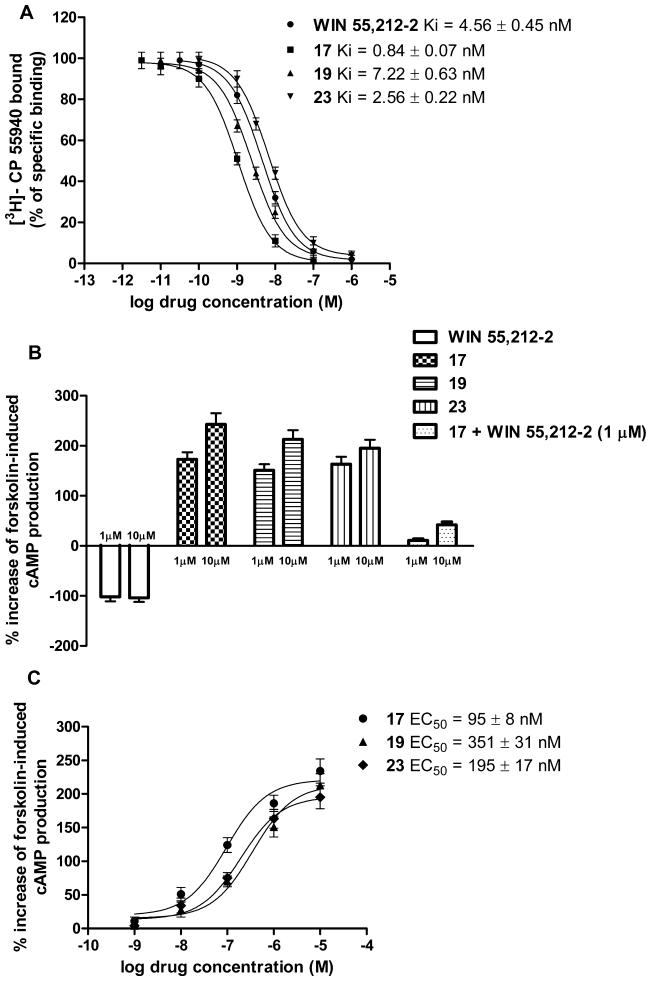

Figure 1. Competition curves on hCB_2 receptors of WIN 55,212-2 and selected novel CB compounds (A). Effect of the same compounds expressed as % of increase of forskolin-stimulated cAMP accumulation in hCB_2CHO cells (B). Concentration–response curves of the novel compounds **17**, **19**, and **23** in cAMP assays (C). Results are the mean ± SEM (n = 4 independent experiments).

These results clearly confirmed our previous studies. To achieve good affinity and selectivity at the CB_2 receptor, the adamantyl group borne by the amide function should be retained.

Interestingly, when the 2-alkyl group was replaced with an aromatic phenyl or substituted phenyl moieties at the C-2 position, affinity for the CB_1 receptors was completely lost (compounds **21**–**39**) except for analogues **21** and **22** bearing the cyclohexyl and cycloheptyl carboxamide groups, respectively, that exhibited moderate affinities for the CB_1 receptor. More significantly, all of the 2-aryl derivatives displayed high affinity for the CB_2 receptor, in contrast with the results obtained with the previous pyrazolo[3,4-*b*]pyridine series where the phenyl or 4-Cl-phenyl moiety on the pyrazole led to loss of affinity at cannabinoid receptors.

As seen with the 3-alkylpyrazolo[3,4-*b*]pyridine series, introduction of an adamant-1-ylcarboxamide, as in compound **23**, improved affinity and selectivity for the CB_2 receptor in comparison to the cyclohexylamide and cycloheptylamide (**21** and **22**, respectively). In contrast, the adamant-1-ylamide in this group of compounds led to a loss of affinity for the CB_1 receptor, showing a significant increase in selectivity and suggesting a difference between the two receptor subtypes in steric tolerance at this position. In particular, the adamant-1-ylcarboxamide **23** is the most selective compound in this series with high affinity for the target receptor (hCB_2 K_i =2.56 nM, SI > 3906).

Introduction of a 2-methylphenyl group at C-2 of the pyrazolopyrimidine moiety, as with compounds **24**–**26**, resulted in a 15- and 41-fold increase of selectivity for the cyclohexyl- and cycloheptylcarboxamide derivatives (SI > 250 and SI > 1119, respectively) relative to the analogous 2-phenyl group (compounds **21** and **22**, respectively). Affinity at the CB_2 receptor was also increased by the 2-methylphenyl derivative when the amide carried a cycloheptyl moiety (compound **25** hCB_2 K_i = 8.93 nM). In contrast, the adamant-1-ylcarboxamide (**26**) retained affinity and selectivity for the CB_2 receptor equivalent to that of the phenyl derivative **23**. Moving the methyl group from the ortho to the para position of the phenyl moiety (compounds **27**–**29**) did not induce a marked alteration in affinity at the CB_2 receptor. The most active compound was the adamantyl carboxamide **29** (hCB_2 K_i = 3.41, SI > 2932), although the cycloheptylamide **28** displayed high affinity with remarkable selectivity over the CB_1 receptor (hCB_2 K_i = 10.3 nM, SI > 970).

We therefore investigated the impact of other phenyl substituents by introducing a chlorine atom at the ortho and para positions of the 2-phenyl moiety (compounds **30**–**35**, Table 1). As shown by the biological assays, these new compounds displayed affinity similar to that of the 2- and 4-methyl compounds (**24**–**29**). The cycloheptyl- and adamant-1-ylcarboxamide derivatives showed high affinity and selectivity for the CB_2 receptor (**31**, **32** hCB_2 K_i = 10.3, 6.21 nM and **34**, **35** hCB_2 K_i = 6.31, 3.88 nM, respectively; hCB_1 K_i > 10 000 nM). These results suggest that electronic effects on the aromatic moiety do not significantly influence the affinity for the cannabinoid CB_2 receptor.

Disubstitution of the aryl moiety at C-2 was also examined, specifically the 2,4-dichlorophenyl (**36**–**38**) and 2,6-dichlorophenyl derivatives (**39**). These modifications resulted in a significant loss of affinity for the CB_2 receptors in comparison with the corresponding unsubstituted or monosubstituted phenyl compounds.

Replacement of the phenyl ring of compounds **21**–**23** by the bioisosteric 2- or 3-furyl groups (compounds **40**–**42** and **43**, respectively) mainly induced a marked improvement in affinity at the CB_2 receptor, as seen with compound **42**, bearing a 6-cyclohexylcarboxamide, in comparison to the parent 2-phenyl analogue **21** (hCB_2 K_i = 9.52 nM vs 42 nM, respectively). All of the 2-furyl compounds showed excellent affinity at the CB_2

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Table 2. Effect of the Novel CB Compounds 10−49 in hCB$_2$CHO Cells on Cyclic AMP Assays at 1 and 10 μM

compd	increase in cAMP production (%)[a] at 1 μM	at 10 μM	compd	increase in cAMP production (%)[a] at 1 μM	at 10 μM
WIN 55,212-2	−102 ± 9	−104 ± 8	**30**	37 ± 4	55 ± 5
10	42 ± 4	54 ± 5	**31**	53 ± 6	69 ± 7
11	44 ± 5	63 ± 6	**32**	114 ± 9	136 ± 12
12	56 ± 6	75 ± 8	**33**	79 ± 9	94 ± 10
13	38 ± 3	52 ± 4	**34**	121 ± 13	162 ± 14
14	48 ± 5	67 ± 5	**35**	144 ± 13	183 ± 15
15	121 ± 11	146 ± 12	**36**	45 ± 4	65 ± 6
16	137 ± 13	159 ± 16	**37**	61 ± 5	77 ± 8
17	173 ± 14	243 ± 22	**38**	87 ± 8	112 ± 10
18	85 ± 9	115 ± 10	**39**	38 ± 4	62 ± 6
19	151 ± 12	213 ± 18	**40**	73 ± 7	103 ± 9
20	114 ± 11	189 ± 16	**41**	42 ± 4	88 ± 9
21	92 ± 8	123 ± 11	**42**	107 ± 11	124 ± 13
22	103 ± 9	134 ± 12	**43**	56 ± 6	92 ± 8
23	163 ± 15	195 ± 17	**44**	89 ± 8	102 ± 9
24	73 ± 6	94 ± 8	**45**	71 ± 6	97 ± 8
25	107 ± 8	125 ± 10	**46**	38 ± 4	68 ± 5
26	155 ± 15	184 ± 17	**47**	32 ± 4	48 ± 4
27	63 ± 7	93 ± 9	**48**	34 ± 4	51 ± 6
28	81 ± 7	107 ± 11	**49**	36 ± 4	60 ± 6
29	138 ± 12	168 ± 16			

[a]The data are espressed as the mean ± SEM of n = 4 independent experiments and represent the % of increase of cAMP production in hCB$_2$CHO cells stimulated with forskolin (1 μM) obtained by novel CB compounds at 1 or 10 μM in comparison with the full agonist WIN 55,212-2 that completely inhibited the forskolin-stimulated cAMP levels.

receptor ranging from 4.92 to 9.52 nM. The 3-furyl compound **43** is substantially equivalent to derivative **42** in in terms of affinity, yet endowed with somewhat lower receptor selectivity.

Among the 2-thienyl derivatives **44**−**46** bearing an adamant-1-yl residue on the amide nitrogen, better affinity was seen with the unsubstituted thiophene derivative **44** (hCB$_2$ K_i = 6.24 nM, SI > 1602) compared to the 4- or 5-methylthiophene analogues (**45** or **46**, respectively) with slightly reduced the affinity at CB$_2$ receptor.

An overall comparison between the binding assays of 2-alkylpyrazolopyrimidine and 2-aryl derivatives indicates that C-2 may be a site for enhancement in selectivity together with an apparent improvement in affinity.

We also evaluated the effect on receptor affinity and selectivity of structural modifications at the 3-position of the pyrazolopyrimidone nucleus (**46**−**48**) within the 2-methyl series. The introduction of a bromine (**47**, hCB$_2$ K_i = 220 nM), a phenyl (**48**, hCB$_2$ K_i = 181 nM), or a 4-methoxyphenyl (**49**, hCB$_2$ K_i = 350 nM) resulted in a significant decrease in affinity for CB$_2$ receptor when compared to the 3-unsubstituted analogue (**12**, hCB$_2$ K_i = 12.7 nM), suggesting a severe steric constraint at this position.

Functional Activity at CB$_2$ Receptor and SAR. The novel compounds were also evaluated in functional assays in order to study their effects on forskolin-stimulated adenylate cyclase production in hCB$_2$ CHO cells at 1 and 10 μM compared to the maximal effect (set at −100%) achieved with the full CB$_2$ agonist WIN 55,212-2. All of the tested compounds show some level of efficacy, as evidenced by their ability to increase forskolin-induced cAMP production, thus characterizing these compounds as inverse agonists (Table 2).

Interestingly, the highest effect was achieved with the compounds **17** and **19** that were able to increase forskolin-induce cAMP production by 243% and 213% at 10 μM, respectively (Figure 1B), yet the two compounds display slightly different affinities at the CB$_2$ receptor (K_i = 0.84 and 7.22 nM, respectively). When tested in the presence of WIN 55,212-2, the novel compound **17** was able to completely abrogate the inhibitory effect of the agonist on forskolin-stimulated cAMP production, confirming its opposite effect with respect to WIN 55,212-2 (Figure 1B).

For the most effective compounds **17**, **19**, and **23**, full dose−response curves were measured and EC_{50} values were determined. The obtained results that confirmed the inverse agonism activity relative to the selected compounds are presented in Figure 1C. The potencies of the novel compounds **17**, **19**, and **23** were 95 ± 8, 351 ± 31, and 195 ± 17 nM, respectively, confirming a good correlation with their affinity for CB$_2$ receptors.

Among the various compounds bearing an alkyl substituent at C-2 (compounds **10**−**20**), when the amide substituent is held constant, there is a trend toward an ethyl substituent at C-2 being more effective than *tert*-butyl, which is more effective than methyl, suggesting that the size of the C-2 substituent may influence the efficacy of these inverse agonists. For instance, in the group of compounds having an adamantylamide, the C-2 ethyl compound (**17**) is one of the most effective compounds evaluated, showing a 243% increase in cAMP levels at 10 μM, while the *tert*-butyl (**20**) and methyl (**12**) analogues show 189% and 75% increases, respectively, at the same concentration. A similar trend is observed among the cyclohexylamides (compounds **15**, **18**, and **10**), while within the cycloheptylamides (compounds **19**, **16**, and **11**) the highest effect is for the *tert*-butyl (**19**) analogue. Interestingly, within each limited series of amides, there appears to be a correlation between the observed efficacy as inverse agonists at 1 or 10 μM and the affinity of the individual compounds for the CB$_2$ receptor.

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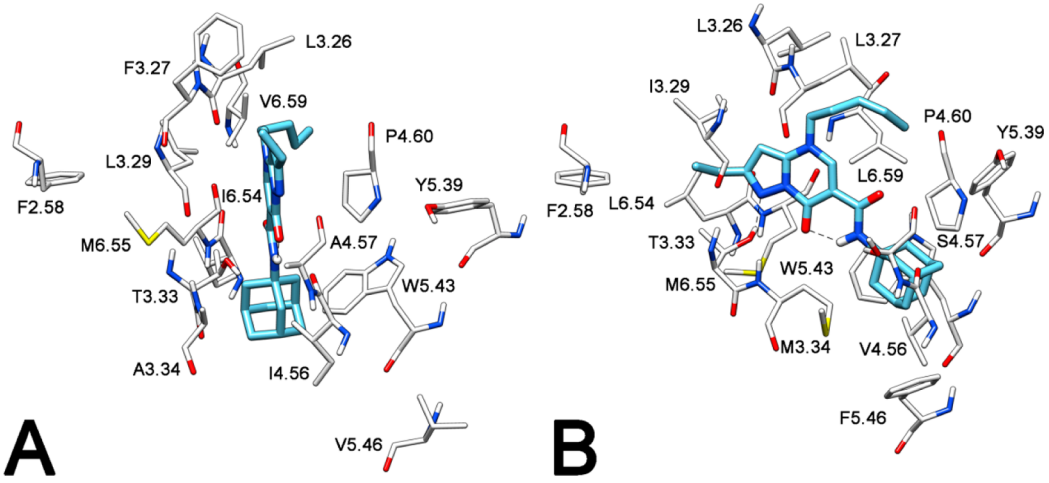

Figure 2. Compound **17** docked into CB_1 (A) and CB_2 (B) receptors.

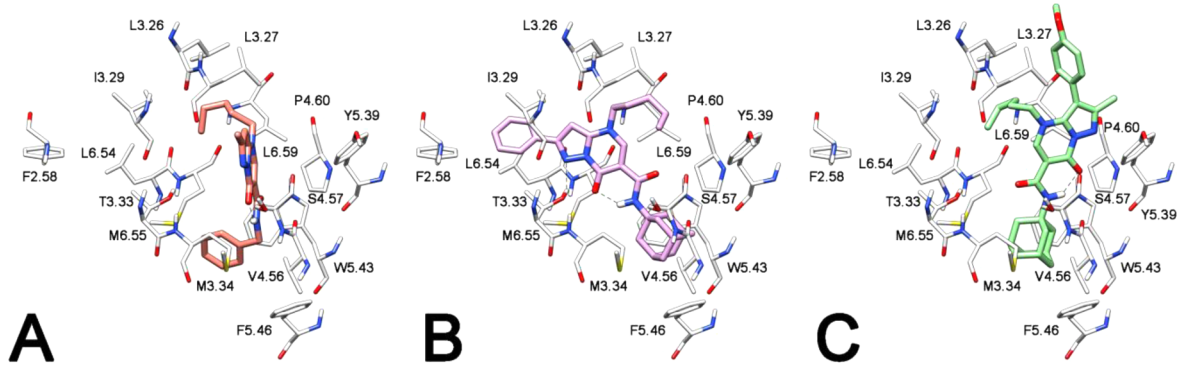

Figure 3. Docking of **13** (A), **23** (B), and **49** (C) into the CB_2 receptor model.

We did not observe similar trends among the compounds bearing a phenyl or substituted phenyl moiety at C-2 (**21**−**39**). The adamantylamides (**23**, **26**, **35**, and **29**) are the most effective, showing 195%, 184%, 168%, and 183% increases in cAMP levels at 10 μM, respectively. These compounds were also found to bind to the CB_2 receptor with very good affinities (K_i < 4 nM). Similary, the cyclohexylamides (**21**, **24**, **33**, **27**, **36**, and **30**) show moderate effects at 10 μM with modest affinity (40−57 nM). Among the cycloheptylamides (**34**, **22**, **25**, **28**, **37**, and **31**) the increase in cAMP levels at 10 μM is correlated with K_i values, while a different trend was observed only for the 2-Cl-phenyl derivative **31** that revealed a modest increase in cAMP (69%) despite its good affinity at CB_2 (K_i = 10.3 nM).

Molecular Modeling Studies. The synthesized ligands were docked, using AUTODOCK 4.0,[32] into the CB_1 and CB_2 receptor models. Figure 2A shows the docking of compound **17** into both receptors. In the CB_1 receptor model, the pyrazolopyrimidine central scaffold and the adamantane ring interact in the lipohilic pocket mainly delimited by L3.29(193), T3.33(197), I4.56(247), A4.57(248), P4.60(251), and W5.43(279), while the *n*-pentyl chain is directed toward the sixth transmembrane domain (TM6) and interacts with L3.26(190) and V6.57(367).

In the CB_2 receptor model, the *n*-pentyl chain interacts with L3.27(108) and L6.57(269) whereas the pyrazolopyrimidine central scaffold is rotated about 90° with respect to the disposition in the CB_1 receptor model (clockwise sense from the extracellular point of view) with the formation of an H-bond with T3.33(114) and lipophilic interactions with I3.29(110), M6.59(465), and L6.59(269). Furthermore, the adamantane ring interacts with V4.56(164), P4.60(168), Y5.39(190), W5.43(194) and feels the effect of a strong interaction with F5.46(197), which is a nonconserved residue (V282 in the CB_1 receptor). The interactions with T3.33(114) and F5.46(197), together with the different disposition of the pyrazolopyrimidine central scaffold, could be the reason for the CB_2 versus CB_1 selectivity of these ligands. Site-directed mutagenesis partially confirms our hypothesis, as mutations in the CB_2 subtype of F5.46(197) cause a substantial decrease of WIN-55,212-2 affinity.[33,34]

The substitution of the adamantane ring with the benzylic group (see **12** vs **13**) causes an important decrease of the CB_2 affinity. As shown in Figure 3A, the docking of compound **13** highlights that the benzyl group is not adapted to interact into the lipophilic pocket delimited by V4.56(164), P4.60(168), Y5.39(190), W5.43(194), and F5.46(197), causing the ligand to assume a disposition very similar to that observed for **17** in the CB_1 receptor, with the loss of the H-bond interaction with T3.33(114).

The addition of a phenyl ring in position 2 of the pyrazolopyrimidine scaffold seems to be well tollerated, as shown by the CB_2 activity of compound **23**. The docking of this compound into the CB_2 receptor model highlights that **23** and **17** interact in a very similar manner, showing the same lipophilic interactions and the H-bond with T3.33(114) (see Figure 3B). With regard to the phenyl group, it interacts with F2.58(87) and is oriented toward an empty space delimited by

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TM2 and TM7 that could correspond to the anandamide binding pocket.[35]

Finally, the addition of a bulky group in position 3 of the pyrazolopyrimidine scaffold determines a general decrease of the CB_2 affinity. As shown in Figure 3C, the docking of compound **49** into the CB_2 receptor model highlights that, because of the steric hindrance produced by L3.26(107) and I3.29(110), the presence of a 4-methoxyphenyl group in position 3 determines a positional change of the pyrazolopyrimidine scaffold with the loss of the H-bond with T3.33(114) and the lipophilic interactions with I3.29(110) and M6.59(465).

CONCLUSION

A series of 7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide derivatives was designed and synthesized as high affinity CB_2 receptor ligands, tested in radioligand binding studies, and functionally characterized in cAMP accumulation assays.

A major focus of the optimization effort was to increase selectivity of our previous series of heteroarylpyridine/pyrimidine derivatives to avoid or reduce the potential CB_1-associated CNS adverse effects of this novel series. The best affinity values associated with high selectivity were obtained with compounds **23**, **26**, **29**, **35**, **42**, and **44** (2.56 nM < hCB_2 K_i < 6.24 nM, 1602 < SI < 3906) bearing an aryl group at the C-2 position and an adamant-1-yl moiety on the C-6 amide function.

The effect of novel compounds on forskolin induced elevation of cyclic adenosine monophosphate (cAMP) levels in Chinese hamster ovary (CHO) cells transfected with human CB_2 receptor was assessed. The potencies of the compounds **17**, **19**, and **23** were also measured in functional assays. These results reveal that the novel series behaved as CB_2 receptor inverse agonists. A good correlation between receptor affinity (expressed as K_i) and efficacy (represented by % increase in cAMP levels at different concentrations of test compound) was observed; thus, the wide range of relative efficacies seen with this limited series of compounds offers the potential opportunity to more closely examine the structural requirements for delineation between neutral antagonists and potent inverse agonists. When compared to the triaryl bis-sulfones described by the Schering group, the compounds herein display similar levels of affinity and selectivity for the CB_2 receptor[17] while significantly exceeding the affinity and selectivity reported recently for the related triarylsulfonamide derivatives.[18] Thus, this novel series of compounds offers an attractive starting point for further optimization, representing novel pharmacological tools to evaluate the therapeutic potential of CB_2 inverse agonists in various disease settings. To achieve these ambitious objectives, an evaluation of the systemic bioavailability and metabolic stability will be required, tasks beyond the scope of this present study.

EXPERIMENTAL SECTION

Pharmacology. Competition binding experiments were performed by using [^{3}H]CP-55,940 (specific activity, 180 Ci/mmol) that was obtained from Perkin-Elmer Life and Analytical Sciences (U.S.). Human CB_1 and CB_2 receptors expressed in CHO cells were purchased from Perkin-Elmer Life and Analytical Sciences (U.S.). All other reagents were of analytical grade and obtained from commercial sources.

Competition Binding Experiments on CB_1 and CB_2 Receptors. To study CB_1 receptors, rat brain (male Sprague–Dawley rats, Charles River) was removed, frozen in liquid nitrogen, and stored at −80 °C until ready for use. The thawed rat brain tissue was resuspended in 50 mM Tris-HCl buffer, pH 7.4, at 4 °C. The suspension was homogenized with a Polytron, centrifuged for 10 min at 2000*g*, and the supernatant was centrifuged again for 20 min at 40000*g*. The pellet was resuspended in a buffer containing 50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, 0.5% fatty acid free BSA, pH 7.4, at 30 °C.

Competition binding experiments to rat CB_1 receptors were performed using [^{3}H]CP-55,940 (1.0 nM), a membrane suspension containing 40 μg of protein/100 μL, and different concentrations (1 nM to 10 μM) of the examined compounds.[36]

To investigate CB_2 receptors, [^{3}H]CP-55,940 binding assay was performed by using previously frozen rat spleen (male Sprague–Dawley rats, Charles River) that was homogenized in 50 mM Tris-HCl buffer, pH 7.4, at 4 °C with a Polytron and centrifuged for 10 min at 2000*g*, and the supernatant was centrifuged for 20 min at 40000*g*. The pellet was resuspended in a buffer containing 50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, 0.5% fatty acid free BSA, pH 7.4, at 30 °C. Competition binding experiments to rat CB_2 receptors were performed using [^{3}H]CP-55,940 (0.5 nM), a membrane suspension containing 80 μg of protein/100 μL and different concentrations (1 nM to 10 μM) of examined compounds.[36]

CHO cells expressing human CB_1 and CB_2 receptors were grown adherently and maintained in Ham's F12 containing 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL), and Geneticin (G418, 0.4 mg/ml) at 37 °C in 5% CO_2/95% air.[11,37,38] For membrane preparations, the culture medium was removed and the cells were washed with PBS, then scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris-HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with a Polytron and centrifuged for 10 min at 1000*g*, and the supernatant was then centrifuged for 30 min at 100000*g*. The membrane pellet was suspended in 50 mM Tris-HCl buffer, 0.5% BSA (pH 7.4) containing 5 mM $MgCl_2$, 2.5 mM EDTA or 1 mM EDTA for hCB_1 or hCB_2 receptor, respectively.

Competition binding experiments were performed using 0.5 nM [^{3}H]CP-55,940 and different concentrations (1 nM to 10 μM) of the examined compounds or a reference agonist (WIN 55,212-2) for an incubation time of 90 or 60 min at 30 °C for CB_1 or CB_2 receptors, respectively.

Bound and free radioactivities were separated by filtering the assay mixture through Whatman GF/C glass fiber filters using a Brandel cell harvester (Brandel Instruments, Unterföhring, Germany). The filter bound radioactivity was counted on a Perkin-Elmer 2810 TR scintillation counter (Perkin-Elmer Life and Analytical Sciences, U.S.).

Cyclic AMP Assay for Human CB_2 Receptors. CHO cells transfected with human CB_2 receptors were washed with phosphate-buffered saline, diluted trypsin and centrifuged for 10 min at 200*g*. The pellet containing CHO cells (1×10^6 cells/assay) was suspended in 0.5 mL of incubation mixture: NaCl 150 mM, KCl 2.7 mM, NaH_2PO_4 0.37 mM, $MgSO_4$ 1 mM, $CaCl_2$ 1 mM, HEPES 5 mM, $MgCl_2$ 10 mM, glucose 5 mM, pH 7.4 at 37 °C. Then 0.5 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as a phosphodiesterase inhibitor was added and the mixture preincubated for 10 min in a shaking bath at 37 °C.[39] The effect of the novel CB compounds was studied in the presence of forskolin, 1 μM, in comparison with the well-known CB agonist WIN 55,212-2. The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000*g* for 10 min at 4 °C, and the supernatant was extracted four times with water saturated diethyl ether. The final aqueous solution was tested for cyclic AMP levels by a competition protein binding assay. Samples of cyclic AMP standard (0–10 pmol) were added to each test tube containing the incubation buffer (Trizma base 0.1 M, aminophylline 8.0 mM, 2-mercaptoethanol 6.0 mM, pH 7.4) and [^{3}H]cyclic AMP in a total volume of 0.5 mL. The binding protein previously prepared from beef adrenals was added to the samples previously incubated at 4 °C for 150 min, and after the addition of charcoal, they were centrifuged at 2000*g* for 10 min. The clear supernatant was counted by using a Perkin-Elmer 2810 TR scintillation counter (Perkin-Elmer Life and Analytical Sciences, U.S.).

Data Analysis. The protein concentration was determined according to a Bio-Rad method (Bradford, 1976) with bovine albumin as reference standard. Inhibitory binding constants, K_i, were calculated from the IC_{50} values according to the Cheng and Prusoff equation:[40] $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where $[C^*]$ is the concentration of the radioligand and K_D^* its dissociation constant. A weighted nonlinear least-squares curve fitting program, LIGAND,[41] was used for computer analysis of the inhibition experiments. All the data are expressed as the mean ± SEM of $n = 4$ independent experiments. Statistical analysis of the data was performed using unpaired two-sided Student's t test.

Docking Studies. The ligands were built by means of Maestro[42] and were then minimized in a water environment (using the generalized Born/surface area model) by means of Macromodel.[43] They were minimized using the conjugate gradient, the MMFFs force field, and a distance-dependent dielectric constant of 1.0 until they reached a convergence value of 0.05 kcal $Å^{-1}$ mol^{-1}.

The ligands were docked using AUTODOCK 4.0[32] in the previously reported CB_1 and CB_2 receptor models[44] optimized on the basis of the recently deposited PDB structures of adenosine[45] and rhodopsine receptors.[46] AUTODOCK TOOLS[47] was used to identify the torsion angles in the ligands, to add the solvent model, and to assign partial atomic charges (Gasteiger for the ligands and Kollman for the receptors). The regions of interest used by AUTODOCK were defined by considering the previously published WIN 55,212-2 docked into the CB_1 and CB_2 receptor[44] as the central group of a grid of 54, 50, and 52 points in the x, y, and z directions. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the energetic map calculations. Since the compounds can form an intramolecular H-bond and our previous study suggested that the interaction was quite strong,[44] this H-bond was considered to be maintained during interaction in the binding site. For this reason, during the AUTODOCK protocol, we blocked the torsions involved in this intramolecular bond to prevent the loss of this interaction. By use of the Lamarckian genetic algorithm, the compounds were subjected to 100 runs of the AUTODOCK search using 500 000 steps of energy evaluation and default values for the other parameters. Cluster analysis was performed on the results using an rms tolerance of 1.0 Å. The cluster with the best average of estimated free energy was chosen.

Chemistry. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer or a Varian Mercury Plus 400 spectrometer. Chemical shifts (δ) are given in ppm, and the spectra were recorded in appropriate deuterated solvents, as indicated. Positive ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument with BE geometry. Melting points were determined on a Buchi-Tottoli apparatus and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. The purity of the tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Department of Chemistry of the University of Ferrara, Italy, with a Yanagimoto MT-5 CHN recorder elemental analyzer. All tested compounds yielded data consistent with a purity of at least 95% compared with the theoretical values. Reaction progress and product mixtures were routinely monitored by TLC on silica gel (precoated Merck F_{254} plates), and compounds were visualized with aqueous $KMnO_4$. Flash chromatography was performed using 230−400 mesh silica gel and a mixture of ethyl acetate/petroleum ether or ethyl acetate/methanol as eluent. Organic solutions were dried over anhydrous Na_2SO_4. All chemicals and reagents were purchased from Aldrich (Sigma-Aldrich) or Lancaster (Alfa Aesar, Johnson Matthey Company).

Synthesis of Ethyl 4,7-Dihydro-7-oxo-2-(substituted)-pyrazolo[1,5-*a*]pyrimidine-6-carboxylate (7a−o). General Procedure. A mixture of the appropriate 3-substituted-5-aminopyrazole (**6a−o**, 10 mmol), diethyl ethoxymethylenemalonate (10 mmol), and acetic acid (10 mL) was heated at reflux for 4 h. After the reaction was complete (from TLC), the solvent was evaporated, water was added, and the crude product was filtered and washed with cold ethanol.

Ethyl 4,7-Dihydro-2-methyl-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7a). White solid; mp, 295 °C; yield, 75%. MS: m/z 222.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.98 (bs, 1H), 8.52 (s, 1H), 6.13 (s, 1H), 4.22 (q, J = 7 Hz, 2H), 2.23 (s, 3H), 1.27 (t, J = 7.2 Hz, 3H).

Ethyl 4,7-Dihydro-2-ethyl-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylate (7b). White solid; mp, 291 °C (dec); yield, 70%. MS: m/z 235.8 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.95 (bs, 1H), 8.52 (s, 1H), 6.16 (s, 1H), 4.22 (q, J = 7 Hz, 2H), 2.67 (q, J = 7.6 Hz, 2H), 1.31−1.18 (m, 6H).

Ethyl 2-*tert*-Butyl-4,7-dihydro-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7c). White solid; mp, >300 °C; yield, 75%. MS: m/z 264.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.07 (bs, 1H), 8.47 (s, 1H), 6.15 (s, 1H), 4.17 (q, J = 7 Hz, 2H), 1.27−1.21 (m, 12H).

Ethyl 4,7-Dihydro-7-oxo-2-phenylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7d). White solid; mp, >300 °C; yield, 70%. MS: m/z 284.3 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.08 (bs, 1H), 8.56 (s, 1H), 8.00−7.98 (m, 2H), 7.50−7.42 (m, 3H), 6.77 (s, 1H), 4.23 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 6.8 Hz, 3H).

Ethyl 4,7-Dihydro-2-(2-methylphenyl)-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7e). White solid; mp, >300 °C; yield, 65%. MS: m/z 298.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.12 (bs, 1H), 8.59 (s, 1H), 7.64−7.62 (m, 1H), 7.34−7.29 (m, 3H), 6.56 (s, 1H), 4.24 (q, J = 7 Hz, 2H), 2.52 (s, 3H), 1.29 (t, J = 7.4 Hz, 3H).

Ethyl 4,7-Dihydro-2-(4-methylphenyl)-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7f). White solid; mp, >300 °C; yield, 68%. MS: m/z 298.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.02 (bs, 1H), 8.56 (s, 1H), 7.88 (dd, J = 8 Hz, 2H), 7.29 (dd, J = 8 Hz, 2H), 6.58 (s, 1H), 4.24 (q, J = 7 Hz, 2H), 2.36 (s, 3H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 2-(2-Chlorophenyl)-4,7-dihydro-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7g). White solid; mp, >300 °C; yield, 60%. MS: m/z 318.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.18 (bs, 1H), 8.64 (s, 1H), 7.87−7.45 (m, 4H), 6.55 (s, 1H), 4.25 (q, J = 7 Hz, 2H), 1.30 (t, J = 7.2 Hz, 3H).

Ethyl 2-(4-Chlorophenyl)-4,7-dihydro-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7h). Pale white solid; mp, >300 °C; yield, 70%. MS: m/z 318.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.06 (bs, 1H), 8.57 (s, 1H), 8.03 (dd, J = 8.6 Hz, 2H), 7.54 (dd, J = 8.6 Hz, 2H), 6.81 (s, 1H), 4.24 (q, J = 7 Hz, 2H), 1.29 (t, J = 7.2 Hz, 3H).

Ethyl 2-(2,4-Dichlorophenyl)-4,7-dihydro-7-oxopyrazolo-[1,5-*a*]pyrimidine-6-carboxylate (7i). White solid; mp, >300 °C; yield, 60%. MS: m/z 353.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.18 (bs, 1H), 8.65 (s, 1H), 7.93−7.80 (m, 2H), 7.60−7.54 (m, 1H), 6.75 (s, 1H), 4.25 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 2-(2,6-Dichlorophenyl)-4,7-dihydro-7-oxopyrazolo-[1,5-*a*]pyrimidine-6-carboxylate (7j). White solid; mp, 197 °C; yield, 28%. MS: m/z 353.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.18 (bs, 1H), 8.64 (s, 1H), 7.60−7.56 (m, 2H), 7.21−7.16 (m, 1H), 6.41−6.37 (s, 1H), 4.25 (q, J = 7 Hz, 2H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 4,7-Dihydro-2-(furan-2-yl)-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7k). White solid; mp, >300 °C; yield, 80%. MS: m/z 273.9 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.16 (bs, 1H), 8.59 (s, 1H), 7.84 (s, 1H), 7.06−7.01 (m, 1H), 6.66−6.56 (m, 2H), 4.24 (q, J = 7.2 Hz, 2H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 4,7-Dihydro-2-(furan-3-yl)-7-oxopyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7l). White solid; mp, >300 °C; yield, 50%. MS: m/z 273.9 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.16 (bs, 1H), 8.57 (s, 1H), 8.32 (s, 1H), 7.88 (m, 1H), 6.97−6.89 (m, 1H), 6.56 (s, 1H), 4.24 (q, J = 7 Hz, 2H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 4,7-Dihydro-7-oxo-2-(thiophen-2-yl)pyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (7m). White solid; mp, >300 °C; yield, 70%. MS: m/z 290.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.16 (bs, 1H), 8.57 (s, 1H), 7.71−7.62 (m, 2H), 7.18−7.14 (m 1H), 6.70 (s, 1H), 4.24 (q, J = 7.Hz, 2H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 4,7-Dihydro-2-(4-methylthiophen-2-yl)-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylate (7n). White solid; mp, >300 °C; yield, 59%. MS: m/z 304.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.12 (bs, 1H), 8.56 (s, 1H), 7.52 (s, 1H), 7.20 (s,

1H), 6.65 (s, 1H), 4.24 (q, J = 7.2 Hz, 2H), 2.25 (s, 3H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 4,7-Dihydro-2-(5-methylthiophen-2-yl)-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylate (7o). White solid; mp, >300 °C; yield, 23%. MS: m/z 304.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.16 (bs, 1H), 8.54 (s, 1H), 7.47 (d, J = 3.4 Hz, 1H), 6.85 (d, J = 2.8 Hz, 1H), 6.61 (s, 1H), 4.24 (q, J = 7.2 Hz, 2H), 2.49 (s, 3 H), 1.28 (t, J = 7.2 Hz, 3H).

Synthesis of Ethyl 3-Bromo-4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylate (7p). A solution of bromine (38.5 mmol, 2.12 mL) in glacial acetic acid (5 mL) was added dropwise and under vigorous stirring to a mixture of ethyl 4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxylate (**7a**, 11 mmol) in glacial acetic acid (40 mL). The reaction mixture was stirred for 3 h at room temperature. The precipitate was removed by filtration and washed with water. Yellow solid; mp, 163 °C; yield, 65%. MS: m/z 301.7 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 13.38 (bs, 1H), 8.36 (s, 1H), 4.23 (q, J = 7.2 Hz, 2H), 2.29 (s, 3H), 1.28 (t, J = 7.4 Hz, 3H).

Synthesis of Ethyl 4,7-Dihydro-7-oxo-4-pentyl-2-(substituted)-pyrazolo[1,5-*a*]pyrimidine-6-carboxylates (8a–p). General Procedure. A mixture of ethyl 4,7-dihydro-7-oxo-2-(substituted)-pyrazolo[1,5-*a*]pyrimidine-6-carboxylates (**7a–o**, 1.3 mmol) and K_2CO_3 (4 mmol) in dry DMF (10 mL) was heated at 100 °C. After 1 h, 1-pentyl bromide (2 mmol) was added and the mixture was stirred at 80–100 °C for 10–18 h. The solution was evaporated to dryness in vacuo and treated with water. The precipitate was collected and was purified by crystallization (ethyl acetate/hexane).

Ethyl 4,7-Dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (8a). White solid; mp, 93 °C; yield, 65%. MS: m/z 292.4 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.63 (s, 1H), 6.41 (s, 1H), 4.23 (q, J = 7.2 Hz, 2H), 4.13 (t, J = 7.2 Hz, 2H), 2.32 (s, 3H), 1.75–1.63 (m, 2H), 1.30–1.26 (m, 7H), 0.86 (t, J = 6.8 Hz, 3H).

Ethyl 2-Ethyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (8b). White solid; mp, 106 °C; yield, 74%. MS: m/z 306.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.63 (s, 1H), 6.47 (s, 1H), 4.25–4.14 (m, 4H), 2.69 (q, J = 7.6 Hz, 2H), 1.81–1.72 (m, 2H), 1.32–1.20 (m, 10H), 0.86 (t, J = 6.6 Hz, 3H).

Ethyl 2-*tert*-Butyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8c). White solid; mp, 87 °C; yield, 80%. MS: m/z 334.5 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.62 (s, 1H), 6.56 (s, 1H), 4.25–4.11 (m, 4H), 1.79–1.68 (m, 2H), 1.31–1.24 (m, 16H), 0.86 (t, J = 6.4 Hz, 3H).

Ethyl 4,7-Dihydro-7-oxo-4-pentyl-2-phenylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylate (8d). White solid; mp, 130 °C; yield, 60%. MS: m/z 354.6 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.01–7.99 (m, 2H), 7.52–7.44 (m, 3H), 7.18 (s, 1H), 4.27–4.20 (m, 4H), 1.83–1.76 (m, 2H), 1.34–1.28 (m, 7H), 0.87 (t, J = 3.4 Hz, 3H).

Ethyl 4,7-Dihydro-2-(2-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8e). White solid; mp, 100 °C; yield, 74%. MS: m/z 368.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.71 (s, 1H), 7.67–7.64 (m, 1H), 7.34–7.31 (m, 3H), 6.93 (s, 1H), 4.31–4.20 (m, 4H), 2.52 (s, 3H), 1.82–1.76 (m, 2H), 1.33–1.26 (m, 7H), 0.87 (t, J = 6.4 Hz, 3H).

Ethyl 4,7-Dihydro-2-(4-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8f). White solid; mp, 194 °C; yield, 71%. MS: m/z 368.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.68 (s, 1H), 7.89 (dd, J = 8 Hz, 2H), 7.31 (dd, J = 8 Hz, 2H), 7.12 (s, 1H), 4.27–4.20 (m, 4H), 2.36 (s, 3H), 1.85–1.77 (m, 2H), 1.33–1.26 (m, 7H), 0.87 (t, J = 6.4 Hz, 3H).

Ethyl 2-(2-Chlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8g). White solid; mp, 91 °C; yield, 78%. MS: m/z 388.9 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.73 (s, 1H), 7.89–7.81 (m, 1H), 7.60–7.47 (m, 3H), 7.03 (s, 1H), 4.28–4.21 (m, 4H), 1.89–1.80 (m, 2H), 1.34–1.25 (m, 7H), 0.89–0.83 (m, 3H).

Ethyl 2-(4-Chlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8h). White solid; mp, 196–197 °C; yield, 80%. MS: m/z 388.7 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.70 (s, 1H), 8.02 (dd, J = 8.4 Hz, 2H), 7.57 (dd, J = 8.4 Hz, 2H), 7.21 (s, 1H), 4.31–4.17 (m, 4H), 1.3 (m, 2H), 1.36–1.26 (m, 7H), 0.91–0.84 (t, J = 6.6 Hz, 3H).

Ethyl 2-(2,4-Dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8i). White solid; mp, >300 °C; yield, 58%. MS: m/z 423.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.72 (s, 1H), 7.88–7.77 (m, 2H), 7.59–7.54 (m, 1H), 7.04 (s, 1H), 4.30–4.19 (m, 4H), 1.79 (m, 2H), 1.32–1.25 (m, 7H), 0.87–0.81 (t, J = 6.6 Hz, 3H).

Ethyl 2-(2,6-Dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8j). White solid; mp, >300 °C; yield, 55%. MS: m/z 423.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.76 (s, 1H), 7.67–7.54 (m, 3H), 6.83 (s, 1H), 4.28–4.18 (m, 4H), 1.80–1.69 (m, 2H), 1.32–1.25 (m, 7H), 0.87–0.81 (t, J = 6.4 Hz, 3H).

Ethyl 4,7-Dihydro-2-(furan-2-yl)-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylate (8k). White solid; mp, 98–99 °C; yield, 75%. MS: m/z 344.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.69 (s, 1H), 7.86–7.78 (m, 1H), 7.04 (m, 1H), 6.92 (s, 1H), 6.68–6.67 (m, 1H), 4.27–4.20 (m, 4H), 1.89–1.78 (m, 2H), 1.34–1.26 (m, 7H), 0.86 (t, J = 6.6 Hz, 3H).

Ethyl 4,7-Dihydro-2-(furan-3-yl)-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylate (8l). White solid; mp, 127–129 °C; yield, 80%. MS: m/z 344.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.68 (s, 1H), 8.29–8.21 (m, 1H), 7.81 (m, 1H), 6.95 (s, 1H), 6.89 (s, 1H), 4.30–4.14 (m, 4H), 1.89–1.78 (m, 2H), 1.34–1.26 (m, 7H), 0.87 (t, J = 6.2 Hz, 3H).

Ethyl 4,7-Dihydro-7-oxo-4-pentyl-2-(thiophen-2-yl)-pyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8m). Pale white solid; mp, 171 °C; yield, 46%. MS: m/z 360.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.69 (s, 1H), 7.69–7.64 (m, 2H), 7.21–7.17 (m, 1H), 7.06 (s, 1H), 4.31–4.16 (m, 4H), 1.82 (m, 2H), 1.36–1.26 (m, 7H), 0.87 (t, J = 6.6 Hz, 3H).

Ethyl 4,7-Dihydro-2-(4-methylthiophen-2-yl)-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8n). Pale yellow solid; mp, 211–213 °C; yield, 90%. MS: m/z 374.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.68 (s, 1H), 7.50 (s, 1H), 7.23 (s, 1H), 7.02 (s, 1H), 4.27–4.15 (m, 4H), 2.26 (s, 3H), 1.81–1.75 (m, 2H), 1.35–1.24 (m, 7H), 0.87 (t, J = 6.4 Hz, 3H).

Ethyl 4,7-Dihydro-2-(5-methylthiophen-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8o). Yellow solid; mp, 200 °C; yield, 65%. MS: m/z 374.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.67 (s, 1H), 7.47 (d, J = 3.4 Hz, 1H), 6.99 (s, 1H), 6.87–6.79 (m, 1H), 4.27–4.18 (m, 4H), 2.48 (s, 3H), 1.82–1.64 (m, 2H), 1.34–1.26 (m, 7H), 0.87 (t, J = 6.6 Hz, 3H).

Ethyl 3-Bromo-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylate (8p). White solid; mp, 163 °C; yield, 55%. MS: m/z 370.1–372.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.45 (s, 1H), 4.23 (q, J = 7.2 Hz, 2H), 4.15 (t, J = 7.2 Hz, 2H), 2.24 (s, 3H), 1.75–1.68 (m, 2H), 1.30–1.26 (m, 7H), 0.85 (t, J = 6.8 Hz, 3H).

Synthesis of 4,7-Dihydro-2-(substituted)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acids (9a–p). General Procedure. The appropriate 4,7-dihydro-7-oxo-4-pentyl-2-substituted-pyrazolo[1,5-*a*]pyrimidine-6-carboxylates (**8a–p**, 1.7 mmol) was stirred in a mixture of aqueous 10% sodium hydroxide (5 mL) and methanol (5 mL) at room temperature for 3 h. The solution was adjusted to pH 4 with aqueous 10% hydrochloric acid. The resulting precipitate was collected by filtration and washed with H_2O and cold methanol.

4,7-Dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylic Acid (9a). White solid; mp, 134 °C; yield, 68%. MS: m/z 264.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.89 (bs, 1H), 8.80 (s, 1H), 6.53 (s, 1H), 4.19 (t, J = 7.4 Hz, 2H), 2.35 (s, 3H), 1.77–1.68 (m, 2H), 1.29–1.26 (m, 4H), 0.85 (t, J = 6.6 Hz, 3H).

4,7-Dihydro-2-ethyl-7-oxo-4-pentylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylic Acid (9b). White solid; mp, 126 °C; yield, 60%. MS: m/z 278.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.80 (bs, 1H), 8.77 (s, 1H), 6.59 (s, 1H), 4.20 (t, J = 7.4 Hz, 2H),

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2.69−2.58 (m, 2H), 1.77−1.69 (m, 2H), 1.32−1.22 (m, 7H), 0.89−0.82 (t, J = 6.6 Hz, 3H).

2-*tert*-Butyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (9c). White solid; mp, 110 °C; yield, 58%. MS: m/z 306.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.78 (bs, 1H), 8.78 (s, 1H), 6.71 (s, 1H), 4.21 (t, J = 7.4 Hz, 2H), 1.78−1.66 (m, 2H), 1.33−1.29 (m, 13H), 0.89 (t, J = 7.4 Hz, 3H).

4,7-Dihydro-7-oxo-4-pentyl-2-phenylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (9d). White solid; mp, 185 °C; yield, 58%. MS: m/z 326.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.78 (bs, 1H), 8.28 (s, 1H), 8.03−8.01 (m, 2H), 7.51−7.46 (m, 3H), 7.28 (s, 1H), 4.26 (t, J = 7.2 Hz, 2H), 1.84−1.71 (m, 2H), 1.34−1.30 (m, 4H), 0.88−0.85 (t, J = 6.8 Hz, 3H).

4,7-Dihydro-2-(2-methylphenyl)-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylic Acid (9e). White solid; mp, 159 °C; yield, 56%. MS: m/z 340.5 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.72 (bs, 1H), 8.84 (s, 1H), 7.69−7.45 (m, 1H), 7.35−7.30 (m, 3H), 7.05 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 2.53 (s, 3H), 1.83−1.69 (m, 2H), 1.34−1.31 (m, 4H), 0.86 (t, J = 6.6 Hz, 3H).

4,7-Dihydro-2-(4-methylphenyl)-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylic Acid (9f). White solid; mp, 215 °C; yield, 60%. MS: m/z 340.5 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.76 (bs, 1H), 8.82 (s, 1H), 7.92 (dd, J = 8.2 Hz, 2H), 7.33 (dd, J = 7.8 Hz, 2H), 7.24 (s, 1H), 4.26 (t, J = 7.4 Hz, 2H), 2.37 (s, 3H), 1.84−1.77 (m, 2H), 1.37−1.32 (m, 4H), 0.87 (t, J = 6.6 Hz, 3H).

2-(2-Chlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylic Acid (9g). White solid; mp, 167 °C; yield, 64%. MS: m/z 360.7 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.71 (bs, 1H), 8.84 (s, 1H), 7.85−7.83 (m, 1H), 7.61−7.48 (m, 3H), 7.12 (s, 1H), 4.30 (t, J = 7.4 Hz, 2H), 1.82−1.75 (m, 2H), 1.34−1.30 (m, 4H), 0.86 (t, J = 6.4 Hz, 3H).

2-(4-Chlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylic Acid (9h). White solid; mp, 199 °C; yield, 63%. MS: m/z 360.7 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.70 (bs, 1H), 8.82 (s, 1H), 8.04 (dd, J = 8.8 Hz, 2H), 7.59 (dd, J = 8.4 Hz, 2H), 7.30 (s, 1H), 4.26 (t, J = 7.4 Hz, 2H), 1.82−1.73 (m, 2H), 1.35−1.32 (m, 4H), 0.86 (t, J = 6.4 Hz, 3H).

2-(2,4-Dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylic Acid (9i). White solid; mp, 175 °C; yield, 47%. MS: m/z 395.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.65 (bs, 1H), 8.84 (s, 1H), 7.92−7.57 (m, 3H), 7.13 (s, 1H), 4.29 (t, J = 6.6 Hz, 2H), 1.81−1.71 (m, 2H), 1.33−1.30 (m, 4H), 0.86 (t, J = 6.4 Hz, 3H).

2-(2,6-Dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxylic Acid (9j). White solid; mp, 178−179 °C; yield, 45%. MS: m/z 395.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.65 (bs, 1H), 8.86 (s, 1H), 7.68−7.52 (m, 3H), 6.91 (s, 1H), 4.24 (t, J = 7.6 Hz, 2H), 1.81−1.74 (m, 2H), 1.33−1.30 (m, 4H), 0.86 (t, J = 6.6 Hz, 3H).

4,7-Dihydro-2-(furan-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylic Acid (9k). Pale white solid; mp, 164−166 °C; yield, 40%. MS: m/z 316.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.71 (bs, 1H), 8.81 (s, 1H), 7.88−7.73 (m, 1H), 7.10−7.08 (m, 1H), 7.02 (s, 1H), 6.69 (t, J = 7.2 Hz, 1H), 4.26−4.13 (m, 2H), 1.77−1.68 (m, 2H), 1.36−1.25 (m, 4H), 0.86 (t, J = 6.4 Hz, 3H).

4,7-Dihydro-2-(furan-3-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]-pyrimidine-6-carboxylic Acid (9l). White solid; mp, 191 °C (dec); yield, 71%. MS: m/z 316.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.71 (bs, 1H), 8.81 (s, 1H), 8.34−8.33 (m, 1H), 7.83−7.75 (m, 1H), 6.99−6.89 (m, 2H), 4.23 (t, J = 7 Hz, 2H), 1.83−1.74 (m, 2H), 1.35−1.27 (m, 4H), 0.87 (t, J = 6.6 Hz, 3H).

4,7-Dihydro-7-oxo-4-pentyl-2-(thiophen-2-yl)pyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (9m). White solid; mp, 157 °C; yield, 94%. MS: m/z 332.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.73 (bs, 1H), 8.81 (s, 1H), 7.72−7.68 (m, 2H), 7.22−7.16 (m, 2H), 4.24 (t, J = 7 Hz, 2H), 1.83−1.69 (m, 2H), 1.34−1.27 (m, 4H), 0.87 (t, J = 6.4 Hz, 3H).

4,7-Dihydro-2-(4-Methylthiophen-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (9n). Pale white solid; mp, 162−164 °C; yield, 40%. MS: m/z 345.6 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.71 (bs, 1H), 8.80 (s, 1H), 7.54 (s, 1H), 7.26 (s, 1H), 7.12 (s, 1H), 4.23 (t, J = 7 Hz, 2H), 2.26 (s, 3H), 1.82−1.73 (m, 2H), 1.35−1.26 (m, 4H), 0.87 (t, J = 6.6 Hz, 3H).

4,7-Dihydro-2-(5-Methylthiophen-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (9o). White solid; mp, 185 °C; yield, 76%. MS: m/z 345.6 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.73 (bs, 1H), 8.79 (s, 1H), 7.51 (d, J = 3.4 Hz, 1H), 7.09 (s, 1H), 6.89 (d, J = 2.8 Hz, 1H), 4.23 (t, J = 7.2 Hz, 2H), 2.49 (s, 3H), 1.82−1.76 (m, 2H), 1.34−1.21 (m, 4H), 0.87 (t, J = 6.2 Hz, 3H).

3-Bromo-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (9p). White solid; mp, 222 °C; yield, 68%. MS: m/z 342.2−344.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.71 (bs, 1H), 8.73 (s, 1H), 4.39 (t, J = 7.8 Hz, 2H), 2.32 (s, 3H), 1.79−1.77 (m, 2H), 1.35−1.32 (m, 4H), 0.87 (t, J = 6.8 Hz, 3H).

Synthesis of 4,7-Dihydro-7-oxo-4-pentyl-2-(substituted)-pyrazolo[1,5-*a*]pyrimidine-6-carboxamides (10, 11, 13−16, 18, 19, 21, 22, 24, 25, 27, 28, 30, 31, 33, 34, 36, 37, 40, 41). General Procedure. A mixture of 4,7-dihydro-7-oxo-4-pentyl-2-(substituted)-pyrazolo[1,5-*a*]pyrimidine-6-carboxylic acids (**9a−k**, **9p**, 0.48 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC, 1.44 mmol), and 1-hydroxybenzotriazole (HOBt, 1.44 mmol) in dry DMF (5 mL), was stirred for 10 min at room temperature. The appropriate amine was added, and the mixture was stirred for 12 h at room temperature.The reaction mixture was concentrated and purified by column chromatography on silica gel.

***N*-Cyclohexyl-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (10).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 1:1, as eluent); mp, 148 °C; yield, 45%. MS: m/z 345.2 (M + H). ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.11 (bd, J = 8 Hz, 1H), 8.48 (s, 1H), 5.96 (s, 1H), 4.02−3.94 (m, 3H), 2.45 (s, 3H), 1.91−1.32 (m, 16H), 0.91 (t, J = 6.6 Hz, 3H). Anal. ($C_{19}H_{28}N_4O_2$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (11).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 1:1, as eluent); mp, 149 °C; yield, 42%. MS: m/z 359.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.00 (bd, J = 8 Hz, 1H), 8.66 (s, 1H), 6.44 (s, 1H), 4.18 (t, J = 7 Hz, 2H), 4.02−3.90 (m, 1H), 2.34 (s, 3H), 1.84−1.41 (m, 14H), 1.39−1.28 (m, 4H), 0.85 (t, J = 6.6 Hz, 3H). Anal. ($C_{20}H_{30}N_4O_2$) C, H, N .

***N*-Benzyl-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (13).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 3:7, as eluent); mp, 144 °C; yield, 50%. MS: m/z 353.19 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.34 (bt, J = 6 Hz, 1H), 8.72 (s, 1H), 7.35−7.29 (m, 5H), 6.46 (s, 1H), 4.55 (d, J = 6 Hz, 2H), 4.19 (t, J = 7.4 Hz, 2H), 2.34 (s, 3H), 1.82−1.68 (m, 2H), 1.31−1.26 (m, 4H), 0.85 (t, J = 6.6 Hz, 3H). Anal. ($C_{20}H_{24}N_4O_2$) C, H, N.

4,7-Dihydro-2-methyl-*N*-(4-methyl-cyclohexyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (14). White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 3:7, as eluent); mp, 149 °C; yield, 62%. MS: m/z 359.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.11 (bd, J = 8 Hz, 1H), 8.68 (s, 1H), 6.45 (s, 1H), 4.22−4.08 (m, 3H), 3.81−3.64 (m, 1H), 2.34 (s, 3H), 1.99−0.81 (m, 20H). Anal. ($C_{20}H_{30}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-2-ethyl-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxamide (15).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 1:1, as eluent); mp, 157 °C; yield, 40%. MS: m/z 359.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.01 (bd, J = 7.8 Hz, 1H), 8.67 (s, 1H), 6.50 (s, 1H), 4.19 (t, J = 7.4 Hz, 2H), 3.89−3.72 (m, 1H), 2.71 (q, J = 7.6 Hz, 2H), 1.99−1.21 (m, 19H), 0.85 (t, J = 6.6 Hz, 3H). Anal. ($C_{20}H_{30}N_4O_2$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-2-ethyl-7-oxo-4-pentylpyrazolo-[1,5-*a*]pyrimidine-6-carboxamide (16).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, 116 °C; yield, 40%. MS: m/z 373.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.01 (bd, J = 7.6 Hz, 1H), 8.65 (s, 1H), 6.49 (s, 1H), 4.18 (t, J = 7.2 Hz, 2H), 4.03−3.81 (m, 1H), 2.67 (q, J = 7.6

Hz, 2H), 1.82−1.20 (m, 21H), 0.84 (t, J = 6.6 Hz, 3H). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

2-*tert*-Butyl-*N*-cyclohexyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (18). White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 3:7, as eluent); mp, 191 °C; yield, 40%. MS: m/z 387.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.01 (bd, J = 7.8 Hz, 1H), 8.66 (s, 1H), 6.60 (s, 1H), 4.20 (t, J = 7 Hz, 2H), 3.97−3.84 (m, 1H), 1.97−1.29 (m, 25H), 0.86 (t, J = 6.6 Hz, 3H). Anal. ($C_{22}H_{34}N_4O_2$) C, H, N.

2-*tert*-Butyl-*N*-cycloheptyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (19). White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 3:7, as eluent); mp, 186 °C; yield, 40%. MS: m/z 401.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.05 (bd, J = 8.2 Hz, 1H), 8.65 (s, 1H), 6.60 (s, 1H), 4.20 (t, J = 7.6 Hz, 2H), 4.11−3.91 (m, 1H), 1.83−1.76 (m, 4H), 1.55−1.28 (m, 23H), 0.86 (t, J = 6.4 Hz, 3H). Anal. ($C_{23}H_{36}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-7-oxo-4-pentyl-2-phenylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (21).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 2:8, as eluent); mp, 156 °C; yield, 59%. MS: m/z 407.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.99 (bd, J = 7.2 Hz, 1H), 8.73 (s, 1H), 8.04−8.00 (m, 2H), 7.57−7.45 (m, 3H), 7.21 (s, 1H), 4.27 (t, J = 7.2 Hz, 2H), 3.93−3.77 (m, 1H), 1.87−1.31 (m, 16H), 0.87 (t, J = 6.4 Hz, 3H). Anal. ($C_{24}H_{30}N_4O_2$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-7-oxo-4-pentyl-2-phenylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (22).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 2:8, as eluent); mp, 190 °C; yield, 55%. MS: m/z 421.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.04 (bd, J = 7.8 Hz, 1H), 8.73 (s, 1H), 8.04−7.99 (m, 2H), 7.57−7.45 (m, 3H), 7.21 (s, 1H), 4.27 (t, J = 6.6 Hz, 2H), 4.02−3.89 (m, 1H), 1.87−1.83 (m, 4H), 1.63−1.57 (m, 10H), 1.37−1.28 (m, 4H), 0.87 (t, J = 6.6 Hz, 3H). Anal. ($C_{25}H_{32}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-2-(2-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (24).** White solid (purified by crystallization using methanol); mp, 122 °C; yield, 52%. MS: m/z 421.5 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.02 (bd, J = 7.8 Hz, 1H), 8.74 (s, 1H), 7.76−7.61 (m, 1H), 7.35−7.34 (m, 3H), 6.98 (s, 1H), 4.28 (t, J = 7 Hz, 2H), 3.92−3.85 (m, 1H), 2.53 (s, 3H), 1.82−1.30 (m, 16H), 0.86 (t, J = 6.6 Hz, 3H). Anal. ($C_{25}H_{32}N_4O_2$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-2-(2-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (25).** White solid (purified by crystallization using methanol); mp, 147 °C; yield, 45%. MS: m/z 435.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.03 (bd, J = 7.8 Hz, 1H), 8.73 (s, 1H), 7.67 (m, 1H), 7.35−7.32 (m, 3H), 6.97 (s, 1H), 4.28 (t, J = 7 Hz, 2H), 4.03 (m, 1H), 2.54 (s, 3H), 1.85−1.27 (m, 18H), 0.86 (t, J = 6.6 Hz, 3H). Anal. ($C_{26}H_{34}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-2-(4-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (27).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 2:8, as eluent); mp, 190 °C; yield, 55%. MS: m/z 421.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.00 (bd, J = 7.8 Hz, 1H), 8.72 (s, 1H), 7.90 (dd, J = 7.8 Hz, 2H), 7.32 (dd, J = 7.8 Hz, 2H), 7.16 (s, 1H), 4.26 (t, J = 6.6 Hz, 2H), 3.97−3.83 (m, 1H), 2.37 (s, 3H), 1.86−1.33 (m, 16H), 0.37 (t, J = 6 Hz, 3H). Anal. ($C_{25}H_{32}N_4O_2$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-2-(4-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (28).** White solid (purified by crystallization using methanol); mp, 208 °C; yield, 43%. MS: m/z 435.5 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.03 (bd, J = 7.8 Hz, 1H), 8.71 (s, 1H), 7.90 (dd, J = 7.8 Hz, 2H), 7.32 (dd, J = 7.8 Hz, 2H), 7.16 (s, 1H), 4.25 (t, J = 6.6 Hz, 2H), 4.15−4.07 (m, 1H), 2.37 (s, 3H), 1.85−1.31 (m, 18H), 0.87 (t, J = 6.6 Hz, 3H). Anal. ($C_{26}H_{34}N_4O_2$) C, H, N.

2-(2-Chlorophenyl)-*N*-cyclohexyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (30). White solid (purified by crystallization using methanol); mp, >300 °C; yield, 43%. MS: m/z 441.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.98 (bd, J = 7.8 Hz, 1H), 8.77 (s, 1H), 7.96−7.82 (m, 1H), 7.65−7.61 (m, 1H), 7.52−7.48 (m, 2H), 7.07 (s, 1H), 4.30 (t, J = 7 Hz, 2H), 3.99−3.78 (m, 1H), 1.81−1.21 (m, 16H), 0.89 (t, J = 6.6 Hz, 3H). Anal. ($C_{24}H_{29}ClN_4O_2$) C, H, N.

2-(2-Chlorophenyl)-*N*-cycloheptyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (31). White solid (purified by crystallization using methanol); mp, 117 °C; yield, 41%. MS: m/z 455.7 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.99 (bd, J = 7.8 Hz, 1H), 8.76 (s, 1H), 7.87−7.83 (m, 1H), 7.64−7.61 (m, 1H), 7.52−7.48 (m, 2H), 7.07 (s, 1H), 4.30 (t, J = 7 Hz, 2H), 4.11−4.03 (m, 1H), 1.85−1.30 (m, 18H), 0.86 (t, J = 6.6 Hz, 3H). Anal. ($C_{25}H_{31}ClN_4O_2$).

2-(4-Chlorophenyl)-*N*-cyclohexyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (33). White solid (purified by crystallization using methanol); mp, >300 °C; yield, 44%. MS: m/z 441.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.97 (bd, J = 7.6 Hz, 1H), 8.74 (s, 1H), 8.03 (dd, J = 8.6 Hz, 2H), 7.59 (dd, J = 8.6 Hz, 2H), 7.24 (s, 1H), 4.26 (t, J = 6.8 Hz, 2H), 3.96−3.84 (m, 1H), 1.87−1.33 (m, 16H), 0.87 (t, J = 6.4 Hz, 3H). Anal. ($C_{24}H_{29}ClN_4O_2$) C, H, N.

2-(4-Chlorophenyl)-*N*-cycloheptyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (34). White solid (purified by crystallization using methanol); mp, 184 °C; yield, 41%. MS: m/z 455.6 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 9.01 (bd, J = 7.6 Hz, 1H), 8.73 (s, 1H), 8.03 (dd, J = 8.2 Hz, 2H), 7.59 (dd, J = 8.4 Hz, 2H), 7.24 (s, 1H), 4.25 (t, J = 6.8 Hz, 2H), 4.13−4.05 (m, 1H), 1.84−1.31 (m, 18H), 0.87 (t, J = 6.4 Hz, 3H). Anal. ($C_{25}H_{31}ClN_4O_2$) C, H, N.

***N*-Cyclohexyl-2-(2,4-dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (36).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, 149 °C; yield, 41%. MS: m/z 476.1 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.92 (bd, J = 8 Hz, 1H), 8.76 (s, 1H), 7.91−7.80 (m, 2H), 7.61−7.57 (m, 1H), 7.09 (s, 1H), 4.30 (t, J = 7.2 Hz, 2H), 3.96−3.84 (m, 1H), 1.87−1.30 (m, 16H), 0.85 (t, J = 6.6 Hz, 3H). Anal. ($C_{24}H_{28}Cl_2N_4O_2$) C, H, N.

***N*-Cycloheptyl-2-(2,4-dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (37).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, 134 °C; yield, 41%. MS: m/z 490.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.97 (bd, J = 7.8 Hz, 1H), 8.76 (s, 1H), 7.91−7.80 (m, 2H), 7.63−7.57 (m, 1H), 7.09 (s, 1H), 4.30 (t, J = 7.2 Hz, 2H), 4.12−4.06 (m, 1H), 1.84−1.30 (m, 18H), 0.85 (t, J = 6.6 Hz, 3H). Anal. ($C_{25}H_{30}Cl_2N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-2-(furan-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (40).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 6:4, as eluent); mp, 169 °C; yield, 44%. MS: m/z 397.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.94 (bd, J = 8 Hz, 1H) 8.72 (s, 1H), 7.88−7.87 (m, 1H), 7.06 (d, J = 3.4 Hz, 1H), 6.96 (s, 1H), 6.69−6.67 (m, 1H), 4.26 (t, J = 7.2 Hz, 2H), 3.92−3.79 (m, 1H), 1.87−1.30 (m, 16H), 0.86 (t, J = 6.6 Hz, 3H). Anal. ($C_{22}H_{28}N_4O_2$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-2-(furan-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (41).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 1:1, as eluent); mp, 168 °C; yield, 44%. MS: m/z 411.7 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.99 (bd, J = 8 Hz, 1H) 8.72 (s, 1H), 7.88−7.87 (m, 1H), 7.07 (d, J = 3.4 Hz, 1H), 6.96 (s, 1H), 6.68−6.67 (m, 1H), 4.26 (t, J = 7.2 Hz, 2H), 4.15−4.02 (m, 1H), 1.84−1.30 (m, 18H), 0.86 (t, J = 6.6 Hz, 3H). Anal. ($C_{23}H_{30}N_4O_2$) C, H, N.

Synthesis of *N*-Adamantan-1-yl-4,7-dihydro-7-oxo-4-pentyl-2-(substituted)-pyrazolo[1,5-*a*]pyrimidine-6-carboxamides (12, 17, 20, 23, 26, 29, 32, 35, 38, 39, 42−47). General Procedure. To a stirred solution of the desired 4,7-dihydro-7-oxo-4-pentyl-2-(substituted)-pyrazolo[1,5-*a*]pyrimidine-6-carboxylic acid (**9a−p**, 1.00 mmol) in dry DMF (15 mL) was added diisopropylethylamine (5.42 mmol). The resulting solution was stirred at room temperature for 10 min before adding HBTU (*o*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) (1.45 mmol)

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and stirring for an additional 3 h at room temperature. 1-Adamantylamine (1.45 mmol) was then added, and the solution was stirred for 24 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in ethyl acetate and successively washed with aqueous saturated sodium bicarbonate, water, and brine. The combined organic layers were dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography or crystallization.

***N*-Adamantan-1-yl-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (12).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 1:1, as eluent); mp, 168 °C; yield, 44%. MS: *m*/*z* 397.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.85 (bd, 1H), 8.61 (s, 1H), 6.43 (s, 1H), 4.16 (t, *J* = 6.6 Hz, 2H), 2.34 (s, 3H), 2.05 (s, 9H), 1.82−1.67 (m, 8H), 1.29−1.27 (m, 4H), 0.85 (t, *J* = 6.2 Hz, 3H). Anal. ($C_{23}H_{32}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-2-ethyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (17).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 1:1, as eluent); mp, 149 °C; yield, 45%. MS: *m*/*z* 411.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.86 (bs, 1H), 8.61 (s, 1H), 6.49 (s, 1H), 4.17 (t, *J* = 7 Hz, 2H), 2.71 (q, *J* = 7.6 Hz, 2H), 2.05 (s, 9H), 1.81−1.66 (m, 8H), 1.28−1.21 (m, 7H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{24}H_{34}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-2-*tert*-butyl-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (20).** White solid (purified by crystallization using methanol); mp, 168 °C; yield, 40%. MS: *m*/*z* 439.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.80 (bs, 1H), 8.60 (s, 1H), 6.59 (s, 1H), 4.17 (t, *J* = 7.6 Hz, 2H), 2.04 (s, 9H), 1.82−1.67 (m, 8H), 1.32−1.29 (m, 13H), 0.86 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{26}H_{38}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-4,7-dihydro-7-oxo-4-pentyl-2-phenylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (23).** White solid (purified by flash chromatography, using ethyl acetate as eluent); mp, 206 °C; yield, 48%. MS: *m*/*z* 459.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.87 (bs, 1H), 8.68 (s, 1H), 8.03−7.98 (m, 2H), 7.52−7.45 (m, 3H), 7.20 (s, 1H), 4.25 (t, *J* = 7.2 Hz, 2H), 2.06 (m, 9H), 1.83−1.80 (m, 2H), 1.68 (s, 6H), 1.35−1.28 (m, 4H), 0.87 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{28}H_{34}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-4,7-dihydro-2-(2-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (26).** White solid (purified by crystallization using methanol); mp, 240 °C (dec); yield, 43%. MS: *m*/*z* 473.8 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.67 (bs, 1H), 8.69 (s, 1H), 7.73−7.62 (m, 1H), 7.35−7.28 (m, 3H), 6.96 (s, 1H), 4.26 (t, *J* = 7.2 Hz, 2H), 2.53 (s, 3H), 2.06 (s, 9H), 1.93−1.68 (m, 8H), 1.34−1.31 (m, 4H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{29}H_{36}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-4,7-dihydro-2-(4-methylphenyl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (29).** White solid (purified by crystallization using methanol); mp, 258 °C; yield, 50%. MS: *m*/*z* 473.8 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.86 (bs, 1H), 8.64 (s, 1H), 7.88 (dd, *J* = 7.8 Hz, 2H), 7.30 (dd, *J* = 7.8 Hz, 2H), 7.13 (s, 1H), 4.22 (t, *J* = 7.2 Hz, 2H), 2.35 (s, 3H), 2.05 (s, 9H), 1.91−1.66 (m, 8H), 1.30 (m, 4H), 0.85 (t, *J* = 6 Hz, 3H). Anal. ($C_{29}H_{36}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-2-(2-chlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (32).** White solid (purified by crystallization using methanol); mp, 300 °C; yield, 42%. MS: *m*/*z* 493.9 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.82 (bs, 1H), 8.71 (s, 1H), 7.85−7.83 (m, 1H), 7.63−7.60 (m, 1H), 7.52−7.47 (m, 2H), 7.06 (s, 1H), 4.28 (t, *J* = 7.2 Hz, 2H), 2.07 (s, 9H), 1.92−1.68 (m, 8H), 1.34−1.30 (m, 4H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{28}H_{33}ClN_4O_2$) C, H, N.

***N*-Adamantan-1-yl-2-(4-chlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (35).** White solid (purified by crystallization using methanol); mp, 226 °C; yield, 42%. MS: *m*/*z* 493.9 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.85 (bs, 1H), 8.68 (s, 1H), 8.02 (dd, *J* = 8.6 Hz, 2H), 7.59 (dd, *J* = 8.4 Hz, 2H), 7.23 (s, 1H), 4.23 (t, *J* = 6.6 Hz, 2H), 2.06 (s, 9H), 1.92−1.68 (m, 8H), 1.31−1.25 (m, 4H), 0.87 (t, *J* = 6 Hz, 3H). Anal. ($C_{28}H_{33}ClN_4O_2$) C, H, N.

***N*-Adamantan-1-yl-2-(2,4-dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (38).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 3:7, as eluent); mp, 230 °C; yield, 41%. MS: *m*/*z* 527.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.80 (bs, 1H), 8.72 (s, 1H), 7.91−7.80 (m, 2H), 7.62−7.58 (m, 1H), 7.09 (s, 1H), 4.28 (t, *J* = 6.6 Hz, 2H), 2.06 (s, 9H), 1.93−1.68 (m, 8H), 1.34−1.30 (m, 4H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{28}H_{32}Cl_2N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-2-(2,6-dichlorophenyl)-4,7-dihydro-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (39).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 3:7, as eluent); mp, 209 °C; yield, 57%. MS: *m*/*z* 527.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.76 (bs, 1H), 8.73 (s, 1H), 7.66−7.52 (m, 3H), 6.84 (s, 1H), 4.24 (t, *J* = 7.2 Hz, 2H), 2.04 (s, 9H), 1.95−1.78 (m, 2H), 1.66 (s, 6H), 1.35−1.23 (m, 4H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{28}H_{32}Cl_2N_4O_2$).

***N*-Adamantan-1-yl-4,7-dihydro-2-(furan-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (42).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, 199 °C; yield, 44%. MS: *m*/*z* 449.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.83 (bs, 1H), 8.67 (s, 1H), 7.88−7.87 (m, 1H), 7.05 (d, *J* = 3 Hz, 1H), 6.95 (s, 1H), 6.69−6.67 (m, 1H), 4.24 (t, *J* = 7.2 Hz, 2H), 2.06 (s, 9H), 1.67 (m, 8H), 1.33−1.30 (m, 4H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{26}H_{32}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-4,7-dihydro-2-(furan-3-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (43).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, 241 °C; yield, 53%. MS: *m*/*z* 449.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.86 (bs, 1H) 8.66 (s, 1H), 8.30 (s, 1H), 7.84−7.81 (m, 1H), 6.96−6.94 (m, 1H), 6.91 (s, 1H), 4.20 (t, *J* = 7.2 Hz, 2H), 2.06 (s, 9H), 1.67 (m, 8H), 1.34−1.28 (m, 4H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{26}H_{32}N_4O_2$) C, H, N.

***N*-Adamantan-1-yl-4,7-dihydro-7-oxo-4-pentyl-2-(thiophen-2-yl)pyrazolo[1,5-*a*]pyrimidine-6-carboxamide (44).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, >300 °C; yield, 64%. MS: *m*/*z* 465.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.84 (bs, 1H) 8.66 (s, 1H), 7.71−7.66 (m, 2H), 7.22−7.17 (m, 1H), 7.09 (s, 1H), 4.22 (t, *J* = 7 Hz, 2H), 2.06 (s, 9H), 1.93−1.68 (m, 8H), 1.36−1.31 (m, 4H), 0.87 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{26}H_{32}N_4O_2S$) C, H, N.

***N*-Adamantan-1-yl-4,7-dihydro-2-(4-methylthiophen-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (45).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, 217 °C; yield, 58%. MS: *m*/*z* 479.3 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.84 (bs, 1H) 8.65 (s, 1H), 7.52 (s, 1H), 7.24 (s, 1H), 7.03 (s, 1H), 4.21 (t, *J* = 6.6 Hz, 2H), 2.26 (s, 3H), 2.06 (s, 9H), 1.92−1.67 (m, 8H), 1.34−1.31 (m, 4H), 0.87 (t, *J* = 6.2 Hz, 3H). Anal. ($C_{27}H_{34}N_4O_2S$) C, H, N.

***N*-Adamantan-1-yl-4,7-dihydro-2-(5-methylthiophen-2-yl)-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (46).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 4:6, as eluent); mp, 234 °C (dec); yield, 48%. MS: *m*/*z* 479.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.85 (bs, 1H) 8.64 (s, 1H), 7.48 (d, *J* = 3.8 Hz, 1H), 7.01 (s, 1H), 6.95 (d, *J* = 3.6 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 2H), 2.49 (s, 3H), 2.06 (s, 9H), 1.92−1.67 (m, 8H), 1.34−1.30 (m, 4H), 0.87 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{27}H_{34}N_4O_2S$) C, H, N.

***N*-Adamantan-1-yl-3-bromo-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (47).** White solid (purified by flash chromatography, using ethyl acetate/petroleum ether, 3:7, as eluent); mp, 222 °C; yield, 45%. MS: *m*/*z* 474.2−476.2 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.70 (bs, 1H) 8.62 (s, 1H), 4.44 (t, *J* = 7.8 Hz, 2H), 2.32 (s, 3H), 2.05 (s, 9H), 1.85−1.71 (m, 2H), 1.67 (s, 6H), 1.36−1.30 (m, 4H), 0.87 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{23}H_{31}BrN_4O_2$) C, H, N.

Synthesis of *N*-Adamantan-1-yl-4,7-dihydro-2-methyl-7-oxo-4-pentyl-[3-phenyl or 3-(4-methoxyphenyl)]pyrazolo[1,5-*a*]pyrimidine-6-carboxamide (48 or 49). A suspension of *N*-adamantyl-3-bromo-4,7-dihydro-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (**47**, 0.3 mmol), arylboronic acid (0.95 mmol), and K_2CO_3 (0.45 mmol) in toluene (5.5 mL) was thoroughly

deaerated and stirred under argon. Tetrakis(triphenylphosphine)-palladium(0) (0.04 mmol) was then added, and the mixture was heated at 100 °C for 16 h. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate and successively washed with aqueous saturated sodium bicarbonate, water, and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by column chromatography on silica gel, eluting with ethyl acetate/petroleum ether, 3:7.

***N*-Adamantyl-4,7-dihydro-2-methyl-7-oxo-4-pentyl-3-phenylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (48).** White solid; mp, 236 °C; yield, 35%. MS: *m*/*z* 473.4 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.84 (bs, 1H) 8.58 (s, 1H), 7.47−7.43 (m, 5H), 3.87 (t, *J* = 7.8 Hz, 2H), 2.12 (s, 3H), 2.06 (s, 9H), 1.67 (s, 6H), 1.41−1.23 (m, 2H), 1.12−0.92 (m, 2H), 0.71−0.63 (m, 5H). Anal. ($C_{29}H_{36}N_4O_2$) C, H, N.

***N*-Adamantyl-4,7-dihydro-3-(4-methoxyphenyl)-2-methyl-7-oxo-4-pentylpyrazolo[1,5-*a*]pyrimidine-6-carboxamide (49).** White solid; mp, 222 °C (dec); yield, 38%. MS: *m*/*z* 503.5 (M + H). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.82 (bs, 1H), 8.55 (s, 1H), 7.33 (dd, *J* = 8.6 Hz, 2H), 7.02 (dd, *J* = 8.8 Hz, 2H), 3.99−3.82 (m, 2H), 3.79 (s, 3H), 2.10 (s, 3H), 2.04 (s, 9H), 1.66 (s, 6H), 1.39−1.25 (m, 2H), 1.19−1.04 (m, 2H), 0.70−0.63 (m, 5H). Anal. ($C_{30}H_{38}N_4O_3$) C, H, N.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table 1 organized in ascending order by selectivity index (SI) of the novel CB compounds **10**−**49**. 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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■ ABBREVIATIONS USED

CHO, Chinese hamster ovary; DEEM, diethylethoxymethylene malonate; SI, selectivity index; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride; HOBt, hydroxybenzotriazole; HBTU, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate

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