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Design, Synthesis, and Pharmacological Properties of New Heteroarylpyridine/Heteroarylpyrimidine Derivatives as CB_2 Cannabinoid Receptor Partial Agonists

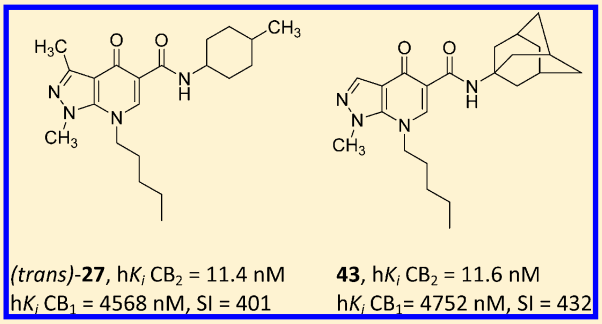
Mojgan Aghazadeh Tabrizi,*[,†] Pier Giovanni Baraldi,*[,†] Giulia Saponaro,[†] Allan R. Moorman,[‡,∥] Romeo Romagnoli,[†] Delia Preti,[†] Stefania Baraldi,[†] Carmen Corciulo,[§] Fabrizio Vincenzi,[§] Pier Andrea Borea,[§] and Katia Varani[§]

[†]Dipartimento di Scienze Farmaceutiche, Via Fossato di Mortara 17-19, Università di Ferrara, 44121, Ferrara, Italy

[‡] King Pharmaceuticals Research and Development, Inc., 4000 Centre Green Way, Suite 300, Cary, North Carolina 27707, United States

[§]Dipartimento di Medicina Clinica e Sperimentale, Sezione di Farmacologia, Università di Ferrara, 44121, Ferrara, Italy

ABSTRACT: Recent developments indicate that CB_2 receptor ligands have the potential to become therapeutically important. To explore this potential, it is necessary to develop compounds with high affinity for the CB_2 receptor. Very recently, we have identified the oxazinoquinoline carboxamides as a novel class of CB_2 receptor full agonists. In this paper we describe the medicinal chemistry of a new series of heteroaryl-4-oxopyridine/7-oxopyrimidine derivatives. Some of the reported compounds showed high affinity and potency at the CB_2 receptor while showing only modest affinity for the centrally expressed CB_1 cannabinoid receptor. Moreover, we found 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, the novel series show dose-dependent effects on the modulation of forskolin-induced cAMP production, revealing different behaviors as full agonists, partial agonists, and inverse agonists.



INTRODUCTION

Cannabinoids are a group of distinct compounds found in *Cannabis sativa*, with cannabinol, cannabidiol, and Δ^9-tetrahydrocannabinol (**1**, THC, Chart 1) being the most representative molecules. The long history of *Cannabis* use has led to the introduction into the clinic of **1** (dronabinol), for the stimulation of appetite in AIDS patients), and of one of its synthetic analogues, nabilone (for the suppression of nausea and vomiting produced by chemotherapy).[1,2] Despite the clinical benefits, the therapeutic use of cannabis is limited by its psychoactive effects including hallucination, addiction, and dependence.[3] The physiological effects of cannabinoids are mediated by at least two G-protein-coupled receptors, CB_1 and CB_2. Autoradiographic studies have demonstrated that CB_1 receptors are expressed primarily in the central nervous system, specifically in the cerebral cortex, hippocampus, basal ganglia, and cerebellum. They are also found to a lesser degree in the reproductive system and other peripheral tissues, including those of the immune system.[4] CB_2 receptors are almost exclusively found in the immune system, with the greatest density in the spleen. It is estimated that the expression level of CB_2 receptors in the immune cells is about 10−100 times higher than that of CB_1. Within the immune system, CB_2 is found in various cell types, including B cells, NK cells, monocytes, microglial cells, neutrophils, T cells, dentritic cells, and mast cells, suggesting that a wide range of immune functions can be regulated through CB_2 modulators.[5]

CB_2 receptors are also present in some central and peripheral neurons,[6,7] and the role of neuronal CB_2 receptors still has to be established. Various strategies have been developed to increase the benefit-to-risk ratio of cannabinoid therapies,[8] one of which is the development of CB_2 selective agonists for circumventing the unwanted consequences of cannabinoid CB_1 receptor activation.[9] Selective agonists of CB_2 receptors have been shown to suppress inflammation in vivo as well as inhibiting disease severity and spasticity in an animal model of multiple sclerosis.[10−12] Additionally, CB_2 agonists have been shown to inhibit inflammatory and neuropathic pain, as well as emesis.[13−16]

A large number of CB_2-selective agonists have been discovered and classified according to their chemical structures (Chart 1). The dibenzopyran derivatives include **1**, the main psychoactive constituent of *Cannabis*, and (6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol **2** (HU-210), a synthetic analogue of (−)-Δ^8-tetrahydrocannabinol. Compound **2** displays high affinity for CB_1 and CB_2 receptors and also high

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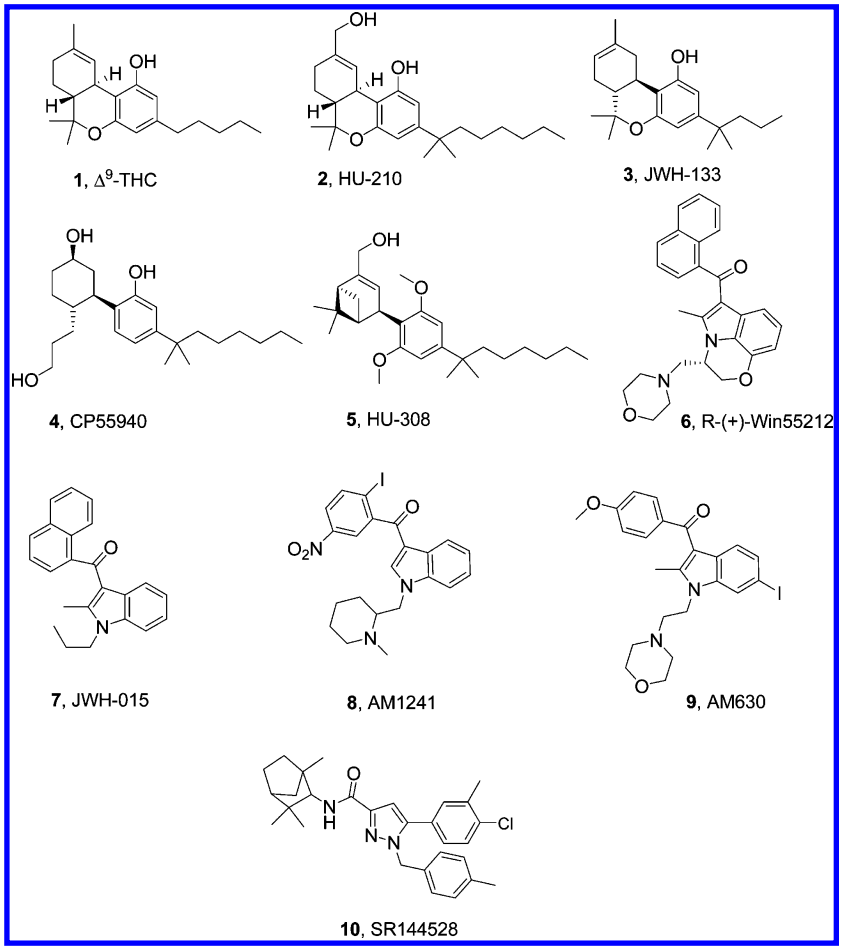
Chart 1. Structures of Representative CB_2 Selective Ligands



potency and relative intrinsic activity as a cannabinoid receptor agonist. A third member of this class is (6a*R*,10a*R*)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran (**3**, JWH-133), frequently used as a pharmacological tool.[17] Another well-known member of the bicyclic and tricyclic analogues of **1** that lack a pyran ring is (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol **4** (CP-55,940, Chart 1). This has been found to have slightly lower CB_1 and CB_2 affinities than **2** in some investigations but does seem to possess HU-210-like CB_1 and CB_2 receptor relative intrinsic activity.[17,18]

Another member of the bicyclic analogues is {4-[4-(1,1dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl}methanol **5** (HU-308) that is selective for the CB_2 receptor subtype, with a selectivity of over 400× for CB_2 vs CB_1.[17]

The aminoalkylindole derivatives are represented by *R*-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-*de*]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanonemesylate **6** (*R*-(+)-WIN55212). This displays CP-55,940-like and HU-210-like relative intrinsic activity at both CB_1 and CB_2 receptors. However, unlike **2** and **4**, it has been found in some investigations to possess slightly higher CB_2 than CB_1 affinity. (2-Methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone **7** (JWH-015), *R*-3-(2-iodo-5-nitrobenzoyl)-1-methyl-2-piperidinylmethyl)-1*H*-indole **8** (AM1241), and methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)-methanone **9** (AM630) are very important members of the aminoalkylindoles, which are frequently used as pharmacological tools.[4,19−21]

Finally, the diarylpyrazole *N*-[(1*S*)-*endo*-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)pyrazole-3-carboxamide **10** (SR144528) is one of the more potent compounds for blocking CB_2 rather than for CB_1 receptor activation. It displays much higher affinity for CB_2 than CB_1 receptors and blocks agonist-induced CB_2 receptor activation in a competitive manner.[17−19]

In recent years, structurally diverse CB_2 selective agonists exhibiting analgesic activity in various pain models have been discovered and recently reviewed.[22−24] These CB_2 receptor agonists include 4-oxo-1,4-dihydroquinoline-3-carboxamide (general structure **A**, Chart 2) and 1,8-naphthyridine-4(1*H*)-one-3-carboxamide derivatives (general structure **B**, Chart 2) that have been described as structurally diverse ligands endowed with high affinity and selectivity toward the CB_2 receptor (Chart 2). Some of these analogues were demonstrated to act as agonists or inverse agonists in functional activity assays, depending on the nature of the substituents on the different positions of the heterocyclic scaffold.[25−27] In light of these considerations and following these previous findings, our group recently reported a series of oxazinoquinolone derivatives (general structure **C**, Chart 2) with high affinity and selectivity at the CB_2 receptor.[28] All of these new compounds behaved as CB_2 full agonists.

Taking into consideration these findings and as part of a new research program to identify novel CB_2 agonists, we designed a

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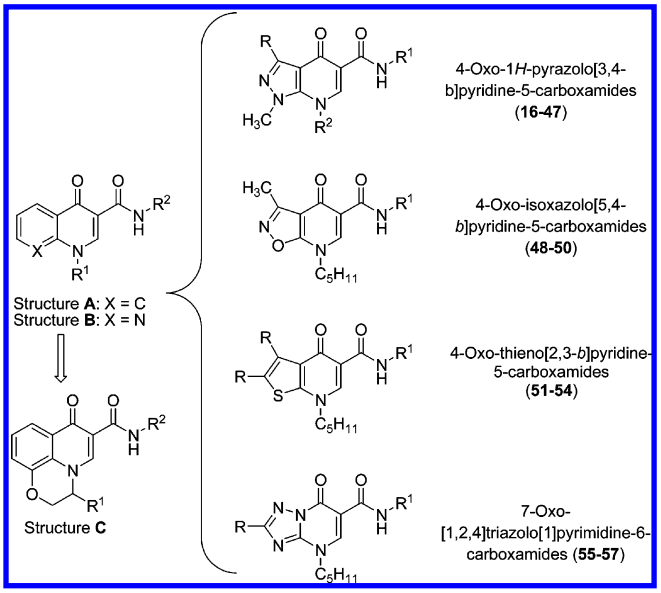
Chart 2. General Structures of 4-Oxo-1,4-dihydroquinoline-3-carboxamide Derivatives (A), 1,8-Naphthyridine-4(1*H*)-on-3-carboxamide (B), 4-Oxo-oxazinoquinoline (C), and the New Synthesized Compounds 16−57



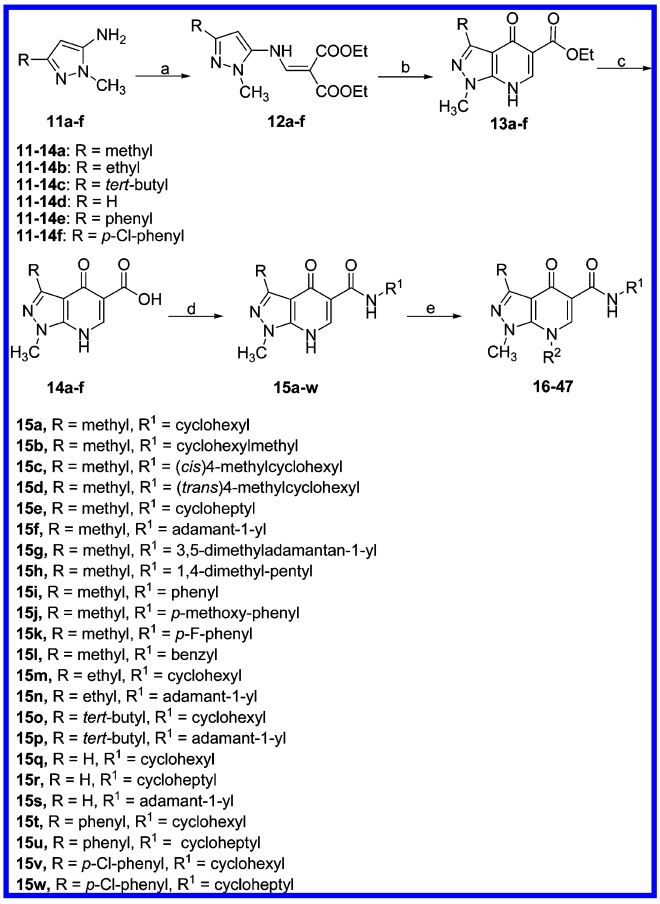
new chemical structure to investigate the biological activity toward cannabinoid receptors. Initially, our goal was to synthesize a series of 4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide derivatives to evaluate the effect of replacing the phenyl ring of general structures **A** or **B** with different 1-methyl-5-substituted pyrazolo nuclei (Chart 2). Indeed, the condensed benzene ring of 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives was replaced with the pyrazolo nucleus, resulting in the 4,7-dihydro-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamides. Aliphatic or aromatic carboxamide groups in position 5 have been selected on the basis of other cannabinoid pharmacophores, such as those present in the quinolone, naphthyridine, and oxazinoquinolone derivatives. A hydrophobic substituent in position 7 was preferred, while position 3 of the pyrazole ring was investigated with different alkyl or aryl moieties.

Subsequentely, we designed additional CB_2 ligands by replacing of the pyrazolo ring with different heterocycles via a combination of structure−activity relationships (SARs). The 7-pentylisoxazolo[5,4-*b*]pyridine, 7-pentylthieno[2,3-*b*]pyridine, and 4-pentyl[1,2,4]triazolo[1,5-*a*]pyrimidine carboxamides (as illustrated in Chart 2) were found to be potent CB_2 receptor ligands, and the consequences of such modifications deeply affected the functionality of these compounds. These newly synthesized compounds were tested in competition binding assays toward both rat CB_1 (rCB_1) and rat CB_2 (rCB_2) receptors expressed in native tissues as rat brain or spleen and toward human CB_1 (hCB_1) and CB_2 (hCB_2) receptors expressed in CHO cells. Affinity data (K_i, nM) were used to calculate the selectivity of these novel compounds for the CB_2 receptors. Most of these ligands were also examined in cyclic AMP assays on hCB_2 CHO cells, with the aim of evaluating the potency values (EC_{50}, nM) and efficacy (E_{max}, %) relative to the efficacy of the reference compound WIN 55,212 (10 μM) attributed as 100%.

CHEMISTRY

The synthetic routes to obtain the target 4-oxo-4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide derivatives **16−47** are outlined in Scheme 1. All substituents are summarized in Table 1. The required aminopyrazoles **11a−f** were prepared by known procedures (see Experimental Section).

Scheme 1[a]



[a]Reagents: (a) diethyl ethoxymethylenemalonate, 120 °C, 2 h; (b) diphenyl ether, 250 °C, 3 h; (c) NaOH 10%, 6 h, rt; (d) amine, EDC, HOBt, DMF, 16 h, rt *or* amine, HBTU, DIEA, DMF, 16 h, rt; (e) K_2CO_3, DMF, alkyl bromide, 16 h, 100 °C.

Replacement of the ethoxy group of diethyl ethoxymethylenemalonate by the 5-amino function of the appropriate pyrazoles **11a−f** afforded the intermediate diethyl 5-pyrazolylaminomethylenemalonates **12a−f**, which were cyclized in excellent yields, giving the ethyl 3-substituted-4-oxo-1-methyl-4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates **13a−f**.[29] In a first attempt, alkylation at N7 in anhydrous DMF in the presence of K_2CO_3 with appropriate alkyl bromides was performed on the esters **13a−f**, yielding 1:1 mixtures of the N-/O-alkylated compounds. To increase the regioselectivity of N7 alkylation, intermediates **13a−f** were transformed to the corresponding 4-oxo-4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic acids **14a−f**[30] by hydrolysis in 10% aqueous NaOH. Subsequently, the amides **15a−w** were obtained by a coupling reaction mediated by an acid-activating agent between selected amines and the 4-oxopyrazolo[3,4-*b*]pyridinecarboxylic acids **14a−f**. Alkylation of the resulting 4-oxo-4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamides **15a−w** yielded the N-

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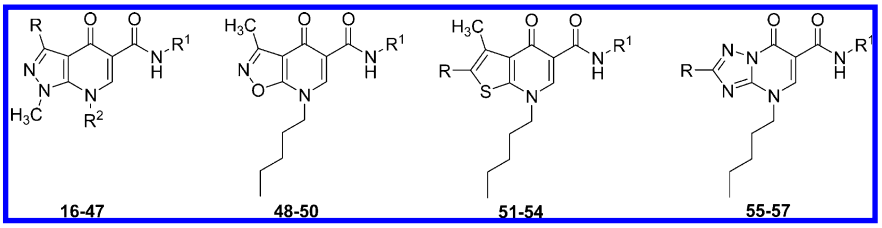
Table 1. Affinity (K_i, nM) and Selectivity Index (SI) on Rat and Human CB_1 and CB_2 Receptors of the Novel CB Compounds 16−57[a]



				K_i (nM)				
compd	R	R^1	R^2	rCB_1 [b]	rCB_2 [c]	hCB_1 [d]	hCB_2 [e]	hCB_1/hCB_2 SI
6	R-(+)-WIN 55,212-2			14.4 ± 1.3	7.73 ± 0.68	11.3 ± 1.2	4.95 ± 0.43	2.3
16	Me	cyclohexyl	pentyl	1420 ± 138	100 ± 12	500 ± 49	85 ± 9	5.9
17	Me	cyclohexyl	butyl	1533 ± 145	145 ± 13	1256 ± 127	138 ± 12	9.1
18	Me	cyclohexyl	propyl	832 ± 78	593 ± 18	758 ± 72	412 ± 38	1.8
19	Me	cyclohexyl	allyl	>10000 (27%)	>10000 (46%)	>10000 (34%)	>10000 (42%)	
20	Me	cyclohexyl	2-ethoxyethyl	>10000 (10%)	>10000 (1%)	>10000 (14%)	>10000 (1%)	
21	Me	cyclohexyl	4-cyanobutyl	>10000 (25%)	>10000 (5%)	>10000 (28%)	>10000 (11%)	
22	Me	cyclohexyl	hexyl	3220 ± 310	480 ± 32	3870 ± 322	325 ± 33	12
23	Me	cyclohexyl	benzyl	4560 ± 428	1100 ± 58	4248 ± 415	956 ± 94	4.4
24	Me	cyclohexyl	4-CH_3-benzyl	>10000 (38%)	>10000 (29%)	>10000 (35%)	>10000 (33%)	
25	Me	cyclohexyl	2-(morpholin-4-yl)ethyl-	>10000 (13%)	>10000 (32%)	>10000 (16%)	>10000 (36%)	
26	Me	cyclohexyl-CH_2	pentyl	1040 ± 95	508 ± 22	1124 ± 126	372 ± 35	3
27	Me	*trans*-4-CH_3-cyclohexyl	pentyl	4800 ± 570	13.3 ± 1.5	4568 ± 422	11.4 ± 1.2	401
28	Me	*cis*-4-CH_3-cyclohexyl	pentyl	750 ± 73	11.8 ± 1.3	723 ± 63	10.6 ± 1.1	68
29	Me	cycloheptyl	pentyl	1620 ± 165	35 ± 3	252 ± 22	11.2 ± 1.2	23
30	Me	adamant-1-yl	pentyl	832 ± 82	11.4 ± 1.7	800 ± 84	9.51 ± 0.93	84
31	Me	3,5-di-Me-adamant-1-yl	pentyl	3260 ± 352	16.7 ± 1.8	1050 ± 95	9.83 ± 1.01	107
32	Me	1,4-di-Me-pentyl	pentyl	2256 ± 216	262 ± 25	1795 ± 164	212 ± 20	8.5
33	Me	phenyl	pentyl	1020 ± 95	740 ± 38	865 ± 82	524 ± 53	1.7
34	Me	4-OCH_3-phenyl	pentyl	>10000 (46%)	>10000 (24%)	>10000 (38%)	>10000 (33%)	
35	Me	4-F-phenyl	pentyl	>10000 (48%)	>10000 (27%)	>10000 (44%)	>10000 (37%)	
36	Me	benzyl	pentyl	>10000 (42%)	4517 ± 188	>10000 (38%)	3652 ± 355	>2.7
37	Et	cyclohexyl	pentyl	1120 ± 126	516 ± 25	957 ± 92	469 ± 42	2
38	Et	adamant-1-yl	pentyl	2670 ± 253	97 ± 12	440 ± 35	37 ± 4	12
39	*t*-Bu	cyclohexyl	pentyl	>10000 (19%)	4033 ± 260	>10000 (23%)	3526 ± 227	>2.8
40	*t*-Bu	adamant-1-yl	pentyl	3430 ± 322	417 ± 33	3753 ± 385	363 ± 34	10
41	H	cyclohexyl	pentyl	5650 ± 525	250 ± 20	5150 ± 480	237 ± 21	22
42	H	cycloheptyl	pentyl	5200 ± 550	130 ± 14	4550 ± 435	103 ± 12	44
43	H	adamant-1-yl	pentyl	5342 ± 493	13.3 ± 1.2	4752 ± 381	11.6 ± 1.1	432
44	Ph	cyclohexyl	pentyl	>10000 (18%)	>10000 (1%)	>10000 (23%)	>10000 (1%)	
45	Ph	cycloheptyl	pentyl	>10000 (1%)	>10000 (1%)	>10000 (1%)	>10000(1%)	
46	*p*-Cl-Ph	cyclohexyl	pentyl	>10000 (15%)	5017 ± 188	>10000 (22%)	3620 ± 326	>2.8
47	*p*-Cl-Ph	cycloheptyl	pentyl	>10000 (12%)	4050 ± 132	>10000 (17%)	2830 ± 264	>3.5
48		cyclohexyl		187 ± 15	38 ± 4	155 ± 16	8.74 ± 0.92	17.7
49		cycloheptyl		184 ± 19	50 ± 4	145 ± 13	18.5 ± 1.7	7.8
50		adamant-1-yl		1495 ± 153	26 ± 3	1468 ± 138	21 ± 2	69.9
51	H	cyclohexyl		98 ± 9	14.8 ± 1.4	86 ± 7	12.3 ± 1.1	7.2
52	H	cycloheptyl		67 ± 5	8.23 ± 0.81	52 ± 4	6.95 ± 0.71	7.5
53	H	adamant-1-yl		16.5 ± 1.5	1.25 ± 0.13	13.7 ± 1.2	1.12 ± 0.09	12.5
54	Me	adamant-1-yl		52 ± 5	4.26 ± 0.42	43 ± 4	3.27 ± 0.29	15.9
55	H	cyclohexyl		nd[f]	nd[f]	nd[f]	nd[f]	
56	H	adamant-1-yl		850 ± 70	21 ± 2	712 ± 64	20 ± 2	35.6
57	phenyl	adamant-1-yl		560 ± 52	4.83 ± 0.46	523 ± 47	3.58 ± 0.31	146

[a]The data are espressed as the mean ± SEM of $n = 4$ independent experiments. [b]Affinity was calculated by using [^{3}H]CP-55,940 as radioligand on rat brain for CB_1 receptors. [c]Affinity was calculated by using [^{3}H]CP-55,940 as radioligand calculated on rat spleen for CB_2 receptors. [d]Affinity was calculated by using [^{3}H]CP-55,940 as radioligand calculated on human CB_1 CHO membranes. [e]Affinity was calculated by using [^{3}H]CP-55,940 as radioligand calculated on human CB_2 CHO membranes [f]nd = not determined.

substituted regioisomers as the major products (80%). This was probably favored because of the stabilization of the pyridine-4-one tautomeric form by intramolecular hydrogen bonding between the NH of the amide group and the 4-carbonyl function of the 1-alkyl-4-oxopyrazolo[3,4-*b*]pyridines.[29] Alkylation at N7 was confirmed by 2D ^{1}H NMR (ROESY) as

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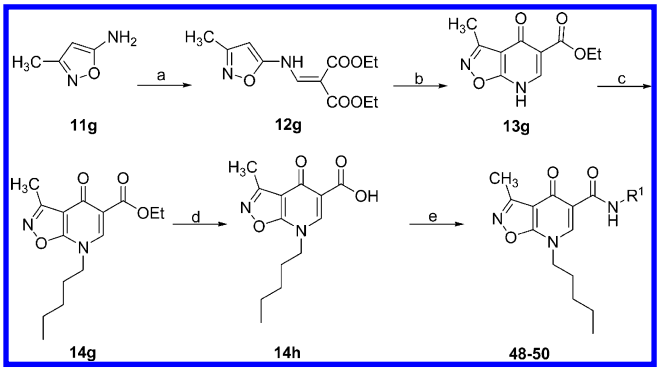
evidenced by the presence of cross-peaks between H6 and *N*-CH_2 protons.

By use of the general procedures shown in Scheme 1, starting from 3-methyl-5-aminoisoxazole[31] **11g** as depicted in Scheme 2, the target isoxazolopyridone derivatives **48**−**50** were prepared.

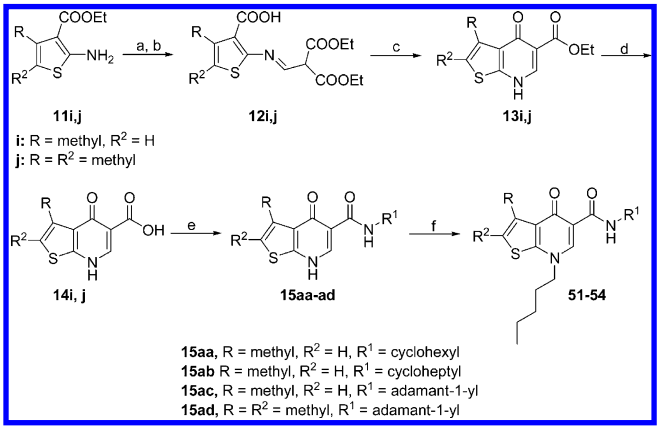
Scheme 2[a]



[a]Reagents: (a) diethyl ethoxymethylenemalonate, 120 °C, 2 h; (b) diphenyl ether, 250 °C, 3 h; (c) K_2CO_3, DMF, 1-pentyl bromide, 100 °C, 16 h; (d) NaOH 10%, 6 h, rt; (e) amine, EDC, HOBt, DMF, 16 h, rt *or* amine, HBTU, DIEA, DMF, 16 h, rt.

The thieno[2,3-*b*]pyridine-4-one derivatives were synthesized according to the reported procedure shown in Scheme 3.

Scheme 3[a]

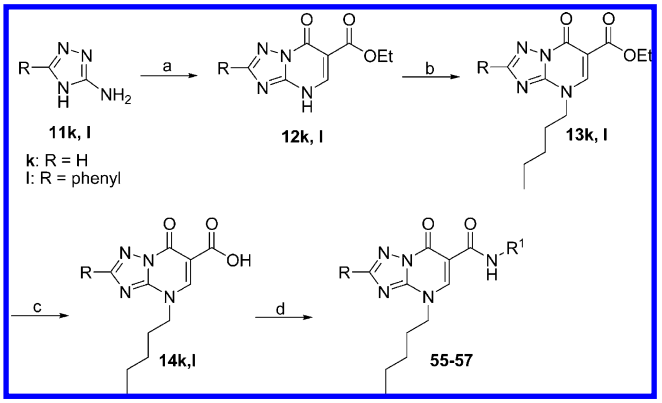


[a]Reagents: (a) diethyl ethoxymethylenemalonate, 2 h, 120 °C; (b) KOH−EtOH, dioxane, 10 min, 70 °C, then 30 min, rt; (c) PPA, 120 °C, 1 h; (d) NaOH 10%, 6 h, rt; (e) amine, EDC, HOBt, DMF, 16 h, rt *or* amine, HBTU, DIEA, DMF, 16 h, rt; (f) K_2CO_3, DMF, 1-pentyl bromide, 16 h, 100 °C.

Reaction of aminothiophenes **11i,j**,[32] prepared from the corresponding ketones using Gewald's methodology, with diethyl ethoxymethylenemalonate followed by selective alkaline hydrolysis of the 3-ethyl ester gave the intermediates **13i,j**.[33] The synthesis of target compounds **51**−**54** was accomplished using a procedure similar to that utilized for preparing the pyrazolopyridinones **16**−**47**.

The synthesis of 1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxamide analogues **55**−**57** is outlined in Scheme 4. The starting 4*H*-1,2,4-triazol-3-amine **11k** was available commercially, while 5-phenyl-4*H*-1,2,4-triazol-3-amine **11l** was prepared by a reported procedure.[34] Refluxing with diethyl ethoxymethylenemalonate in glacial acetic acid afforded 7-oxo-4,7-dihydro-1,2,4-triazolo[1,5-*a*]pyrimidine-6-carboxylic acid ethyl esters **12k,l**.[35] Alkylation of the resulting ester at the N4 position with 1-bromopentane in dry DMF and K_2CO_3 and subsequent hydrolysis of the ester function afforded compounds **14k,l**, which were transformed into the target compounds **55**−**57** in accordance with the previously described synthetic approaches.

Scheme 4[a]



[a]Reagents: (a) diethyl ethoxymethylenemalonate, CH_3COOH, 3 h, reflux; (b) K_2CO_3, DMF, 1-pentyl bromide, 16 h, 100 °C; (c) NaOH 10%, 6 h, rt; (d) amine, EDC, HOBt, DMF, 16 h, rt *or* amine, HBTU, DIEA, DMF, 16 h, rt.

RESULTS AND DISCUSSION

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 (Table 1). These findings suggest a high degree of homology between the human and rat CB_2 receptors and their respective binding sites. While translation of these binding results from rodent to man will require a consideration of numerous other factors, including receptor distribution, signaling pathways, and ADME parameters, evaluation in rat models of inflammatory and/or neuropathic pain can be considered without undue concern for differences in receptor affinity. As noted previously, we hypothesized that replacement of the benzene nucleus of the 4-oxo-1,4-dihydroquinoline-3-carboxamide system with a 1-methyl-5-substituted pyrazolo nucleus would afford novel and selective CB_2 receptor agonists. The initial choice of the cyclohexylcarboxamide group in position 5 and the 1-pentyl chain substituent in position 7 (compound **16**) was based upon the binding results obtained for other cannabinoid pharmacophores. This compound displayed modest affinity and slight selectivity for the cannabinoid CB_2 receptor (hCB_2 K_i = 85 nM; hCB_1 K_i = 500 nM, SI = 5.9). Subsequently, the adopted strategy was to prepare analogues by stepwise introduction of structural modifications on positions 3, 5, and 7 of the pyrazolopyridine template of compound **16** to define the correct structural requirements for binding to the CB_2 cannabinoid receptor.

The structural modifications in position 7 consisted of a set of nine compounds (**17**−**25**, Table 1) synthesized on the basis of previously reported work.[27,36] The 7-allyl derivative (**19**) showed a complete loss of affinity for both CB receptors. The same activity profile was found by introduction of an ethoxyethyl or a cyanobutyl substituent (**20** and **21**, respectively), revealing that the affinity is quite sensitive to

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modifications at the 7-position of the pyrazolopyridine nucleus. Compounds **23** and **24**, bearing benzyl and 4-methylbenzyl substituents, respectively, also exhibited a marked decrease or a complete loss of affinity at both CB receptors. Introduction of a 2-(morpholin-4-yl)ethyl chain (**25**), mimicking the morpholinoethyl substituent found in WIN-55,212-2 (**6**), also resulted in an inactive compound at the CB_2 and CB_1 receptors. It is important to highlight that the benzyl and 2-(morpholin-4-yl)ethyl moieties are widely found in the structures of cannabinoid ligands with agonist properties.[25,27] Because the *n*-pentyl residue appears to be preferred, subsequent modifications of the 4-oxopyrazolopyridine-5-carboxamide nucleus were performed while keeping this N7-substituent constant.

The SAR studies were extended by examining modifications of the cyclohexyl ring on the carboxamide moiety at the 5-position. Compound **26**, bearing a methylene spacer, showed 4-fold lower affinity toward the CB_2 receptor and lower selectivity when compared with compound **16**. Substitution of the cyclohexylamide group at position 5 of the pyrazolopyridine nucleus with 4-methylcyclohexylamide (compounds **27**, **28**) afforded compounds with higher affinity at the CB_2 receptor (hK_i of 11.4 and 10.6 nM, respectively). Indeed, these compounds proved to have some of the highest CB_2 receptor affinities in this series. A stereogenic preference is observed with the trans-isomer **27** exhibiting better selectivity against the CB_1 receptor (SI = 401) compared with the cis-isomer **28** (SI = 68). These data are not in accordance with results previously reported by Tuccinardi et al.,[36] that the cis-isomer of 4-methylcyclohexylcarboxamides synthesized by them showed increases of affinity for the CB_2 receptor when compared with the corresponding *trans*-isomers.

Replacing the cyclohexyl moiety of compound **16** with cycloheptyl, adamantyl, or 3,5-dimethyladamantyl led to a marked increase in affinity, as shown by compounds **29**, **30**, and **31**. In addition to compounds **27** and **28**, these were among the most potent analogues tested, with comparable potencies (hK_i values of 11.2, 9.5, and 9.8 nM respectively), although the highest selectivity among the three against the CB_1 receptor was observed with the most lipophilic compound **31** (SI = 107). Replacing the cyclohexyl moiety with a noncyclic, yet lipophilic chain such as the (1,4-dimethylpentyl)carboxamide induced a dramatic reduction in affinity toward CB_2 receptors (**32**, hK_i = 212 nM), with a concomitant reduction in selectivity.

Replacement of the cyclohexyl group of derivative **16** by the aromatic phenyl moiety (**33**) resulted in a 6-fold decreased affinity for the CB_2 receptor. Substitution on the aromatic moiety in the para-position led to a complete loss of affinity for cannabinoid receptors, as shown by the 4-methoxyphenyl and 4-fluorophenyl derivatives (**34**, **35**, respectively, hK_i > 10 000 nM). Introduction of a methylene spacer between the carboxamide nitrogen and the phenyl ring (compound **36**) afforded a similar result.

Within this series of 4-oxopyrazolopyridine-5-carboxamides, we also examined the effect of structural modifications at the 3-position of the pyrazolopyridine nucleus on receptor affinity and selectivity (compounds **37**−**47**). The 3-ethyl analogues bearing a cyclohexyl (**37**) or adamant-1-yl (**38**) carboxamide displayed lower affinity and poor selectivity at the CB receptors when compared with the parent 3-methyl compounds (**16** and **30**, respectively). Similarly, replacement of the 3-methyl group with a bulky *tert*-butyl moiety, as in compounds **39** and **40**, led to decreased affinity (hK_i values of 3526 and 363 nM, respectively).

Replacing the methyl group at position 3 by a hydrogen yielded compounds **41**−**43**, bearing the cyclohexyl, cycloheptyl, and adamantyl carboxamide groups, respectively. Although these compounds showed decreased affinity for the CB_2 receptor relative to the corresponding 3-methyl compounds (**16**, **29**, and **30**, respectively), they each showed increased selectivity, suggesting an exploitable difference in the bindings sites of the CB_2 and CB_1 receptors. In particular, the adamant-1-ylamide **43** was one of the most potent compounds evaluated and the most selective compound in this series (hK_i =11.6 nM, SI = 432).

Aromatic substituents at the 3-position of the pyrazole, as in compounds **44**−**47**, led to a significant loss of affinity at both the CB_2 and CB_1 receptors (K_i > 10 000 nM for **44** and **45**). Interestingly, the 3-*p*-chlorophenyl derivatives **46** and **47** displayed a slight affinity for the CB_2 receptor. Compared to the 3-phenyl analogues (**44** and **45**), this may suggest that the steric intolerance at the 3-position can be at least partially overcome by electronic effects.

Replacement of the pyrazolo ring of the parent nucleus with differently substituted 5-membered heterocycles allowed us to further extend these SAR studies. In particular various isoxazolo, thiophene, and triazolo derivatives were prepared. The isoxazolo[5,4-*b*]pyridines **48**−**50** each included an *N*7-pentyl chain, a 3-methyl group, and a cyclohexyl, cycloheptyl, or adamantyl susbstituent on the 5-carboxamide group, respectively. Optimal affinity within this group of compounds was obtained with the cyclohexyl moiety on the carboxamide chain (compound **48**, hCB_2 K_i = 8.74 nM, SI = 17.7), which showed significantly greater affinity and selectivity than the corresponding 4-oxopyrazolopyridine derivative (**16**). In contrast, neither the *N*-cycloheptyl (**49**) nor the *N*-adamantyl (**50**) derivatives showed the same relative enhancement in affinity. Although there was an apparent increase in selectivity for the 5-adamantyl derivative **50** (hCB_2 K_i = 21 nM, SI = 69.9), the cycloheptyl derivative (**49**) displayed lower affinity and selectivity relative to analogue **29** (hCB_2 K_i = 18.5 nM, SI = 7.8 vs hCB_2 K_i = 11.2 nM, SI = 23).

All of the thieno[2,3-*b*]pyridine derivatives (**51**−**54**) showed high affinity at the CB receptors (hK_i values from 1.12 to 12.3 nM for the CB_2 receptor). In this group, the highest affinities were obtained with the adamant-1-ylamides (compounds **53**, **54** hK_i = 1.12, 3.27 nM respectively), in analogy to the pyrazolopyridine series, although there was an apparent decrease in selectivity.

Evaluation of the 4-pentyl[1,2,4]triazolo[1,5-*a*]pyrimidine carboxamides **55**−**57** showed further improvements in receptor affinity and selectivity relative to the adamantyl derivatives **30**, **38**, **43**, **50**, **53**, **54**, although the compound with a cyclohexylamide (**55**) lacked sufficient solubility in DMSO/water mixtures to permit evaluation. In particular, the *N*-adamantan-1-yl-4,7-dihydro-7-oxo-4-pentyl-2-phenyl[1,2,4]-triazolo[1,5-*a*]pyrimidine-6-carboxamide (compound **57**) proved to have the greatest affinity and selectivity in this group for the CB_2 receptor, with K_i = 3.58 nM and SI = 146.

We also evaluated the efficacy (E_{max}), expressed as a percentage relative to that of WIN 55,212-2 representing the full CB agonist with E_{max} of 100. The majority of the novel compounds (from **17** to **43** and **56**) showed moderate potency, inhibiting adenylate cyclase activity with EC_{50} values between 41 and 3824 nM. Interestingly, most of these compounds reveal

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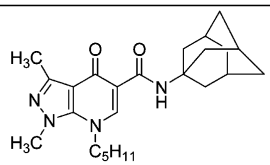
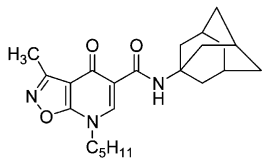
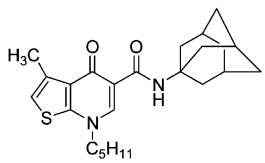
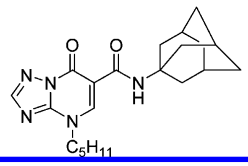
Table 2. Potency (EC_{50}, nM), Efficacies (E_{max}, %) in hCB_2CHO Cells on Cyclic AMP Assays and CLogP of the Novel CB Compounds 16−57

compd	hCB_2 EC_{50} (nM)[a]	E_{max} (%)[b]	CLogP[c]	compd	hCB_2 EC_{50} (nM)[a]	E_{max} (%)[b]	CLogP[c]
6	14.8 ± 1.2	100	4.28	**37**	2348 ± 227	32 ± 2	3.73
16	323 ± 29	52 ± 5	3.20	**38**	162 ± 14	51 ± 4	4.55
17	1895 ± 193	38 ± 3	2.66	**39**			4.42
18			2.13	**40**	1764 ± 167	43 ± 3	5.25
19	587 ± 52	58 ± 4	1.97	**41**	872 ± 79	48 ± 5	2.74
20			1.61	**42**	404 ± 42	56 ± 5	3.30
21			1.23	**43**	41 ± 4	67 ± 6	3.56
22	1203 ± 117	27 ± 2	3.73	**44**			4.34
23	3824 ± 392	36 ± 3	3.41	**45**			4.91
24			3.09	**46**			4.91
25			0.83	**47**			5.48
26	1562 ± 149	41 ± 3	3.73	**48**	14.3 ± 1.2	98 ± 7	2.69
27	58 ± 6	14 ± 1	3.69	**49**	35 ± 4	99 ± 6	3.25
28	52 ± 5	16 ± 1	3.69	**50**	83 ± 8	97 ± 8	3.51
29	51 ± 6	65 ± 6	3.76	**51**	107 ± 10	−64 ± 5	4.50
30	49 ± 5	68 ± 6	4.02	**52**	65 ± 6	−95 ± 9	5.07
31	45 ± 5	71 ± 6	4.98	**53**	31 ± 3	−167 ± 15	5.33
32	827 ± 75	46 ± 4	3.96	**54**	34 ± 3	−178 ± 16	5.79
33	2159 ± 248	38 ± 3	3.42	**55**			2.12
34			3.37	**56**	87 ± 9	60 ± 5	2.94
35			3.87	**57**			5.38
36			3.09				

[a]EC_{50} values were calculated on cAMP experiments performed on human CB_2 CHO cells. [b]Efficacies (E_{max}) for CB_2 of the examined compounds are expressed as a percentage relative to the efficacy of the reference compound WIN 55,212-2 at the 10 μM. E_{max} of ∼100% indicated that the compounds behave as full agonists. E_{max} between 0% and 100% indicated that the compounds behave as partial agonists. Negative E_{max} indicated that the compounds behave as inverse agonists. [c]CLogP (calculated logarithm of the partition coefficient between *n*-octanol and water) values were obtained using ACDLabs/ChemSketch.

Table 3. Comparison between Direct Analogues of Four Core Series

Com	Chemical Structure	EC_{50}	Emax (%)	Functional Class
30		49 ± 5	68 ± 6	Partial Agonist
50		83 ± 8	97 ± 8	Full Agonist
53		31 ± 3	-167 ±15	Inverse Agonist
56		87 ± 9	60 ± 5	Partial Agonist

a low E_{max} activity, suggesting they act as partial agonists (Table 1). As can be seen from Tables 2 and 3, the different structural classes display different behaviors as full agonist, partial agonist, and inverse agonist. The pyrazolopyridines **16**−**47** and

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triazolopyrimidine **56** were found to act as partial agonists, while the thienopyridines **51**−**54** act as inverse agonists. Interestingly, the isoxazolopyridines **48**−**50** act as full agonists. Thus, it appears that the core structure of these molecules defines the type of activity to be seen, while the substituents around the core structure can be used to modulate the potency of that activity. This finding is unexpected in light of the recent findings of Pasquini, et al., where substituted 6-arylquinolones and 5-arylindoles were compared as ligands of the CB_2 receptor. In this case, the authors suggested that the two heterocyclic systems simply act as scaffolds to simply orient the substituents in 3D space, with both classes of compounds exerting the same functional effects.[37] The calculation of log *P* of the synthesized compounds (Table 2) has indicated the absence of a significant correlation between the lipophilicity and biological activities of this series.

CONCLUSION

The purpose of this study was to design and synthesize a novel chemical class of compounds as selective ligands for CB_2 receptor. In this effort, we have discovered a new series of heteroarylpyridine/heteroarylpyrimidine derivatives that were tested on CB_1 and CB_2 receptors. In light of the results presented, this represents a new class of heterocyclic derivatives acting as potent CB_2 receptor partial agonists. The best affinity values were obtained with compounds **30**, **31**, **48**, **52**−**54** (1.12 nM < hCB_2 K_i < 9.83 nM) bearing a lipophilic carboxamido substituent. Compounds containing aromatic moieties on the amide resulted in a decreased affinity for the CB_2 cannabinoid receptors, as shown by **33**−**36**. The preferred N7 side chain was the 1-pentyl moiety. Taken together, the present data could be a suitable starting point for the optimization of novel CB_2 receptor ligands as high quality leads.

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.[38]

To investigate CB_2 receptors, [^{3}H]CP-55,940 binding assay was performed by using 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, 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.[38]

Human CB_1 and CB_2 receptors expressed in CHO cells 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.[39−41] For membrane preparation, 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, 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 receptors, 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 to 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 phosphodiesterase inhibitor was added and the mixture preincubated for 10 min in a shaking bath at 37 °C.[42] The potency of the novel CB compounds was studied in the presence of 1 μM forskolin 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. After the addition of charcoal, the samples 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 with bovine albumin as reference standard. Inhibitory binding constants, K_i, were calculated from the EC_{50} values according to the Cheng and Prusoff equation:[43] $K_i = EC_{50}/(1 + [C^*]/K_D^*)$, where $[C^*]$ is the concentration of the radioligand and K_D^* its dissociation constant. A weighted nonlinear least-squares curve fitting program, LIGAND,[44] was used for computer analysis of the inhibition experiments. All the data are expressed as the mean ± SEM of $n = 4$ independent experiments for the in vitro assays. Statistical analysis of the data was performed using unpaired two-sided Student's *t* test.

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 Chemistry Department 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). The 5-aminopyrazoles were prepared by known procedures: 1,3-dimethyl-1*H*-pyrazol-5-amine (**11a**),[45] 3-ethyl-1-methyl-1*H*-pyrazol-5-amine (**11b**),[46] 3-*tert*-butyl-1-methyl-1*H*-pyrazol-5-amine (**11c**),[47] 1-methyl-3-phenyl-1*H*-pyrazol-5-amine (**11d**),[48] 3-(4-chlorophenyl)-1-methyl-1*H*-pyrazol-5-amine (**11e**),[49] 1-methyl-1*H*-pyrazol-5-amine (**11f**).[30]

Synthesis of 2-[(Pyrazol-3-ylamino)methylene]malonic Acid Diethyl Ester (12a−f) and 2-[(Isoxazol-5-ylamino)methylene]-malonic Acid Diethyl Ester (12g). *General Procedure.* A mixture of the appropriate pyrazol-5-amines **11a−f** or isoxazol-5-amine **11g** (10 g, 0.09 mol) and diethyl ethoxymethylenemalonate (19.4 g, 0.09 mol) was heated at 120 °C for 2 h with stirring. The reaction mixture was poured into *n*-hexane, and the precipitate was collected by filtration to give the desired compounds **12a−g**.

Diethyl 2-[(1,3-Dimethyl-1*H*-pyrazol-5-ylamino)methylene]-malonate (12a). Yield: 86%. ^{1}H NMR (DMSO-d_6): δ 10.50 (bd, J = 13.4 Hz, 1H), 8.79 (d, J = 13.4 Hz, 1H), 6.06 (s, 1H), 4.23 (q, J = 7.2 Hz, 2H), 4.09 (q, J = 7.2 Hz, 2H), 3.63 (s, 3H), 2.10 (s, 3H), 1.30 (t, J = 7.2 Hz, 3H), 1.22 (t, J = 7.2 Hz, 3H).

Diethyl 2-[(3-Ethyl-1-methyl-1*H*-pyrazol-5-ylamino)-methylene]malonate (12b). Yield: 82%. ^{1}H NMR (DMSO-d_6): δ 11.00 (bd, J = 12.8 Hz, 1H), 8.15 (d, J = 12.8 Hz, 1H), 5.87 (s, 1H), 4.27 (m, 4H), 3.79 (s, 3H), 2.57 (q, J = 7.6 Hz, 2H), 1.36−1.17 (m, 9H).

Diethyl 2-[(3-*tert*-Butyl-1-methyl-1*H*-pyrazol-5-ylamino)-methylene]malonate (12c). Yield: 50%. ^{1}H NMR (DMSO-d_6): δ 10.98 (bd, J = 12.6 Hz, 1H), 8.12 (d, J = 12.6 Hz, 1H), 5.90 (s, 1H), 4.27 (m, 4H), 3.79 (s, 3H), 1.43 (s, 9H), 1.39−1.22 (m, 6H).

Diethyl 2-[1-Methyl-1*H*-pyrazol-5-ylamino)methylene]-malonate (12d). Yield: 77%. ^{1}H NMR (DMSO-d_6): δ 10.49 (bd, J = 12.6 Hz, 1H), 7.98 (bd, J = 12.6 Hz, 1H), 7.35 (d, J = 2.0 Hz, 1H), 6.28 (d, J = 2.0 Hz, 1H), 4.12 (m, 4H), 4.01 (s, 3H), 1.27−1.13 (m, 6H).

Diethyl 2-[(3-Phenyl-1-methyl-1*H*-pyrazol-5-ylamino)-methylene]malonate (12e). Yield: 77%. ^{1}H NMR (DMSO-d_6): δ 10.56 (bs, 1H), 8.07 (s, 1H), 7.81−7.77 (m, 2H), 7.34−7.29 (m, 3H), 6.82 (s, 1H), 4.16 (m, 4H), 3.77 (s, 3H), 1.25 (m, 6H).

Diethyl 2-[(3-(4-Chlorophenyl)-1-methyl-1*H*-pyrazol-5-ylamino)methylene]malonate (12f). Yield: 87%. ^{1}H NMR (DMSO-d_6): δ 10.60 (bs, 1H), 8.12 (bs, 1H), 7.80 (dd, J = 8.6 Hz, 2H), 7.45 (dd, J = 6.8 Hz, 2H), 6.85 (s, 1H), 4.12 (m, 4H), 3.76 (s, 3H), 1.24−1.19 (m, 6H).

Diethyl 2-[(3-Methylisoxazol-5-ylamino)methylene]-malonate (12g). Yield: 90%. ^{1}H NMR (DMSO-d_6): δ 11.09 (bs, 1H), 8.10 (s, 1H), 5.95 (s, 1H), 4.23−4.12 (m, 4H), 2.17 (s, 3H), 1.28−1.20 (m, 6H).

Synthesis of (*E*)-2-((3-Ethoxy-2-(ethoxycarbonyl)-3-oxo-prop-1-en-1-yl)amino)-4-methylthiophene-3-carboxylic Acid (12i) and (*E*)-2-((3-Ethoxy-2-(ethoxycarbonyl)-3-oxoprop-1-en-1-yl)amino)-4,5-dimethylthiophene-3-carboxylic Acid (12j). A mixture of **11i** or **11j**[32] (5.4 mmol) and diethyl ethoxymethylenemalonate (1.4 g, 6 mmol) was stirred at 120 °C for 2 h. After being cooled, the mixture was triturated with Et_2O. The yellow precipitate was collected by filtration, washed with ice-cold Et_2O, and dried to give the corresponding triester compounds (85%) as yellow crystalline powders. A solution of KOH (1.5 g, 26.7 mmol) in EtOH (10 mL) was added to a solution of the triester compound in dioxane (15 mL) at 70 °C. After being stirred at 70 °C for 10 min, the mixture was stirred at room temperature for a further 30 min, then acidified with 2 N HCl. The resulting suspension was concentrated in vacuo and the precipitate was collected by filtration and purified by crystallization (ethanol/water) to yield **12i** (93%) and **12j** (95%) as yellow solids.

12i. Mp 186−187 °C. ^{1}H NMR (DMSO-d_6): δ 13.38 (bs, 1H), 12.34 (bd, J = 13.4 Hz, 1H), 8.08 (d, J = 13.6 Hz, 1H), 6.72 (q, ^4J = 1 Hz, 1H), 4.28−4.09 (m, 4H), 2.29 (d, ^4J = 1.2 Hz, 3H), 1.30−1.20 (m, 6H).

12j. Mp 90 °C (dec). ^{1}H NMR (DMSO-d_6): δ 13.25 (bs, 1H), 12.25 (d, J = 13.5 Hz, 1H), 8.15 (d, J = 13.6 Hz, 1H), 4.34−4.25 (m, 4H), 2.28 (s, 6H), 1.39−1.29 (m, 6H).

Synthesis of Ethyl 4,7-Dihydro-7-oxo[1,2,4]triazolo[1,5-*a*]-pyrimidine-6-carboxylate (12k) and Ethyl 4,7-Dihydro-7-oxo-2-phenyl[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (12l). A mixture of **11k** or **11l**[34] (3.1 mmol), glacial acetic acid (5 mL), and diethyl ethoxymethylene malonate (1 g, 4.6 mmol) was heated at reflux for 3 h. After the mixture was cooled, the white precipitate was collected by filtration, washed with ice-cold water, and dried to give the corresponding ester compounds **12k,l** (75%) as a white crystalline powder.

12k. Mp > 300 °C. ^{1}H NMR (DMSO-d_6): δ 14.30 (bs, 1H), 8.95 (s, 1H), 8.84 (s, 1H), 4.20 (q, J = 6.8 Hz, 2H), 1.29 (t, J = 6.8 Hz, 3H).

12l. Mp > 300 °C. ^{1}H NMR (DMSO-d_6): δ 14.00 (bs, 1H), 8.64 (s, 1H), 8.14−8.10 (m, 2H), 7.55 (m, 3H), 4.26 (q, J = 7.0 Hz, 2H), 1.28 (t, J = 6.8 Hz, 3H).

Ethyl 4-Oxo-4,7-dihydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylates (13a−f) and Ethyl 4,7-Dihydro-3-methyl-4-oxoisoxazolo[5,4-*b*]pyridine-5-carboxylate (13g). *General Procedure.* A solution of the appropriate diethyl [(pyrazol-5-ylamino)-methylene]malonate **12a−f** or diethyl [(isoxazol-5-ylamino)-methylene]malonate **12g** (8.89 mmol) in diphenyl ether (4 mL) was heated at 250 °C for 3 h. After the mixture was cooled to room temperature, the ethanol formed was removed under reduced pressure, leaving the crude product as an oily residue. The residue was purified by column chromatography on silica using a mixture of ethyl acetate/petroleum ether as eluent.

Ethyl 1,3-Dimethyl-4,7-dihydro-4-oxo-1*H*-pyrazolo[3,4-*b*]-pyridine-5-carboxylate (13a). White solid, mp 110 °C. Yield: 65%. ^{1}H NMR ($CDCl_3$): δ 12.20 (bs, 1H), 8.81 (s, 1H), 4.44 (q, J = 7.0 Hz, 2H), 4.01 (s, 3H), 2.65 (s, 3H), 1.43 (t, J = 7.0 Hz, 3H).

Ethyl 3-Ethyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (13b). White solid, mp 77 °C. Yield: 82%. ^{1}H NMR ($CDCl_3$): δ 12.24 (bs, 1H), 8.81 (s, 1H), 4.42 (q, J = 7.2 Hz, 2H), 4.01 (s, 3H), 3.04 (q, J = 7.6 Hz, 2H), 1.46−1.23 (m, 6H).

Ethyl 3-*tert*-Butyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxylate (13c). White solid, mp 156 °C. Yield: 85%. ^{1}H NMR ($CDCl_3$): δ 12.24 (bs, 1H), 8.71 (s, 1H), 4.40 (q, J = 7.0 Hz, 2H), 3.92 (s, 3H), 1.43 (s, 9H), 1.32 (m, 3H).

Ethyl 4,7-Dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]-pyridine-5-carboxylate (13d). White solid, mp 139 °C. Yield: 48%. ^{1}H NMR (DMSO-d_6): δ 12.55 (bs, 1H), 8.73 (s, 1H), 8.25 (s, 1H), 4.37 (q, J = 7.2 Hz, 2H), 4.00 (s, 3H), 1.35 (t, J = 7.2 Hz, 3H).

Ethyl 4,7-Dihydro-1-methyl-4-oxo-3-phenyl-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxylate (13e). Pale white solid, mp 110 °C. Yield: 65%. ^{1}H NMR (DMSO-d_6): δ 12.60 (bs, 1H), 8.76 (s, 1H), 8.05−8.00 (m, 2H), 7.48−7.42 (m, 3H), 4.44 (q, J = 7 Hz, 2H), 4.05 (s, 3H), 1.37 (t, J = 7.2 Hz, 3H).

Ethyl 3-(4-Chlorophenyl)-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylate (13f). White solid, mp 172−173 °C. Yield: 72%. ^{1}H NMR ($CDCl_3$): δ 12.55 (bs, 1H), 8.75 (s, 1H), 8.05 (dd, J = 8.6 Hz, 2H), 7.48 (dd, J = 8.6 Hz, 2H), 4.35 (q, J = 7.2 Hz, 2H), 3.99 (s, 3H), 1.30 (m, 3H).

Ethyl 4,7-Dihydro-3-methyl-4-oxoisoxazolo[5,4-*b*]pyridine-5-carboxylate (13g). Pale yellow solid, mp 151 °C. Yield 45%. ^{1}H NMR (DMSO-d_6): δ 12.40 (bs, 1H), 8.80 (s, 1H), 4.39 (q, J = 7.0 Hz, 2H), 2.58 (s, 3H), 1.35 (t, J = 7.0 Hz, 3H).

Synthesis of Oxothienopyridine Carboxylate Derivatives (13i−j). *General Procedure.* A mixture of the required diethyl ester (**12i** or **12j**, 6 mmol) and polyphosphoric acid (10 g) was heated at

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140 °C for 1 h. The mixture was poured into ice and water to form a precipitate that was collected by filtration and washed with cold water.

Ethyl 4,7-Dihydro-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (13i). White solid, mp 189−190 °C (dec). Yield: 65%. ^{1}H NMR (DMSO-d_6): δ 12.04 (bs, 1H), 8.87 (s, 1H), 7.22 (q, ^4J = 1.4 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 2.52 (d, ^4J = 1.4 Hz, 3H), 1.35 (t, J = 7.0 Hz, 3H).

Ethyl 4,7-Dihydro-2,3-dimethyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylate (13j). White solid, mp 178−180 °C (dec). Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 12.01 (bs, 1H), 8.77 (s, 1H), 4.46 (q, J = 7.2 Hz, 2H), 2.51 (s, 3H), 2.43 (s, 3H), 1.44 (t, J = 7.0 Hz, 3H).

Synthesis of Carboxylic Acid Derivatives (14a−f, 14h−l). *General Procedure.* A suspension of the appropriate carboxylic acid ethyl ester **13a−f**, **13i−1**, **14g** (1.27 mmol) in 20 mL of 10% aqueous sodium hydroxide was stirred at room temperature for 6 h. After cooling, the suspension was adjusted to pH 4 with 2 N HCl. The resulting precipitate was collected by filtration, washed with H_2O, and recrystallized from ethanol/water.

4,7-Dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]-pyridine-5-carboxylic Acid (14a). White solid, mp 237−238 °C. Yield: 99%. ^{1}H NMR (DMSO-d_6): δ 15.30 (bs, 1H), 12.23 (bs, 1H), 8.54 (s, 1H), 3.90 (s, 3H), 2.48 (s, 3H).

4,7-Dihydro-3-ethyl-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]-pyridine-5-carboxylic Acid (14b). White solid, mp 235−236 °C. Yield: 80%. ^{1}H NMR (DMSO-d_6): δ 15.80 (bs, 1H), 12.53 (bs, 1H), 8.51 (s, 1H), 3.94 (s, 3H), 2.89 (q, J = 7.6 Hz, 2H), 1.25 (t, J = 7.6 Hz, 3H).

3-*tert*-Butyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]-pyridine-5-carboxylic Acid (14c). White solid, mp 225 °C. Yield: 95%. ^{1}H NMR (DMSO-d_6): δ 15.90 (bs, 1H), 13.83 (bs, 1H), 8.59 (s, 1H), 3.91 (s, 3H), 1.41 (s, 9H).

4,7-Dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid (14d). White solid, mp 236 °C (dec). Yield: 92%. ^{1}H NMR (DMSO-d_6): δ 15.10 (bs, 1H), 12.55 (bs, 1H), 8.65 (s, 1H), 8.19 (s, 1H), 4.00 (s, 3H).

4,7-Dihydro-1-methyl-4-oxo-3-phenyl-1*H*-pyrazolo[3,4-*b*]-pyridine-5-carboxylic Acid (14e). Pale yellow solid, mp 235 °C. Yield: 78%. ^{1}H NMR (DMSO-d_6): δ 14.51 (bs, 1H), 12.60 (bs, 1H),8.67 (s, 1H), 8.22−8.18 (m, 2H), 7.48−7.41 (m, 3H), 4.04 (s, 3H).

3-(4-Chlorophenyl)-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxylic Acid (14f). White solid, mp > 300 °C. Yield: 88% . ^{1}H NMR (DMSO-d_6): δ 15.30 (bs, 1H), 12.50 (bs, 1H), 8.64 (s, 1H), 8.28 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 4.05 (s, 3H).

4,7-Dihydro-3-methyl-4-oxo-7-pentylisoxazolo[5,4-b]-pyridine-5-carboxylic Acid (14h). White solid, mp 155 °C. Yield: 88%. ^{1}H NMR (DMSO-d_6): δ 14.78 (bs, 1H), 8.91 (s, 1H), 4.36 (t, J = 7 Hz, 2H), 2.56 (s, 3H), 1.85−1.78 (m, 2H), 1.30−1.27 (m, 4H), 0.86 (t, J = 6.8 Hz, 3H).

4,7-Dihydro-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylic Acid (14i). White solid, mp 260−261 °C (dec). Yield: 89%. ^{1}H NMR (DMSO-d_6): δ 15.98 (bs, 1H), 14.01 (bs, 1H), 8.76 (s, 1H), 7.15 (d, ^4J = 1.2 Hz, 1H), 2.55 (d, ^4J = 1.2 Hz, 3H).

4,7-Dihydro-2,3-dimethyl-4-oxothieno[2,3-*b*]pyridine-5-carboxylic Acid (14j). White solid, mp 254−255 °C (dec). Yield: 80%. ^{1}H NMR (DMSO-d_6): δ 16.01 (bs, 1H), 13.95 (bs, 1H), 8.71 (s, 1H), 2.46 (s, 3H), 2.37 (s, 3H).

4,7-Dihydro-7-oxo-4-pentyl[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylic Acid (14k). White solid, mp 215 °C (dec). Yield: 75%. ^{1}H NMR (DMSO-d_6): δ 12.10 (bs, 1H), 8.74 (s, 1H), 8.24 (s, 1H), 4.43 (q, J = 7.0 Hz, 2H), 1.82 (m, 2H), 1.36−1.29 (m, 4H), 0.88 (t, J = 6.6 Hz, 3H).

4,7-Dihydro-7-oxo-4-pentyl-2-phenyl[1,2,4]triazolo[1,5-*a*]-pyrimidine-6-carboxylic Acid (14l). White solid, mp > 300 °C (dec). Yield: 75%. ^{1}H NMR (DMSO-d_6): δ 12.90 (bs, 1H), 8.93 (s, 1H), 8.17 (m, 2H), 7.55 (m, 3H), 4.34 (q, J = 7.0 Hz, 2H), 1.89 (m, 2H), 1.36−1.29 (m, 4H), 0.88 (t, J = 6.6 Hz, 3H).

Synthesis of Carboxamide Derivatives (15a−e, 15h−m, 15o, 15q,r, 15t−w, 15aa,ab, 48, 49, 55). *General Procedure.* A solution of the appropriate carboxylic acid (0.48 mmol), 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide (0.52 mmol), and 1-hydroxybenzotriazole (0.52 mmol) in anhydrous dimethylformamide (5 mL) was stirred at room temperature for 10 min. The appropriate amine (0.72 mmol) was added, and stirring continued for 16 h at ambient temperature. The reaction mixture was concentrated in vacuo, then purified by column chromatography on silica gel, eluting with ethyl acetate/methanol unless otherwise noted.

***N*-Cyclohexyl-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxamide (15a).** White solid, mp 105 °C. Yield: 73%. ^{1}H NMR (DMSO-d_6): δ 12.90 (bs, 1H), 10.10 (bd, J = 7.8 Hz, 1H), 8.36 (s, 1H), 3.95 (m, 1H), 3.81 (s, 3H), 2.40 (s, 3H), 1.82−1.46 (m, 10H).

***N*-(Cyclohexylmethyl)-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15b).** White solid, mp 200 °C. Yield: 36%. ^{1}H NMR (DMSO-d_6): δ 12.30 (bs, 1H), 10.10 (bt, J = 7.8 Hz, 1H), 8.42 (s, 1H), 3.83 (s, 3H), 3.81 (m, 1H), 3.14 (t, J = 6.6 Hz, 2H), 2.45 (s, 3H), 1.73−1.18 (m, 10H).

***trans*-4,7-Dihydro-1,3-dimethyl-*N*-(4-methylcyclohexyl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15c) and *cis*-4,7-Dihydro-1,3-dimethyl-*N*-(4-methylcyclohexyl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15d).** The separation of cis and trans isomers was obtained by flash chromatography on silica gel using ethyl acetate/petroleum ether (9:1) as eluent.

15c. Trans-isomer. White solid, mp 270 °C (dec). Yield: 22%. ^{1}H NMR (DMSO-d_6): δ 13.02 (bs, 1H), 9.92 (bd, J = 7.8 Hz, 1H), 8.40 (s, 1H), 3.82 (s, 3H), 3.68 (m, 1H), 2.43 (s, 3H), 1.89−1.64 (m, 4H), 1.32−0.96 (m, 5H), 0.86 (d, J = 6.4 Hz, 3H).

15d. Cis-isomer. White solid, mp 252 °C (dec). Yield: 25% ^{1}H NMR (DMSO-d_6): δ 13.02 (bs, 1H), 10.27 (bd, J = 7.8 Hz, 1H), 8.39 (s, 1H), 4.03 (m, 1H), 3.82 (s, 3H), 2.45 (s, 3H), 1.68−1.51 (m, 7H), 1.19−1.12 (m, 2H), 0.90 (d, J = 6.4 Hz, 3H).

***N*-Cycloheptyl-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15e).** Pale white solid, mp 249 °C (dec). Yield: 65%. ^{1}H NMR (DMSO-d_6): δ 12.90 (bs, 1H), 10.10 (bd, J = 7.8 Hz, 1H), 8.40 (s, 1H), 4.20 (m, 1H), 3.83 (s, 3H), 2.45 (s, 3H), 1.90−1.54 (m, 12H).

4,7-Dihydro-1,3-dimethyl-*N*-(5-methylhexan-2-yl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15h). White solid, mp 289 °C. Yield: 25%. ^{1}H NMR (DMSO-d_6): δ 13.20 (bs, 1H), 9.88 (bd, J = 7.8 Hz, 1H), 8.43 (s, 1H), 3.90 (m, 1H), 3.82 (s, 3H), 2.51 (s, 3H), 1.51−1.41 (m, 3H), 1.11−1.20 (m, 5H), 0.87 (m, 6H).

4,7-Dihydro-1,3-dimethyl-4-oxo-*N*-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15i). White solid, mp 273 °C. Yield: 32%. ^{1}H NMR (DMSO-d_6): δ 13.20 (bs, 1H), 12.40 (bs, 1H), 8.54 (s, 1H), 7.72−7.76 (m, 2H), 7.38−7.30 (m, 2H), 7.10−7.06 (m, 1H), 3.88 (s, 3H), 2.54 (s, 3H).

4,7-Dihydro-1,3-dimethyl-*N*-(4-methoxyphenyl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15j). White solid, mp 171 °C. Yield: 40%. ^{1}H NMR (DMSO-d_6): δ 13.12 (bs, 1H), 12.35 (bs, 1 H), 8.54 (s, 1H), 7.63 (dd, J = 9 Hz, 2H), 6.92 (dd, J = 9 Hz, 2H), 3.87 (s, 3H), 3.74 (s, 3H), 2.50 (s, 3H).

4,7-Dihydro-1,3-dimethyl-*N*-(4-fluorophenyl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15k). White solid, mp > 300 °C. Yield: 40%. ^{1}H NMR (DMSO-d_6): δ ppm 13.12 (bs, 1H), 12.53 (bs, 1 H), 8.55 (s, 1H), 7.77−7.70 (m, 2H), 7.22−7.14 (m, 2H), 3.87 (s, 3H), 2.50 (s, 3H).

***N*-Benzyl-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15l).** White solid, mp 185−186 °C. Yield: 60%. ^{1}H NMR (DMSO-d_6): δ 13.20 (bs, 1H), 10.40 (bt, J = 6 Hz, 1H), 8.42 (s, 1H), 7.34−7.32 (m, 5H), 4.51 (d, J = 6.0 Hz, 2H), 3.84 (s, 3H), 2.45 (s, 3H).

***N*-Cyclohexyl-4,7-dihydro-3-ethyl-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15m).** White solid, mp 297 °C. Yield: 38%. ^{1}H NMR (DMSO-d_6): δ 13.20 (bs, 1H), 10.10 (bd, J = 7.8 Hz, 1H), 8.42 (s, 1H), 3.85 (s, 3H), 3.80 (m, 1H), 2.83 (q, J = 7.6 Hz, 2H), 1.65−1.29 (m, 10H), 1.22−1.18 (m, 3H).

3-*tert*-Butyl-*N*-cyclohexyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15o). White solid, mp 134−135 °C. Yield: 48%. ^{1}H NMR (DMSO-d_6): δ 13.00 (bs, 1H), 10.10 (bd, J = 7.8 Hz, 1H), 8.45 (s, 1H), 3.98 (m, 1H), 3.84 (s, 3H), 1.82−1.29 (m, 19H).

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***N*-Cyclohexyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15q).** White solid, mp 97−99 °C. Yield: 28%. ^{1}H NMR (DMSO-d_6): δ 13.30 (bs, 1H), 10.03 (bd, J = 7.6 Hz, 1H), 8.50 (s, 1H), 7.96 (s, 1H), 4.04 (m, 1H), 3.94 (s, 3H), 1.65−1.24 (m, 10H).

***N*-Cycloheptyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo-[3,4-*b*]pyridine-5-carboxamide (15r).** White solid, mp 214 °C. Yield: 40%. ^{1}H NMR (DMSO-d_6): δ 13.30 (bs, 1H), 10.03 (bd, J = 7.6 Hz, 1H), 8.50 (s, 1H), 7.96 (s, 1H), 3.95 (s, 3H), 3.92 (m, 1H), 1.74−1.23 (m, 12H).

***N*-Cyclohexyl-4,7-dihydro-1-methyl-4-oxo-3-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15t).** White solid, mp 235 °C. Yield: 64%. ^{1}H NMR (DMSO-d_6): δ 13.09 (bs, 1H), 10.10 (bd, J = 7.4 Hz, 1H), 8.53 (s, 1H), 8.21−8.17 (m, 2H), 7.50−7.38 (m, 3H), 4.00 (s, 3H), 3.81 (m, 1H), 1.83−1.30 (m, 10H).

***N*-Cycloheptyl-4,7-dihydro-1-methyl-4-oxo-3-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15u).** White solid, mp 228 °C (dec). Yield: 50%. ^{1}H NMR (DMSO-d_6): δ 13.10 (bs, 1H), 10.15 (bd, J = 7.6 Hz, 1H), 8.49 (s, 1H), 8.21−8.18 (m, 2H), 7.45−7.40 (m, 3H), 4.05−3.99 (m, 4H), 1.85−1.55 (m, 12H).

3-(4-Chlorophenyl)-*N*-cyclohexyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15v). White solid, mp149−150 °C. Yield: 45%. ^{1}H NMR (DMSO-d_6): δ 13.20 (bs, 1H), 10.06 (bd, J = 7.6 Hz, 1H), 8.50 (s, 1H), 8.24 (dd, J = 8.0 Hz, 2H), 7.52 (dd, J = 8.6 Hz, 2H), 3.95 (s, 3H), 3.79 (m, 1H), 1.87−1.29 (m, 10H).

3-(4-Chlorophenyl)-*N*-cycloheptyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15w). White solid, mp 154−155 °C. Yield: 40%. ^{1}H NMR (DMSO-d_6): δ 13.22 (bs, 1H), 10.03 (bd, J = 7.8 Hz, 1H), 8.52 (s, 1H), 8.24 (dd, J = 8.4 Hz, 2H), 7.52 (dd, J = 8.4 Hz, 2H), 4.02 (s, 3H), 3.98−3.96 (m,1 H), 1.85−1.54 (m, 12H).

***N*-Cyclohexyl-4,7-dihydro-3-methyl-4-oxothieno[2,3-*b*]-pyridine-5-carboxamide (15aa).** White solid, mp 247 °C (dec). Yield: 29%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (bs, 1H), 10.35 (bd, J = 7.8 Hz, 1H), 8.56 (s, 1H), 6.88 (q, J = 1.2 Hz, 1H), 3.91−3.78 (m, 1H), 2.52 (d, J = 1.2 Hz, 3H), 1.95−1.19 (m, 10H).

***N*-Cycloheptyl-4,7-dihydro-3-methyl-4-oxothieno[2,3-*b*]-pyridine-5-carboxamide (15ab).** White solid, mp 252 °C (dec). Yield: 25%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.17 (bs, 1H), 10.36 (bd, J = 8 Hz, 1H), 8.56 (s, 1H), 6.89 (q, J = 1.2 Hz, 1H), 4.01−3.97 (m, 1H), 2.52 (d, J = 1.2 Hz, 3H), 1.86−1.48 (m, 12H).

***N*-Cyclohexyl-4,7-dihydro-3-methyl-4-oxo-7-pentylisoxazolo[5,4-*b*]pyridine-5-carboxamide (48).** White solid, mp 187 °C. Yield: 71%. MS: m/z 346.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.83 (bd, J = 8.2 Hz, 1H), 8.63 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 3.82 (m, 1H), 2.52 (s, 3H), 1.85−1.22 (m, 16H), 0.85 (t, J = 6.8 Hz, 3H). Anal. ($C_{19}H_{27}N_3O_3$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-3-methyl-4-oxo-7-pentylisoxazolo[5,4-*b*]pyridine-5-carboxamide (49).** White solid, mp 180 °C. Yield: 75%. MS: m/z 360.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.83 (bd, J = 8.2 Hz, 1H), 8.62 (s, 1H), 4.28 (t, J = 7.2 Hz, 2H), 4.03 (m, 1H), 2.49 (s, 3H), 1.82−1.78 (m, 4H), 1.54 (m, 10H), 1.28−17 (m, 4H), 0.85 (t, J = 6.8 Hz, 3H). Anal. ($C_{20}H_{29}N_3O_3$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-7-oxo-4-pentyl[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (55).** White solid, mp 161 °C. Yield: 30%. MS: m/z 332.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.91 (s, 1H), 8.78 (bd, J = 7.8 Hz, 1H), 8.39 (s, 1H), 4.33 (t, J = 7.2 Hz, 2H), 3.97−3.79 (m, 1H), 1.86−1.25 (m, 16H), 0.86 (t, J = 6.6 Hz, 3H). Anal. ($C_{17}H_{25}N_5O_2$) C, H, N.

Synthesis of Adamantan-1-ylcarboxamides (15f, 15g, 15n, 15p, 15s, 15ac, 15ad, 50, 56, 57). *General Procedure.* To a stirred solution of the appropriate carboxylic acid (0.97 mmol) in anhydrous dimethylformamide (15 mL) was added diisopropylethylamine (5.42 mmol). The resulting solution was stirred at room temperature for 10 min before adding *o*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (1.45 mmol). The solution was stirred for an additional 3 h at room temperature, at which time 1-adamantylamine or 3,5-dimethyl-1-adamantylamine (1.45 mmol) was added. The mixture was then stirred at ambient temperature for an additional 16 h. After removal of the dimethylformamide under reduce pressure, the residue was dissolved in ethyl acetate (100 mL), then washed with saturated aqueous sodium bicarbonate (20 mL), water (10 mL), and brine (10 mL). The organic extract was dried (Na_2SO_4), filtered, and evaporated to dryness, and the residue was purified by flash chromatography on silica gel, eluting with ethyl acetate/petroleum ether.

***N*-(Adamantan-1-yl)-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15f).** White solid, mp 284−285 °C. Yield: 45%.^{1}H NMR (DMSO-d_6): δ 13.00 (bs, 1H), 10.23 (bs, 1H), 8.37 (s, 1H), 3.78 (s, 3H), 2.42 (s, 3H), 2.03 (s, 9H), 1.65 (s, 6H).

4,7-Dihydro-1,3-dimethyl-*N*-(3,5-dimethyladamantan-1-yl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15g). White solid, mp 241−242 °C. Yield: 42%. ^{1}H NMR (DMSO-d_6): δ 13.10 (bs, 1H), 10.26 (bs, 1H), 8.25 (s, 1H), 3.97 (s, 3H), 2.63 (s, 3H), 1.98−1.19 (m, 19H).

***N*-(Adamantan-1-yl)-4,7-dihydro-3-ethyl-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15n).** White solid, mp 279 °C. Yield: 37%. ^{1}H NMR (DMSO-d_6): δ 13.00 (bs, 1H), 10.00 (s, 1H), 8.36 (s, 1H), 3.84 (s, 3H), 2.85 (q, J = 7.6 Hz, 2H), 2.04 (s, 9H), 1.65 (s, 6H), 1.21 (t, J = 7.6 Hz, 3H).

***N*-(Adamantan-1-yl)-3-*tert*-butyl-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15p).** White solid, mp 256−257 °C. Yield: 54%. ^{1}H NMR (DMSO-d_6): δ 13.00 (bs, 1H), 10.10 (bs, 1H), 8.40 (s, 1H), 3.83 (s, 3H), 2.05 (s, 9H), 1.66 (s, 6H), 1.40 (s, 9H).

***N*-(Adamantan-1-yl)-4,7-dihydro-1-methyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (15s).** White solid, mp > 300 °C. Yield: 45%. ^{1}H NMR (DMSO-d_6): δ 12.97 (bs, 1H), 8.70 (s, 1H), 8.47 (bs, 1H), 7.58 (bs, 1H), 4.01 (s, 3H), 2.06 (s, 9H), 1.67 (s, 6H).

***N*-(Adamantan-1-yl)-4,7-dihydro-3-methyl-4-oxothieno[2,3-*b*]pyridine-5-carboxamide (15ac).** White solid, mp 243 °C (dec). Yield: 57%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.02 (bs, 1H), 10.15 (bs, 1H), 8.51 (s, 1H), 6.92 (q, J = 1.2 Hz, 1H), 2.52 (d, J = 1.2 Hz, 3H), 2.03 (m, 9H), 1.65 (s, 6H).

***N*-(Adamantan-1-yl)-4,7-dihydro-2,3-dimethyl-4-oxothieno-[2,3-*b*]pyridine-5-carboxamide (15ad).** White solid, mp 218 °C (dec). Yield: 55%.^{1}H NMR (DMSO-d_6): δ 13.03 (bs, 1H), 10.25 (bs, 1H), 8.47 (s, 1H), 2.50 (s, 3H), 2.32 (s, 3H), 2.04 (m, 9H), 1.66 (s, 6H).

***N*-(Adamantan-1-yl)-4,7-dihydro-3-methyl-4-oxo-7-pentylisoxazolo[5,4-*b*]pyridine-5-carboxamide (50).** White solid, mp 170 °C. MS: m/z 398.3 (M + H). Yield: 59%. ^{1}H NMR (DMSO-d_6): δ 8.36 (s, 1H), 8.22 (bs, 1H), 4.38 (t, J = 6.4 Hz, 2H), 2.58 (s, 3H), 2.05 (m, 9H), 1.81 (m, 2H), 1.65 (m, 6H), 1.40−1.36 (m, 4H), 0.89 (t, J = 7 Hz, 3H). Anal. ($C_{23}H_{31}N_3O_3$) C, H, N.

***N*-(Adamantan-1-yl)-4,7-dihydro-7-oxo-4-pentyl[1,2,4]-triazolo[1,5-*a*]pyrimidine-6-carboxamide (56).** White solid, mp 163 °C. Yield: 62%. MS: m/z 384.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.85 (s, 1H), 8.65 (s, 1H), 8.38 (s, 1H), 4.31 (q, J = 7.4 Hz, 2H), 2.08 (m, 9H), 1.82 (m, 2H), 1.87 (m, 6H), 1.32−1.28 (m, 4H), 0.85 (t, J = 6.2 Hz, 3H). Anal. ($C_{21}H_{29}N_5O_2$) C, H, N.

***N*-(Adamantan-1-yl)-7-oxo-4-pentyl-2-phenyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (57).** White solid, mp 158 °C. Yield: 38%. MS: m/z 460.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.83 (s, 1H), 8.71 (s, 1H), 8.16−8.11 (m, 2H), 7.57−7.53 (m, 3H), 4.37 (q, J = 7.2 Hz, 2H), 2.06 (m, 9H), 1.85 (m, 2H), 1.68 (m, 6H), 1.36−1.33 (m, 4H), 0.87 (t, J = 6.2 Hz, 3H). Anal. ($C_{27}H_{33}N_5O_2$) C, H, N.

Synthesis of N7-Substituted Pyrazolo[3,4-*b*]pyridine-5-carboxamides 16−47, 7-Pentylthieno[2,3-*b*]pyridine-5-carboxamides 51−54, 13k, l, and 14g. *General Procedure.* To a solution of the suitable carboxamide or carboxylic acid ethyl ester (0.27 mmol) in anhydrous *N*,*N*-dimethylformamide (5 mL) was added K_2CO_3 (0.81 mmol), and the mixture was heated at 80 °C for 1 h. Alkyl bromide (0.81 mmol) was added, and the mixture was stirred at 100 °C for 16 h. After the mixture was cooled to room temperature, the solvent was concentrated in vacuo and the residue partitioned between ethyl acetate (30 mL) and water (5 mL). The organic phase was

evaporated and purified by flash chromatography on silica gel, eluting with ethyl acetate/petroleum ether.

***N*-Cyclohexyl-4,7-dihydro-1,3-dimethyl-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (16).** White solid, mp 69 °C. Yield: 42%. MS: *m*/*z* 359.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.00 (bd, *J* = 7.6 Hz, 1H), 8.37 (s, 1H), 4.22 (t, *J* = 7.6 Hz, 2H), 4.10 (s, 3H), 3.99 (m, 1H), 2.61 (s, 3H), 2.00−1.27 (m, 16H), 0.88 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{20}H_{30}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-1,3-dimethyl-7-propyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (17).** White solid, mp 158 °C. Yield: 40%. MS: *m*/*z* 331.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.06 (bd, *J* = 8.0 Hz, 1H), 8.44 (s, 1H), 4.39 (t, *J* = 7.0 Hz, 2H), 4.06 (s, 3H), 3.90 (m, 1H), 2.44 (s, 3H), 1.82−1.29 (m, 12 H), 0.89 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{18}H_{26}N_4O_2$) C, H, N.

7-Allyl-*N*-(cyclohexyl)-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (18). White solid, mp 212 °C. Yield: 55%. MS: *m*/*z* 329.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.04 (bd, *J* = 7.8 Hz, 1H), 8.41 (s, 1H), 6.23−6.20 (m, 1H), 5.27−5.24 (m, 1H), 5.16−5.14 (m, 2H), 4.88−4.79 (m, 1H), 4.00 (s, 3H), 3.97 (m, 1H), 2.45 (s, 3H), 1.64−1.20 (m, 10H). Anal. ($C_{18}H_{24}N_4O_2$) C, H, N.

7-Butyl-*N*-(cyclohexyl)-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (19). White solid. mp 160 °C. Yield: 35% MS: *m*/*z* 345.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (bd, *J* = 7.8 Hz, 1H), 8.43 (s, 1H), 4.43 (t, *J* = 7.0 Hz, 2H), 4.07 (s, 3H), 3.80 (m, 1H), 2.50 (s, 3H), 1.84−1.23 (m, 14H), 0.94 (t, *J* = 7.0 Hz, 3H). Anal. ($C_{19}H_{28}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-1,3-dimethyl-7-(2-ethoxyethyl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (20).** White solid, mp 98 °C. Yield: 40%. MS: *m*/*z* 361.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.02 (bd, *J* = 7.8 Hz, 1H), 8.41 (s, 1H), 4.35−4.31 (m, 2H), 3.93 (s, 3H), 3.73−3.69 (m, 3H), 3.47 (q, *J* = 7 Hz, 2H), 2.57 (s, 3H), 1.97−1.42 (m, 5H), 1.32−1.27 (m, 5H), 1.09 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{19}H_{28}N_4O_3$) C, H, N.

7-(4-Cyanobutyl)-*N*-(cyclohexyl)-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (21). White solid, mp 209 °C. Yield: 22%. MS: *m*/*z* 370.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (bd, *J* = 7.8 Hz, 1H), 8.46 (s, 1H), 4.47 (t, *J* = 7.2 Hz, 4H), 4.07(s, 3H), 3.90 (m, 1H), 2.48 (s, 3H), 1.88−1.29 (m, 16H). Anal. ($C_{20}H_{27}N_5O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-1,3-dimethyl-7-hexyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (22).** White solid, mp 81 °C. Yield: 40%. MS: *m*/*z* 373.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.20 (bd, *J* = 7.6 Hz, 1H), 8.43 (s, 1H), 4.42 (t, *J* = 7.6 Hz, 2H), 4.06 (s, 3H), 3.85 (bm, 1H), 2.41 (s, 3H), 1.95−1.27 (m, 18H), 0.88 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

7-Benzyl-*N*-cyclohexyl-4,7-dihydro-1,3-dimethyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (23). White solid, mp 240 °C. Yield: 40%. MS: *m*/*z* 379.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (bd, *J* = 7.6 Hz, 1H), 8.55 (s, 1H), 7.40−7.06 (m, 5H), 5.81 (s, 2H), 3.81 (s, 3H), 3.80 (bm, 1H), 2.45 (s, 3H), 1.84−1.27 (m, 10H). Anal. ($C_{22}H_{26}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-1,3-dimethyl-7-(4-methylbenzyl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (24).** White solid, mp 234 °C. Yield: 40%. MS: *m*/*z* 393.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.05 (bd, *J* = 7.6 Hz, 1H), 8.52 (s, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 2H), 5.75 (s, 2H), 3.83 (s, 3H), 3.80 (br m, 1H), 2.45 (s, 3H), 2.28 (s, 3H), 1.83-1. 26 (m, 10H). Anal. ($C_{23}H_{28}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-1,3-dimethyl-7-[2-(morpholin-4-yl)ethyl]-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (25).** White solid, mp 128 °C. Yield: 42%. MS: *m*/*z* 402.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.06 (bd, *J* = 7.6 Hz, 1H), 8.41 (s, 1H), 4.52 (t, *J* = 7.0 Hz, 2H), 4.08 (s, 3H), 3.90 (br m, 1H), 3.49 (m, 4H), 2.59 (t, *J* = 7.0 Hz, 2H), 2.44 (s, 3H), 1.99−1.22 (m, 14H). Anal. ($C_{21}H_{31}N_5O_3$) C, H, N.

***N*-(Cyclohexylmethyl)-4,7-dihydro-1,3-dimethyl-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (26).** White solid, mp 87 °C. Yield: 40%. MS: *m*/*z* 373.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.10 (bt, *J* = 7.8 Hz, 1H), 8.37 (s, 1H), 4.21 (t, *J* = 7.6 Hz, 2H), 4.10 (s, 3H), 3.27 (t, *J* = 6.6 Hz, 2H), 2.61 (s, 3H), 1.85−1.37 (m, 17H), 0.92 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

***trans*-4,7-Dihydro-1,3-dimethyl-*N*-(4-methylcyclohexyl)-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (27).** White solid, mp 153 °C. Yield: 57%. MS: *m*/*z* 373.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.95 (bd, *J* = 7.2 Hz, 1H), 8.43 (s, 1H), 4.42 (t, *J* = 7 Hz, 2H), 4.07 (s, 3H), 3.73 (bm, 1H), 2.44 (s, 3H), 2.00−1.66 (m, 6H), 1.31−0.83 (m, 15H). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

***cis*-4,7-Dihydro-1,3-dimethyl-*N*-(4-methylcyclohexyl)-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (28).** White solid, mp 116 °C. Yield: 57%. MS: *m*/*z* 373.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.28 (bd, *J* = 7.8 Hz, 1H), 8.44 (s, 1H), 4.42 (t, *J* = 7.6 Hz, 2H), 4.07 (m, 4H), 2.46 (s, 3H), 1.75−1.09 (m, 15H), 0.93−0.83 (m, 6H). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

***N*-Cycloheptyl-4,7-dihydro-1,3-dimethyl-7-pentyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (29).** White solid, mp 80 °C. Yield: 40%. MS: *m*/*z* 373.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.10 (bd, *J* = 7.8 Hz, 1H), 8.42 (s, 1H), 4.39 (t, *J* = 7.8 Hz, 2H), 4.06 (s, 3H), 3.99 (m, 1H), 2.44 (s, 3H), 1.82−1.27 (m, 18H), 0.89 (t, *J* = 6.8 Hz, 3H). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

***N*-(Adamantan-1-yl)-4,7-dihydro-1,3-dimethyl-7-pentyl-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (30).** White solid, mp 143 °C. Yield: 25%. MS: *m*/*z* 411.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.90 (bs, 1H), 8.38 (s, 1H), 4.39 (t, *J* = 7.8 Hz, 2H), 4.06 (s, 3H), 2.43 (s, 3H), 2.03 (s, 9H), 1.65 (m, 8H), 1.32 (m, 4H), 0.86 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{24}H_{34}N_4O_2$) C, H, N.

4,7-Dihydro-1,3-dimethyl-*N*-(3,5-dimethyladamantan-1-yl)-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (31). White solid, mp 201 °C. Yield: 25%. MS: *m*/*z* 439.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.97 (bd, *J* = 7.6 Hz, 1H), 8.35 (s, 1H), 4.41 (t, *J* = 7.2 Hz, 2H), 4.06 (s, 3H), 2.43 (s, 3H), 1.92−1.14 (m, 25H), 0.84 (m, 3H). Anal. ($C_{26}H_{38}N_4O_2$) C, H, N.

4,7-Dihydro-1,3-dimethyl-7-pentyl-*N*-(5-methylhexan-2-yl)-4-oxo-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (32). White solid, mp 195 °C. Yield: 25%. MS: *m*/*z* 375.4 (M + H). ^{1}H NMR ($CDCl_3$): δ 9.88 (bd, *J* = 7.8 Hz, 1H), 8.37 (s, 1H), 4.16 (m, 3H), 4.10 (s, 3H), 2.61 (s, 3H), 1.68−1.54 (m, 14H), 0.89 (m, 9H). Anal. ($C_{21}H_{34}N_4O_2$) C, H, N.

4,7-Dihydro-1,3-dimethyl-4-oxo-7-pentyl-*N*-phenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (33). White solid, mp 138 °C. Yield: 40%. MS: *m*/*z* 353.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 12.45 (bs, 1H), 8.61 (s, 1H), 7.70 (m, 2H), 7.35 (m, 2H), 7.07 (m, 1H), 4.48 (t, *J* = 7.2 Hz, 2H), 4.01 (s, 3H), 2.48 (s, 3H), 1.85 (m, 2H), 1.31 (m, 4H), 0.87 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{20}H_{24}N_4O_2$) C, H, N.

4,7-Dihydro-1,3-dimethyl-*N*-(4-methoxyphenyl)-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (34). White solid, mp 167 °C. Yield: 40%. MS: *m*/*z* 384.7 (M + H). ^{1}H NMR (DMSO-d_6): δ 12.35 (bs, 1 H), 8.62 (s, 1H), 7.63 (dd, *J* = 9 Hz, 2H), 6.92 (dd, *J* = 9 Hz, 2H), 4.84 (t, *J* = 7.4 Hz, 2H), 3.89 (s, 3H), 3.76 (s, 3H), 2.49 (s, 3H), 1.81 (m, 2H), 1.32−1.27 (m, 4H), 0.87 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{21}H_{26}N_4O_3$) C, H, N.

4,7-Dihydro-1,3-dimethyl-*N*-(4-fluorophenyl)-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (35). White solid, mp 176 °C. Yield: 40%. MS: *m*/*z* 371.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 12.46 (bs, 1 H), 8.61 (s, 1H), 7.77−7.70 (m, 2H), 7.22−7.14 (m, 2H), 4.84 (t, *J* = 7.4 Hz, 2H), 4.09 (s, 3H), 2.49 (s, 3H), 1.79 (m, 2H), 1.35−1.28 (m, 4H), 0.87 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{20}H_{23}FN_4O_2$) C, H, N.

***N*-Benzyl-4,7-dihydro-1,3-dimethyl-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (36).** White solid, mp 137 °C. Yield: 40%. MS: *m*/*z* 367.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.39 (bt, *J* = 6.0 Hz, 1H), 8.49 (s, 1H), 7.34−7.32 (m, 5H), 4.51 (d, *J* = 6.0 Hz, 2H), 4.21 (t, *J* = 7.2 Hz, 2H), 4.07 (s, 3H), 2.43 (s, 3H), 1.75 (m, 2H), 1.39−1.28 (m, 4H), 0.88 (t, *J* = 6.8 Hz, 3H). Anal. ($C_{21}H_{26}N_4O_2$) C, H, N.

***N*-Cyclohexyl-4,7-dihydro-3-ethyl-1-methyl-4-oxo-7-pentyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carboxamide (37).** White solid, mp 146 °C. Yield: 25%. MS: *m*/*z* 373.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.10 (bd, *J* = 7.8 Hz, 1H), 8.44 (s, 1H), 4.40 (t, *J* = 7.6 Hz, 2H), 4.08 (s, 3H), 3.98 (m, 1H), 2.85 (q, *J* = 7.6 Hz, 2H), 1.79−1.30 (m, 16H), 1.22 (m, 3H), 0.83 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{21}H_{32}N_4O_2$) C, H, N.

N-(Adamantan-1-yl)-4,7-dihydro-3-ethyl-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (38). White solid, mp 130−131 °C. Yield: 20%. MS: *m/z* 425.4 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.95 (s, 1H), 8.39 (s, 1H), 4.41 (t, *J* = 6.8 Hz, 2H), 4.08 (s, 3H), 2.86 (q, *J* = 6.0 Hz, 2H), 2.03 (s, 9H), 1.76 (m, 2H), 1.65 (s, 6H), 1.31 (m, 4H), 1.20 (t, *J* = 6.8 Hz, 3H), 0.88 (t, *J* = 6.4 Hz, 3H). Anal. ($C_{25}H_{36}N_4O_2$) C, H, N.

3-tert-Butyl-N-cyclohexyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (39). White solid, mp 177 °C. Yield: 30%. MS: *m/z* 401.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.20 (bd, *J* = 7.8 Hz, 1H), 8.47 (s, 1H), 4.43 (t, *J* = 7.6 Hz, 2H), 4.09 (s, 3H), 3.97 (m, 1H), 1.99−1.58 (m, 12H), 1.41 (s, 9H), 1.33−1.29 (m, 4H), 0.86 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{23}H_{36}N_4O_2$) C, H, N.

N-(Adamantan-1-yl)-3-tert-butyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (40). White solid, mp 192 °C. Yield: 32%. MS: *m/z* 453.2 (M + H). ^{1}H NMR ($CDCl_3$): δ 10.07 (bs, 1H), 8.34 (s, 1H), 4.19 (t, *J* = 7.6 Hz, 2H), 4.10 (s, 3H), 2.17−2.09 (m, 9H), 1.72−1.70 (m, 8H), 1.47 (s, 9H), 1.36 (m, 4H), 0.91 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{27}H_{40}N_4O_2$) C, H, N.

N-Cyclohexyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (41). White solid, mp 146 °C. Yield: 33%. MS: *m/z* 345.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.69 (bd, *J* = 7.6 Hz, 1H), 8.48 (s, 1H), 7.98 (d, *J* = 6.0 Hz, 1H), 4.72 (t, *J* = 6.2 Hz, 2H), 4.01 (s, 3H), 3.99 (m, 1H), 1.90−1.27 (m, 16H), 0.91 (t, *J* = 7.4 Hz, 3H). Anal. ($C_{19}H_{28}N_4O_2$) C, H, N.

N-Cycloheptyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (42). White solid, mp 131 °C. Yield: 28%. MS: *m/z* 359.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.69 (bd, *J* = 7.8 Hz, 1H), 8.47 (s, 1H), 7.85 (d, *J* = 7.6 Hz, 1H), 4.72 (t, *J* = 6.0 Hz, 2H), 4.00 (s, 3H), 3.99 (m, 1H), 1.7−1.36 (m, 18H), 0.88 (t, *J* = 7.2 Hz, 3H). Anal. ($C_{20}H_{30}N_4O_2$) C, H, N.

N-(Adamantan-1-yl)-4,7-dihydro-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (43). White solid, mp 79 °C. Yield: 35%. MS: *m/z* 397.6 (M + H). ^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H), 8.47 (s, 1H), 7.58 (bs, 1H), 4.73 (t, *J* = 7.2 Hz, 2H), 4.01 (s, 3H), 2.06 (s, 9H), 1.98−1.82 (m, 2H), 1.67 (s, 6H), 1.59−1.35 (m, 4H), 0.92 (t, *J* = 7 Hz, 3H). Anal. ($C_{23}H_{32}N_4O_2$) C, H, N.

N-Cyclohexyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-3-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (44). White solid, mp 176 °C. Yield: 45%. MS: *m/z* 421.1 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.01 (bd, *J* = 7.4 Hz, 1H), 8.54 (s, 1H), 8.04−8.00 (m, 2H), 7.45−7.41 (m, 3H), 4.51 (m, 2H), 4.23 (s, 3H), 3.83 (m, 1H), 1.85−1.28 (m, 16H), 0.88 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{25}H_{32}N_4O_2$) C, H, N.

N-Cycloheptyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-3-phenyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (45). White solid, mp 208 °C. Yield: 33%. MS: *m/z* 435.2 (M + H). ^{1}H NMR ($CDCl_3$) 10.06 (bd, *J* = 7.6 Hz, 1H), 8.44 (s, 1H), 8.04−7.98 (m, 2H), 7.46−7.43 (m, 3H), 4.24 (m, 6H), 1.95−1.27 (m, 18H), 0.94 (m, 3H). Anal. ($C_{26}H_{34}N_4O_2$) C, H, N.

3-(4-Chlorophenyl)-N-cyclohexyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (46). White solid, mp 203 °C. Yield: 25%. MS: *m/z* 455.3 (M + H). ^{1}H NMR (DMSO-d_6): δ 9.94 (bd, *J* = 7.6 Hz, 1H), 8.53 (s, 1H), 8.08 (dd, *J* = 6.8 Hz, 2H), 7.50 (dd, *J* = 7.0 Hz, 2H), 4.48 (t, *J* = 7.2 Hz, 2H), 4.21 (s, 3H), 3.89 (m, 1H), 1.82−1.25 (m, 16H), 0.85 (t, *J* = 6.8 Hz, 3H). Anal. ($C_{25}H_{31}ClN_4O_2$) C, H, N.

3-(4-Chlorophenyl)-N-cycloheptyl-4,7-dihydro-1-methyl-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (47). White solid, mp 219 °C. Yield: 25%. MS: *m/z* 470.2 (M + H). ^{1}H NMR (DMSO-d_6): δ 10.00 (bd, *J* = 7.8 Hz, 1H), 8.54 (s, 1H), 8.09 (dd, *J* = 8.2 Hz, 2H), 7.51 (dd, *J* = 8.2 Hz, 2H), 4.50 (t, *J* = 7.0, 2H), 4.23 (s, 3H), 3.99 (m, 1H), 1.56−1.23 (m, 18H), 0.85 (t, *J* = 6.8 Hz, 3H). Anal. ($C_{26}H_{33}ClN_4O_2$) C, H, N.

N-Cyclohexyl-4,7-dihydro-3-methyl-4-oxo-7-pentylthieno[2,3-b]pyridine-5-carboxamide (51). White solid, mp 130 °C. Yield: 32%. MS: *m/z* 361.2 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.23 (bd, *J* = 7.8 Hz, 1H), 8.64 (bs, 1H), 7.05 (q, ^4J = 1.2 Hz, 1H), 4.25 (t, *J* = 7.2 Hz, 2H), 3.97−3.78 (m, 1H), 2.52 (d, ^4J = 1.8 Hz, 3H), 1.99−1.17 (m, 16H), 0.84 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{20}H_{28}N_2O_2S$) C, H, N.

N-Cycloheptyl-4,7-dihydro-3-methyl-4-oxo-7-pentylthieno-[2,3-b]pyridine-5-carboxamide (52). White solid, mp 113 °C. Yield: 28%. MS: *m/z* 375.2 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.26 (bd, *J* = 7.6 Hz, 1H), 8.63 (bs, 1H), 7.05 (q, ^4J = 1.2 Hz, 1H), 4.24 (t, *J* = 7.4 Hz, 2H), 4.05−3.98 (m, 1H), 2.54 (d, ^4J = 1.2 Hz, 3H), 1.86−1.79 (m, 4H), 1.62−1.28 (m, 14 H), 0.85 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{21}H_{30}N_2O_2S$) C, H, N.

N-(Adamantan-1-yl)-4,7-dihydro-3-methyl-4-oxo-7-pentylthieno[2,3-b]pyridine-5-carboxamide (53). White solid, mp 159 °C. Yield: 37%. MS: *m/z* 413.2 (M + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.08 (bs, 1H), 8.54 (s, 1H), 6.61 (q, ^4J = 1.2 Hz, 1H), 4.03 (t, *J* = 7.6 Hz, 2H), 2.66 (d, ^4J = 1.2 Hz, 1H), 2.09 (s, 9H), 1.97−1.82 (m, 2H), 1.66 (s, 6H), 1.30−1.28 (m, 4H), 0.86 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{24}H_{32}N_2O_2S$) C, H, N.

N-(Adamantan-1-yl)-4,7-dihydro-2,3-dimethyl-4-oxo-7-pentylthieno[2,3-b]pyridine-5-carboxamide (54). White solid, mp 215 °C. Yield: 35%. MS: *m/z* 427.2 (M + H). ^{1}H NMR ($CDCl_3$): δ 10.18 (bs, 1H), 8.49 (s, 1H), 3.99 (t, *J* = 7.6 Hz, 2H), 2.56 (s, 3H), 2.37 (s, 3H), 2.18 (s, 9H), 2.09−1.92 (m, 2H), 1.71 (s, 6H), 1.37−1.30 (m, 4H), 0.90 (t, *J* = 6.6 Hz, 3H). Anal. ($C_{25}H_{34}N_2O_2S$) C, H, N.

Ethyl 4,7-Dihydro-7-oxo-4-pentyl[1,2,4]triazolo[1,5-a]-pyrimidine-6-carboxylate (13k). White solid, mp 85−86 °C. Yield: 85%. ^{1}H NMR ($CDCl_3$): δ 8.47 (s, 1H), 8.09 (s, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 4.29 (q, *J* = 7.2 Hz, 2H), 1.93−1.85 (m, 2H), 1.43−1.35 (m, 7H), 0.91 (t, *J* = 6.6 Hz, 3H).

Ethyl 4,7-Dihydro-7-oxo-4-pentyl-2-phenyl[1,2,4]triazolo-[1,5-a]pyrimidine-6-carboxylate (13l). White solid, mp 112−113 °C. Yield: 80%. ^{1}H NMR (DMSO-d_6): δ 8.87 (s, 1H), 8.16−8.11 (m, 2H), 7.58−7.53 (m, 3H), 4.36−4.3 (m, 4H), 1.92−1.85 (m, 2H); 1.37−1.27 (m, 7H); 0.91 (t, *J* = 6.4 Hz, 3H).

Ethyl 4,7-Dihydro-3-methyl-4-oxo-7-pentylisoxazolo[5,4-b]-pyridine-5-carboxylate (14g). White solid, mp 91 °C. Yield: 42%. ^{1}H NMR (DMSO-d_6): δ ppm 8.46 (s, 1H), 4.24−4.17 (m, 4H), 2.48 (s, 3H), 1.77 (m, 2H), 1.32−1.25 (m, 7H), 0.86 (t, *J* = 7.2 Hz, 3H).

AUTHOR INFORMATION

Corresponding Author

*For M.A.T.: phone, 39-(0)532455926; fax, 39-(0)53245593; e-mail, tbj@unife.it. For P.G.B.: phone, 39-(0)532455921; fax, 39-(0)532455953; e-mail, Baraldi@unife.it.

Present Address

∥Alta Vetta Pharmaceutical Consulting, LLC, 3 Skipwith Court, Durham, North Carolina 27707.

Notes

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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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