

Synthesis and Structure Activity Relationship Investigation of Triazolo[1,5-*a*]pyrimidines as CB₂ Cannabinoid Receptor Inverse Agonists

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Abstract

CB₂ cannabinoid receptor ligands are known to be therapeutically important for the treatment of numerous diseases. Recently, we have identified the heteroaryl-4-oxopyridine/7-oxopyrimidine derivatives as highly potent and selective CB₂ receptor ligands, showing that the pharmacodynamics of the new compounds was controlled by the nature of the heterocycle core. In this paper we describe the synthesis and biological evaluation of 7-oxo-4-pentyl-4,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide derivatives that led to the identification of novel CB₂ receptor inverse agonists. Determination of efficacy (E_{\max}) revealed that introduction of structural modifications at position 2 of triazolopyrimidine template changes the functional activity from partial to inverse agonism. The molecular docking analysis of the novel structures is reported.

Keywords

Cannabinoid; CB₂ receptor; inverse agonists; efficacy; molecular docking.

Introduction

The endocannabinoid system includes a group of neuromodulatory lipids and their receptors that are involved in a diversity of physiological and pathological conditions. The main endocannabinoids, or endogenous cannabis-like substances, are small molecules resulting from arachidonic acid, N-arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoyl-glycerol (2-AG) [1]. Two diverse types of G protein-coupled receptors (GPCRs) have been discovered and named as central cannabinoid (CB₁) and peripheral cannabinoid (CB₂) receptors. The CB₁ and CB₂ receptors are negatively coupled to adenylyl cyclase, exert an inhibitory effect on cyclic AMP (cAMP) production, and alter the mitogen-activated protein kinase (MAPK) pathway [2].

CB₂ receptors were originally supposed to be present in periphery, primarily to immune cells, but at present it has been reported that these receptors are localized neuronally in different species. They are expressed in hematopoietic cells, natural killer cells, macrophages and neutrophils, and represent an attractive target for the treatment of the inflammatory status [3]. These receptors also mediate a significant protection in brain microglia against neurotoxicity by modulating the release of anti- or pro-inflammatory cytokines [4]. CB₂ receptors appear to be implicated in allergic dermatitis where their modulation mediates an inflammatory response obtained from antigen-specific effector T cells upon frequent allergen contact [5]. Contrasting preclinical studies are reported regarding the involvement of CB₂ receptors and allergic dermatitis, supposing the potential use of both CB₂ agonists or inverse agonists/antagonists in the pharmacological therapy [6].

In recent years, pharmaceutical companies and academic research laboratories have challenged to identify novel CB₂ agonists without CB₁ central effects by focusing on the discovery of high affinity and selective agonists for CB₂ receptors with a therapeutic potential in the treatment of different pathologies such as neurodegenerative diseases, pain transduction and perception, ischemic stroke, severe inflammation, autoimmune diseases, osteoporosis and cancers [4,7].

From the pharmacological point of view, CB₂ agonists demonstrate various ways of interacting with the receptor site, suggesting different binding and functional properties well represented by pharmacodynamic concepts of full, partial and inverse agonism [8–11].

The therapeutic potential of the cannabinoids has encouraged the development of several CB₂ receptor selective inverse agonists (Chart 1). In particular, the immunomodulatory activities associated with cannabinoid CB₂ receptor inverse agonists have been demonstrated by oral administration of the inverse agonists **1** (JTE-907) and **2** (SR144528) that inhibited carrageenin-induced paw oedema in mice [12]. The efficacy of triaryl bissulphone **3** (Sch414319), a CB₂ receptor inverse agonist, was investigated *in vivo* in diverse disease models, showing its ability to modulate immune cell mobility and bone damage in antigen-induced mono-articular arthritis in the rat [13]. Additional reports suggest that compound **4** (MH), an inverse agonists of CB₂ receptor, showed anti-inflammatory and antiosteoclastogenic properties [14]. Moreover, N,N'-((4-(dimethylamino)phenyl)methylene)bis(2-phenylacetamide) derivatives such as **5** were found to be CB₂ inverse agonists and potential therapeutic agents for the treatment of antiosteoporosis [15].

The 4-oxo-1,8-naphthyridine-3-carboxamide and 4-oxo-1,4-dihydroquinoline-3-carboxamide cannabinoid based ligands are reported with high affinity and selectivity toward CB₂ receptors. 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. Within this series, the 8-methoxy derivative **6** (hCB₂ K_i = 0.6 nM, hCB₁ K_i > 10000 nM) (Chart 1) behaved as an inverse agonist and showed antihyperalgesic effects [16].

Chart 1 should be inserted here

Our group previously reported the synthesis and cannabinoid receptor binding of 4-oxopyrazolopyridines as CB₂ partial agonists [10]. In this family, compound **7** (Chart 2) displayed high affinity and selectivity for CB₂ receptor. Changing the orientation of the pyrazole ring led to

the 7-oxopyrazolopyrimidines, such as compound **8** that exhibited selective inverse agonist activity at CB₂ receptor [11]. Replacement of the pyrazolo ring of the parent nucleus with differently substituted 5-membered heterocycles allowed to the isoxazolo/thienopyridines that were found to be CB₂ full agonists. In this context, the triazolopyrimidines **9**, **10** (Chart 2) were found to be potent CB₂ receptor ligands and in functional assays compound **9** behaved as partial agonist.

In light of these results, we sought to explore the influence of substituent arrangement at the C-2 position of triazolo heterocycle on the activity versus CB₂ receptor. In this attempt, we have synthesized a new series of 2-substituted-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide derivatives based on the previously well-known pharmacophores in the series of heteroaryl pyridine/pyrimidine compounds. Consequently, the *n*-pentyl chain was retained at the N-4 position of heterocycle and the cyclohexyl, cycloheptyl, and adamantyl moieties were introduced on the carboxamide moiety (Chart 2). All the newly synthesized compounds were tested on membranes prepared from Chinese hamster ovary (CHO) cells expressing human CB₁ (hCB₁) and CB₂ (hCB₂) receptors, in order to estimate their affinity and selectivity for CB₂ receptor. The cyclic AMP assays on hCB₂ CHO cells to test the functionality of these ligands is reported. Moreover, a molecular docking analysis was carried out into the three-dimensional model of CB receptors recently constructed, in order to clarify the structure activity relationships (SARs) experimentally observed.

Chart 2 should be inserted here

Chemistry

Synthesis of derivatives **15-41** was carried out by the general methodology shown in Scheme 1. Cyclocondensation of aminotriazoles **11a-m** with diethyl ethoxymethylenmalonate (DEEM) in glacial acetic acid gave the ethyl 4,7-dihydro-2-substituted-7-oxo-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylates **12a-m** in good yields. Alkylation of the bicyclic nucleus with 1-pentylbromide in the presence of K₂CO₃ led to the formation of **13a-m**. The subsequent hydrolysis with 10% aqueous NaOH yielded the related carboxylic acids **14a-m**, which were converted to the corresponding car-

boxamide derivatives **15-41** by coupling reaction with appropriate amines using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) or *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate in dimethylformamide. The starting 1,2,4-triazol-3-amines **11a-b** were commercially available, while derivatives **11c-m** were prepared following two different procedures. According to method A via cyclocondensation of amidoguanidines, a suitable hydrazide was reacted with *S*-methylisothiourea hemisulfate upon heating in water to give the amidoguanidine intermediate. The subsequent cyclization in 10% aqueous NaOH at 100 °C yielded aminotriazoles **11c-h** [17,18]. The aminotriazoles **11i-m** were prepared according to method B, by the reaction of *N*-cyanocarbonimidodithioic acid dimethyl ester with one molar equivalent of an appropriate amine followed by the reaction with hydrazine monohydrate in refluxing acetonitrile (as except for **11i** that was prepared without adding hydrazine) [19].

Scheme 1 should be inserted here

Biological results and discussion

All of the newly synthesized compounds were examined in [³H]CP-55,940 competition binding experiments for their affinity and selectivity toward the human recombinant CB₁ and CB₂ receptors. The results are reported in Table 1 together with K_i values of previously published triazolopyrimidines **9**, **10** and the CB₂ agonist WIN55,212-2. According to our previous pharmacophore investigations, compounds bearing cyclohexyl, cycloheptyl, or adamantanyl moieties on the carboxamide function had shown the highest affinity for CB₂ receptor. We had also confirmed that the presence of the 1-pentyl chain on the pyridine/pyrimidine nucleus was required for CB₂ receptor affinity. Consequently, we introduced structural modifications at position 2 of the 7-oxo-triazolo[1,5-*a*]pyrimidine-6-carboxamide scaffold, in order to analyze the SARs of the new series. Examination of the binding data for the novel compounds reveals some potent and highly selective ligands for the CB₂ receptor, e.g. compounds **23**, **26-28**, **32** and **39** (Table 1). It is worth noting that the new derivatives although showed decreased in affinity for CB₂ receptor in comparison to the reference compound (**10**), highlighted a higher selectivity profile versus CB₁ receptor. Introduction of cyclo-

heptyl and 3,5-dimethyladamantan-1-yl groups (**15** and **16**, respectively) produced drastic decrease in affinity and selectivity at CB₂ receptor compared with the parent compound **9** bearing the adamantyl moiety. The same results were obtained by the 2-phenyl derivatives **19-21**, when the adamantly carboxamide was replaced, showing lower affinity and selectivity in comparison with **10**. The best affinity and selectivity profile was observed by compound **21** ($K_i\text{CB}_2 = 8.4 \text{ nM}$, $K_i\text{CB}_1 = 653 \text{ nM}$, $K_i\text{CB}_1 / K_i\text{CB}_2 = 77$).

Introduction of a methyl chain at position 2, such as in compounds **17** and **18**, resulted in a substantial reduction of affinity and a total loss of selectivity for both derivatives. Intriguingly, these results are in contrast with those of the previously pyrazolo[3,4-*b*]pyridone series [10], where the most active compounds bore a methyl group or an hydrogen atom, indicating a probable different binding mode of this new series to the receptor. Subsequently, analogues by introduction of 4-substituted-phenyl at the C-2 of the triazolopyrimidine nucleus were prepared, while the cyclohexyl, cycloheptyl, and adamantyl moieties on the carboxamide were maintained (compounds **22-28**, Table 1). The 4-chlorophenyl group at C-2 of the triazolopyrimidine nucleus (compounds **22** and **23**) resulted in a decreased of affinity and selectivity for the cyclohexylcarboxamide **22** compared to the parent phenyl analogue **19**. The adamantylcarboxamide derivative **23** resulted in a 10-fold decrease in affinity for the CB₁ receptor (**23**: $K_i\text{CB}_1 = 5216 \text{ nM}$ versus **10**: $K_i\text{CB}_1 = 523 \text{ nM}$), showing higher selectivity and comparable affinity for CB₂ receptor relative to that of analogue compound **10** (**23**: $K_i\text{CB}_2 = 11.3 \text{ nM}$, **10**: $K_i\text{CB}_2 = 3.5 \text{ nM}$).

Changing of the chlorine atom with an electron donating group, such as methoxy, resulted in derivatives **24** and **25**, which showed lower affinity for the CB₂ receptors ($K_i\text{CB}_2 = 96$ and 47 nM , respectively) in comparison with the phenyl analogue (**10**). Introduction of a 4-methylphenyl group at C-2, as with compounds **26-28**, increased selectivity in comparison with **10**, and the best results were obtained with adamantylamide derivative **28** that showed high affinity and selectivity at CB₂ receptor ($K_i\text{CB}_2 = 6.8 \text{ nM}$, $K_i\text{CB}_1 / K_i\text{CB}_2 = 223$). These results seem to indicate that electronic effects on the aromatic moiety do not significantly affect the binding for CB₂ receptors confirming

our previously results found for the pyrazolopyrimidine series [11]. Replacement of the 2-phenyl ring of **10** with a bioisosteric heterocycle such as 2-furyl (**29**) resulted in a decrease of both affinity and selectivity at the CB receptors (Table 1, $K_iCB_2 = 45$ nM, $K_iCB_1/K_iCB_2 = 26$). Substitution of the 5-membered heterocycle with a 6-membered aromatic ring such as 3-pyridyl moiety (**30**) determined a dramatic loss of affinities at both CB receptors ($K_iCB_2 = 228$ nM, $K_iCB_1 > 10000$ nM).

Replacement of the aromatic ring of the C-2 position by additional substitution on the heterocyclic nucleus had a profound effect on affinity versus CB₁ receptor as shown by compounds **31-44** with a $K_iCB_1 > 10000$ nM (Table 1). The introduction of a thiomethyl at the C-2 position (compounds **31**, **32**) significantly alters affinity at the CB₂ receptor relative to that of parent compounds **19** and **10**, respectively ($K_i = 716$ and 47 versus 43 and 3.5 nM), although the selectivity increased about 1.5-fold for **32** ($K_iCB_1/K_iCB_2 > 213$ versus $K_iCB_1/K_iCB_2 = 146$). Among the 2-morpholino derivatives, the highest affinity and selectivity was observed with the 6-adamantylcarboxamide **35** ($K_iCB_2 = 53$ nM, $K_iCB_1/K_iCB_2 > 188$), while neither the 2-piperazine compounds (**36**, **37**) nor the diallylamine derivatives (**40**, **41**) showed affinity against the CB₂ receptor.

The enhancement in affinity and selectivity was obtained with the 2-N-methylbenzyl chain and adamantyl moiety on the 6-carboxamide group (**39**, $K_iCB_2 = 21$ nM, $K_iCB_1/K_iCB_2 = 476$). This compound was the most selective of whole series probably because of different binding mode into CB₂ receptor due to the shape and volume of the C-2 substituent as shown by molecular modeling studies (Figure 3).

Human CB₂ receptor functional assay

The most active compounds of this series and previously derivative **9** were analyzed in cAMP assays for evaluation of their effect on cAMP production. The new ligands revealed an inverse agonist behavior, as they were able to significantly increase cAMP production, with the exception of compound **16** which acted as partial agonist (Table 2). The greatest effect was purchased by compounds **21**, **23** and **28**, that increased the second messenger production by 243%, 223% and 256% at 10 μ M,

respectively. It is noteworthy that the same compounds were also identified as the most active derivatives in binding assays, revealing a good correlation between affinity and efficacy of the developed series. The remaining compounds provided a comparable trend, since cAMP production increase was obtained within 105% and 135% at 1 μ M, and from 152% to 185% at 10 μ M, respectively. Interestingly, the new 2-unsubstituted triazolopyrimidine **16** acted as partial agonist as same as its parent compound **9** with values of efficacies (E_{\max}) = 57 and 60%, respectively. Moreover, the potency value of **16** measured in functional assays is in the nanomolar range (EC_{50} = 88 nM) and strictly correlated with the binding affinity value. The difference in the functional activity between the 2-substituted derivatives and the compounds lacking this substituent indicates that introduction of structural modifications at C-2 position plays a crucial role in establishing the functionality of the novel cannabinoid ligands switching their activity to inverse agonism.

Tables 1 and 2 should be inserted here

Molecular modeling studies

The synthesized compounds were docked into the inactive CB₂ and CB₁ receptor models by using AUTODOCK4.2 [20], and for each ligand, the top-scored pose belonging to the cluster of solutions with the best average of estimated free energy was considered. The resulting ligand-protein complexes were then energy minimized with AMBER11 [21] in a lipid bilayer and water environment, since molecular dynamic simulation studies carried out on the potent AMG3 CB ligand suggested that the lipid bilayer environment can affect the binding of ligands to CB receptors [22,23]. Figure 1A shows the minimized structures of the receptor-ligand complexes obtained by docking compound **28**, presenting the highest CB₂ affinity, into both CB₂ and CB₁ receptor models. In the CB₂ receptor model the triazolopyrimidine core of the ligand is placed between the α -helices belonging to the third and the sixth transmembrane domains (TM3 and TM6), predominantly interacting with M6.55(265) and L6.59(269) through hydrophobic interactions and forming an H-bond with

T3.33(114). The *n*-pentyl chain interacts into a sort of small lipophilic channel mainly delimited by L3.26(107), L3.27(108) and L6.59(269), and is directed towards the TM4 α -helix, taking contacts with P4.60(168) and L4.61(169). The adamantyl ring interacts with V4.56(164), P4.60(168), Y5.39(190), W5.43(194) and feels the effects of a strong van der Waals interaction with F5.46(197), which is a nonconserved residue (V5.46 in the CB₁ receptor) and could thus contribute to the selectivity of these ligands for CB₂ versus CB₁ receptor, consistently with site-directed mutagenesis studies [24,25]. Finally, the 4-CH₃-phenyl moiety directed toward the α -helices of the second and the seventh transmembrane domains (TM2 and TM7) is located in a small lipophilic cavity mainly delimited by F2.57(87), I3.29(110), L6.54(264) and F7.35(281). In the CB₁ receptor model, compound **28** shows a binding pose in which it is orthogonally oriented with respect to its disposition into the CB₂ receptor (Figure 1B), thus interacting only with four transmembrane domains (TM3-TM6). More in details, the 4-CH₃-phenyl moiety of the ligand is placed between TM3 and TM4 α -helices, surrounded by L3.26(190), F3.27(191) and L4.61(252) with which it establishes van der Waals contacts, whereas the *n*-pentyl chain points toward TM6 interacting mainly with V6.59(367). The central scaffold and the adamantyl ring of the ligand are placed in the hydrophobic pocket primarily constituted by L3.29(193), T3.33(197), I4.56(247), A4.57(248), P4.60(251), and W5.43(279), thus forming lipophilic interactions with these residues. Due to its disposition into CB₁ receptor the ligand not only lacks the strong van der Waals interactions with residue 5.46 (which is V282 instead of F197, as in CB₂) but it is not even able to form any H-bond with T3.33(197) through its triazolopyrimidine core. These two differences, together with the fewer hydrophobic contacts established within the CB₁ receptor, could be the main responsible for the selectivity for CB₂ versus CB₁ receptor shown by compound **28** and, in general, for this series of ligands.

Figure1 should be inserted here

Figure2 should be inserted here

Ligands such as compound **18**, bearing no substituents or only a methyl group in position 2 of the central scaffold, show a general decreased CB₂ affinity with respect to ligands presenting phenyl or aryl groups in the same positions. The energy minimized complex of compound **18** into CB₂ receptor (Figure 2A) shows a very similar binding mode for this ligand with respect to the one predicted for compound **28**. The two ligands share an almost identical disposition of their *n*-pentyl chains and adamantyl ring, which thus take van der Waals contacts with the same residues. Anyway, compound **18** is not able to fill the small lipophilic cavity mainly delimited by F2.57(87), I3.29(110), L6.54(264) and F7.35(281), showing only limited contacts with I3.29(110) and L6.64(264). This could be at the basis of the reduced affinity for CB₂ receptor showed by this ligand and its analogues lacking of larger substituents in C-2 position. Compound **18** shares with compound **28** also a similar binding pose into CB₁ receptor (Figure 2B). Anyway, the smaller C-2 substituent of this ligand allows it to adopt a slightly different orientation where the methyl group points toward the side chain of L4.61(252) and the triazole ring of the central scaffold is closer to P4.60(251). In this disposition, the ligand is able to form an H-bond with T3.33(197) through the carbonyl oxygen of its central scaffold; this interaction could constitute the reason of the highest affinity of this compound for CB₁ receptor with respect to what observed for compound **28** and generally for all those ligands bearing bulkier C-2 substituents that wouldn't allow such disposition due to steric bumps with F3.27(191) and L4.61(252) side chains. Compound **39**, bearing a *N*-benzyl-*N*-methyl group in 2 position of the central scaffold showed a strong affinity for CB₂ receptor and the highest selectivity versus CB₁ receptor among this series of compounds. As shown in Figure 3A, the ligand adopts a different binding mode into CB₂ receptor with respect to the previously analyzed ligands, due to the shape and volume of the C-2 substituent that cannot be placed into the small hydrophobic pocket formed by F2.57(87), I3.29(110), L6.54(264) and F7.35(281). Nevertheless, the compound is still able to establish the strong van der Waals interactions with F5.46(197) through its adamantyl ring, which shows the same location within the receptor as for compounds **18** and **28**. Moreover, the ligand forms an H-bond with T3.33(114) through the carbonyl oxygen of its amide moiety, thus main-

taining both the interactions that are thought to be responsible of the CB₂ versus CB₁ selectivity of these ligands. Finally, the C-2 substituent of the compound is efficiently positioned into the hydrophobic channel mainly delimited by L3.26(107), L3.27(108), L4.61(169) and Y5.39(190), forming lipophilic interactions with all these residues and, in particular, a strong π - π stacking with Y5.39(190). The energy minimized complex of compound **39** docked into CB₁ receptor model (Figure 3B) shows that the ligand does not interact as in the CB₂ receptor model. The compound shows a binding mode similar to that observed for **28** and **18** into the CB₁ receptor model, with a slight rotation of the central core in order to place the bulky benzyl moiety between TM3 and TM4 α -helices. Adopting this disposition the ligand is not able to form any H-bond with T3.33(197) and although it maintains a good interaction with F3.27(191) through the benzyl group, it shows reduced contacts with L3.26(190) and V6.59(367), as well as weaker hydrophobic interactions with T3.33(197), A3.34(198) and I4.65(247) through the adamantyl ring, with respect to compounds **28** and **18**. This could explain the low CB₁ affinity of compound **39** and thus its strong CB₂ versus CB₁ selectivity.

Figure 3 should be inserted here

Conclusions

In order to progress the CB₂ receptor affinity and selectivity and to develop structure activity relationship for the series of triazolo[1,5-*a*]pyrimidine-6-carboxamide, we synthesized and evaluated twenty-seven new derivatives belonging to this chemical class. Structural modifications around the central scaffold, especially at C-2 and C-6 positions, led us to achieve greater selectivity, with respect to the parent compounds **9** and **10**. The best affinity values associated with high selectivity were obtained with compounds **23** (K_i CB₂ = 11.3 nM; K_i CB₁/ K_i CB₂ = 462) and **39** (K_i CB₂ = 21 nM; SI K_i CB₁/ K_i CB₂ > 476) bearing an adamantan-1-yl moiety on the carboxamide function and a 4-Cl-phenyl or N-benzyl-N-methylamine residues at C-2, respectively. cAMP experiments on CB₂ receptors expressed in CHO cells surprisingly revealed a different behavior, basing on the substitu-

tions at C-2. Compound **16** as its parent **9** lacking the substituent in position C-2 of the triazolepyrimidine nucleus showed partial agonist activities. 2-Substituted derivatives provided an inverse agonism behavior, with great efficacy levels from 152% to 246% of increase in cAMP production. Thus, this novel series of compounds proposes an attractive starting point for more optimization, representing novel pharmacological tools to evaluate the therapeutic potential of CB₂ inverse agonists in diverse disease settings.

Chemistry

Materials and methods

Positive ion electrospray ionization (ESI) mass spectra were performed with an Agilent 1100 Series LC/MSD model. All melting points are uncorrected and were assigned by a Buchi-Tottoli apparatus. The proton nuclear magnetic resonance (¹H NMR) spectra were recorded on either a Bruker AC 200 (200MHz) or a Varian Mercury Plus 400 (400MHz) spectrometer. Chemical shifts are reported in δ values (parts per million), using appropriate deuterated solvents (DMSO-*d*₆ and CDCl₃). The purity of tested compounds was determined by combustion elemental analyses performed by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a Yanagimoto MT-5 CHN recorder elemental analyzer. All tested compounds obtained data consistent with a purity of at least 95% as compared with the theoretical values.

Reactions were monitored by TLC (Thin Layer Chromatography) on silica gel (precoated Merck F254 plates), and compounds visualization was performed by UV light. Flash chromatography was accomplished using 230-400 mesh silica gel and a mixture of ethyl acetate and petroleum ether or ethyl acetate and methanol in different ratios as eluting phase. Organic extracts were dried over anhydrous sodium sulfate (Na₂SO₄). All starting materials, reagents and solvents were obtained from Sigma-Aldrich or Alfa Aesar.

General procedure A for the synthesis of compounds 11c-h

A solution of *S*-methyliothiourea hemisulfate salt (22 mmol), appropriate hydrazide (22 mmol), and water (35 mL) was refluxed for 6 h. The solvent was concentrated under reduced pressure and the residue was washed with ethanol (3 x 10 mL). The resulting solid was poured into 10 mL of 10% NaOH and heated to reflux for additional 6 h. The reaction was cooled to room temperature and adjusted to pH 5 using 1N HCl. The precipitate was filtered, washed with cold water and dried.

5-Phenyl-2H-1,2,4-triazol-3-amine (11c). Following general procedure A, compound **11c** was isolated as a white solid. Yield 82%, mp = 233-234 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.58 (br s, 1H); 7.97-7.85 (m, 2H); 7.62-7.35 (m, 3H); 5.97 (br s, 2H).

5-(4-Chlorophenyl)-2H-1,2,4-triazol-3-amine (11d). Following general procedure A, compound **11d** was isolated as a white solid. Yield 90%, mp = 229-230 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.75 (br s, 1H); 7.95-7.89 (dd, *J* = 8.6 Hz, 2H); 7.58-7.52 (dd, *J* = 8.6 Hz, 2H); 5.11 (br s, 2H).

5-(4-Methoxyphenyl)-2H-1,2,4-triazol-3-amine (11e). Following general procedure A, compound **11e** was isolated as pale yellow solid. Yield 87%, mp > 300 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 11.85 (br s, 1H); 7.80-7.78 (dd, *J* = 9 Hz, 2H); 6.96-6.94 (dd, *J* = 8.4 Hz, 2H); 5.98 (br s, 2H); 3.77 (s, 3H).

5-p-Tolyl-2H-1,2,4-triazol-3-amine (11f). Following general procedure A, compound **11f** was isolated as a white solid. Yield 78%, mp = 175-176 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.21 (br s, 1H); 7.78-7.73 (dd, *J* = 8.2 Hz, 2H); 7.21-7.17 (dd, *J* = 8 Hz, 2H); 5.91 (br s, 2H); 2.31 (s, 3H).

5-(Furan-2-yl)-2H-1,2,4-triazol-3-amine (11g). Following general procedure A, compound **11g** was isolated as a white solid. Yield 82%, mp >300 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.11 (br s, 1H); 7.60 (d, *J* = 1 Hz, 1H); 6.67 (d, *J* = 3.4 Hz, 1H); 6.53 (dd, *J* = 3.4 Hz, 1H); 6.07 (br s, 2H).

5-(Pyridin-3-yl)-2H-1,2,4-triazol-3-amine (11h). Following general procedure A, compound **11h** was isolated as a white solid. Yield 75%, mp = 203-204 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ:

12.23 (br s, 1H); 9.04-9.03 (d, $J = 1.6$ Hz, 1H); 8.54-8.52 (dd, $J = 6.4$ Hz, 1H); 8.18-8.15 (dt, $J = 8$ Hz, 1H); 7.44-7.41 (dd, $J = 8$ Hz, 1H); 6.17 (br s, 2H).

*General procedure B for the synthesis of compounds **11i-m***

A solution of *N*-cyanocarbonimidodithioic acid dimethyl ester (17.4 mmol), acetonitrile (10 mL), and appropriate amine (17.4 mmol) was refluxed for 2 h. After cooling to room temperature, hydrazine monohydrate (25.6 mmol) was added and the reaction mixture was further refluxed for additional 5 h. The solvent was evaporated under reduced pressure and the resulting product recrystallized from ethyl acetate.

*5-(Methylthio)-2H-1,2,4-triazol-3-amine (**11i**)*. Following general procedure B, compound **11i** was isolated as a white solid. Yield 90%, mp = 134 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 11.78 (br s, 1H); 5.95 (br s, 2H); 2.39 (s, 3H).

*5-Morpholino-2H-1,2,4-triazol-3-amine (**11j**)*. Following general procedure B, compound **11j** was isolated as a white solid. Yield 82%, mp = 161-162 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 10.98 (br s, 1H); 5.71 (br s, 2H); 3.64-3.59 (m, 4H); 3.13-3.08 (m, 4H).

*5-(4-Methylpiperazin-1-yl)-2H-1,2,4-triazol-3-amine (**11k**)*. Following general procedure B, compound **11k** was isolated as a pale yellow solid. Yield 80%, mp = 103-104 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 10.95 (br s, 1H); 5.72 (br s, 2H); 3.16-3.11 (m, 4H); 2.34-2.29 (m, 4H); 2.17 (s, 3H).

*N-benzyl-N-methyl-1H-1,2,4-triazole-3,5-diamine (**11l**)*. Following general procedure B, compound **11l** was isolated as a white solid. Yield 86%, mp = 163-164 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 10.85 (br s, 1H); 7.27-7.23 (m, 5H); 5.75 (br s, 2H); 4.42 (s, 2H); 2.70 (s, 3H).

*N,N-Diallyl-1H-1,2,4-triazole-3,5-diamine (**11m**)*. Following general procedure B, compound **11m** was isolated as a white solid. Yield 95%, mp = 244-245 °C. ^1H NMR (400 MHz, DMSO- d_6) δ : 10.75 (br s, 1H); 5.82-5.69 (m, 2H); 5.54 (br s, 2H); 5.16-5.02 (m, 4H); 3.81-3.78 (d, $J = 5.6$ Hz, 4H).

*General procedure C for the synthesis of ethyl 4,7-dihydro-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylates **12a-m***

A solution of aminotriazole **11a-m** (3.1 mmol), glacial acetic acid (5 mL), and diethyl ethoxymethylenemalonate (4.6 mmol) was refluxed for 3 h. After cooling to room temperature, the resulting precipitate was collected by filtration, washed with cold water and dried. The residue was stirred with cold ethyl ether (20 mL) and filtered to afford the desired product.

*Ethyl 4,7-dihydro-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (**12a**)*. Following general procedure C, compound **12a** was isolated as a white solid. Yield 76%, mp > 300 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 14.30 (br s, 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).

*Ethyl 4,7-dihydro-2-methyl-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (**12b**)*. Following general procedure C, compound **12b** was isolated as a pale yellow solid 70%, mp = 237 °C dec. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.9 (br s, 1H); 8.58 (s, 1H); 4.28-4.17 (q, *J* = 7.4 Hz, 2H); 2.38 (s, 3H); 1.31-1.27 (t, *J* = 7 Hz, 3H).

*Ethyl 4,7-dihydro-2-phenyl-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (**12c**)*. Following general procedure C, compound **12c** was isolated as a white solid. Yield 67%, mp > 300 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 14.00 (br s, 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 2-(4-chlorophenyl)-4,7-dihydro-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (**12d**)*. Following general procedure C, compound **12d** was isolated as a pale yellow solid. Yield 60%, mp > 300 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 13.41 (br s, 1H); 8.65 (s, 1H); 8.14-8.10 (dd, *J* = 8.4 Hz, 2H); 7.63-7.59 (dd, *J* = 8.6 Hz, 1H); 4.29-4.22 (q, *J* = 7 Hz, 2H); 1.33-1.25 (t, *J* = 7.4 Hz, 3H).

*Ethyl 2-(4-methoxyphenyl)-4,7-dihydro-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (**12e**)*. Following general procedure C, compound **12e** was isolated as a pale yellow solid. Yield 65%, mp > 300 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.25 (br s, 1H); 8.61 (s, 1H); 8.06-8.04 (dd,

$J = 8.8$ Hz, 2H); 7.10-7.08 (dd, $J = 8.8$ Hz, 1H); 4.25-4.23 (q, $J = 7.2$ Hz, 2H); 3.83 (s, 3H); 1.31-1.27 (t, $J = 7.2$ Hz, 3H).

Ethyl 4,7-dihydro-7-oxo-2-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (12f). Following general procedure C, compound **12f** was isolated as a pale yellow solid. Yield 70%, mp = 268 °C dec. ^1H NMR (200 MHz, DMSO- d_6) δ : 13.91 (br s, 1H); 8.63 (s, 1H); 8.03-7.99 (dd, $J = 8$ Hz, 2H); 7.37-7.32 (dd, $J = 8.2$ Hz, 2H); 4.27-4.23 (q, $J = 7$ Hz, 2H); 2.38 (s, 3H); 1.33-1.26 (t, $J = 7.4$ Hz, 3H).

Ethyl 2-(furan-2-yl)-4,7-dihydro-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (12g). Following general procedure C, compound **12g** was isolated as a white solid. Yield 75%, mp > 300 °C. ^1H NMR (200 MHz-DMSO- d_6) δ : 13.24 (br s, 1H); 8.59 (s, 1H); 7.88-7.87 (d, $J = 1$ Hz, 1H); 7.11-7.09 (d, $J = 3.4$ Hz, 1H); 6.67-6.65 (dd, $J = 3.4$ Hz, 1H); 4.23-4.20 (q, $J = 7.2$ Hz, 2H); 1.31-1.24 (t, $J = 7$ Hz, 3H).

Ethyl 4,7-dihydro-7-oxo-2-(pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (12h). Following general procedure C, compound **12h** was isolated as a white solid. Yield 65%, mp = 250 °C. ^1H NMR (400 MHz, DMSO- d_6) δ : 11.98 (br s, 1H); 9.27 (d, $J = 1.6$ Hz, 1H); 8.74-8.72 (dd, $J = 6.4$ Hz, 1H); 8.67 (s, 1H); 8.48-8.46 (dt, $J = 8$ Hz, 1H); 7.62-7.60 (dd, $J = 8$ Hz, 1H); 4.28-4.23 (q, $J = 7.2$ Hz, 2H); 1.31-1.27 (t, $J = 7.2$ Hz, 3H).

Ethyl 4,7-dihydro-2-(methylthio)-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (12i). Following general procedure C, compound **12i** was isolated as a white solid. Yield 70%, mp = 277 °C dec. ^1H NMR (200 MHz, DMSO- d_6) δ : 12.41 (br s, 1H); 8.56 (s, 1H); 4.27-4.20 (q, $J = 7$ Hz, 2H); 2.60 (s, 3H); 1.31-1.24 (t, $J = 7.2$ Hz, 3H).

Ethyl 4,7-dihydro-2-morpholino-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (12j). Following general procedure C, compound **12j** was isolated as a white solid. Yield 75%, mp = 283-284 °C dec. ^1H NMR (200 MHz, DMSO- d_6) δ : 12.5 (br s, 1H); 8.45 (s, 1H); 4.23-4.20 (q, $J = 7.2$ Hz, 2H); 3.70-3.66 (m, 4H); 3.41-3.37 (m, 4H); 1.30-1.23 (t, $J = 7.2$ Hz, 3H).

Ethyl 4,7-dihydro-2-(4-methylpiperazin-1-yl)-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (12k). Following general procedure C, compound **12k** was isolated as a white solid. Yield 78%, mp = 220 °C dec. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 9.95 (br s, 1H); 8.41 (s, 1H); 4.17-4.07 (q, *J* = 7 Hz, 2H); 3.78-3.45 (m, 4H); 2.76 (s, 3H); 2.52-2.49 (m, 4H); 1.26-1.19 (t, *J* = 7.2 Hz, 3H).

Ethyl 2-(N-benzyl-N-methylamino)-4,7-dihydro-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylates (12l). Following general procedure C, compound **12l** was isolated as a white solid. Yield 69%, mp = 284 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 13.37 (br s, 1H); 8.42 (s, 1H); 7.30-7.28 (m, 5H); 4.64 (s, 2H); 4.25-4.17 (q, *J* = 7 Hz, 2H); 2.95 (s, 3H); 1.30-1.23 (t, *J* = 7 Hz, 3H).

Ethyl 2-(diallylamino)-4,7-dihydro-7-oxo-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (12m). Following general procedure C, compound **12m** was isolated as a white solid. Yield 73%, mp = 240-241 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 13.91 (br s, 1H); 8.41 (s, 1H); 5.93-5.76 (m, 2H); 5.21-5.13 (m, 4H); 4.26-4.16 (q, *J* = 7.2 Hz, 2H); 4.02-3.99 (d, *J* = 6.2 Hz, 4H); 1.30-1.23 (t, *J* = 7.2 Hz, 3H).

General procedure D for the synthesis of ethyl 4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylates 13a-m

To a solution of appropriate carboxylic acid ethyl ester **12a-m** (0.54 mmol) in anhydrous *N,N*-dimethylformamide was added K₂CO₃ (1.62 mmol), and the mixture was stirred at 70 °C for 1 h. 1-Pentyl bromide (1.62 mmol) was added, and the mixture was heated at 100 °C for 16 h. The reaction mixture was cooled to room temperature, the solvent was evaporated under reduced pressure and the residue partitioned between water (20 mL) and ethyl acetate (60 mL). The organic phase was dried with Na₂SO₄, and after removal of the solvent, the residue was purified by flash column chromatography, eluting with ethyl acetate/petroleum ether or ethyl acetate/methanol.

Ethyl 4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13a). Following general procedure D, compound **13a** was isolated as a white solid. Eluent: petroleum ether-ethyl ac-

etate 3/2. Yield 75%, mp = 85-86 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.88 (s, 1H), 8.33 (s, 1H), 4.32-4.22 (m, 4H), 1.95-1.79 (m, 2H), 1.33-1.26 (m, 7H), 0.89-0.82 (t, *J* = 6.8 Hz, 3H).

*Ethyl 4,7-dihydro-2-methyl-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (13b).*

Following general procedure D, compound **13b** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 2/3. Yield 78%, mp = 151-152 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.82 (s, 1H), 4.30-4.21 (m, 4H), 2.39 (s, 3H), 1.91-1.77 (m, 2H), 1.32-1.25 (m, 7H), 0.89-0.84 (t, *J* = 6.8 Hz, 3H).

*Ethyl 4,7-dihydro-7-oxo-4-pentyl-2-phenyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (13c).*

Following general procedure D, compound **13c** was isolated as a pale yellow solid. Eluent: petroleum ether-ethyl acetate 2/3. Yield 75%, mp = 112-113 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.87 (s, 1H), 8.16-8.11 (m, 2H), 7.56-7.53 (m, 3H), 4.37-4.23 (m, 4H), 1.93-1.81 (m, 2H), 1.37-1.26 (m, 7H), 0.91-0.85 (t, *J* = 6.6 Hz, 3H).

*Ethyl 2-(4-chlorophenyl)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (13d).* Following general procedure D, compound **13d** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 7/3. Yield 78%, mp = 155-156 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.88 (s, 1H); 8.16-8.11 (dd, *J* = 8.6 Hz, 2H); 7.64-7.60 (dd, *J* = 8.4 Hz, 2H); 4.32-4.23 (m, 4H); 1.96-1.83 (m, 2H); 1.36-1.27 (m, 7H); 0.91-0.84 (t, *J* = 6.8 Hz, 3H).

*Ethyl 4,7-dihydro-2-(4-methoxyphenyl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (13e).* Following general procedure D, compound **13e** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 80%, mp = 147-148 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.85 (s, 1H); 8.07-8.05 (dd, *J* = 8.8 Hz, 2H); 7.10-7.08 (dd, *J* = 8.8 Hz, 2H); 4.33-4.25 (m, 4H); 3.83 (s, 1H); 1.94-1.81 (m, 2H); 1.34-1.29 (m, 7H); 0.90-0.86 (t, *J* = 7.2 Hz, 3H).

*Ethyl 4,7-dihydro-7-oxo-4-pentyl-2-*p*-tolyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxylate (13f).*

Following general procedure D, compound **13f** was isolated as a pale yellow solid. Eluent: petroleum ether-ethyl acetate 1/4. Yield 82%, mp = 216-217 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 8.86

(s, 1H), 8.03-8.01 (dd, $J = 8$ Hz, 2H), 7.36-7.34 (dd, $J = 7.6$ Hz, 2H), 4.32-4.25 (m, 4H), 2.38 (s, 3H), 1.91-1.72 (m, 2H), 1.35-1.29 (m, 7H), 0.89-0.86 (t, $J = 6.8$ Hz, 3H).

Ethyl 2-(furan-2-yl)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13g). Following general procedure D, compound **13g** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 2/3. Yield 77%, mp = 151-152 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 8.86 (s, 1H); 7.94-7.93 (d, $J = 1.6$ Hz, 1H); 7.20-7.18 (d, $J = 3.4$ Hz, 1H); 6.72-6.69 (dd, $J = 3.4$ Hz, 1H); 4.33-4.23 (m, 4H); 1.93-1.79 (m, 2H); 1.33-1.26 (m, 7H); 0.90-0.84 (t, $J = 6.6$ Hz, 3H).

Ethyl 4,7-dihydro-7-oxo-4-pentyl-2-(pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13h). Following general procedure D, compound **13h** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 77%, mp = 104-105 °C. ^1H NMR (400 MHz, DMSO- d_6) δ : 9.46 (d, $J = 1.6$ Hz, 1H); 8.72-8.70 (dd, $J = 6.4$ Hz, 1H); 8.57-8.55 (dt, $J = 8$ Hz, 1H); 8.47 (s, 1H); 7.42-7.39 (dd, $J = 8$ Hz, 1H); 4.45-4.39 (q, $J = 7.2$ Hz, 2H); 4.34-4.30 (t, $J = 7.6$ Hz, 2H); 2.01-1.97 (m, 2H); 1.43-1.39 (m, 7H); 0.95-0.92 (t, $J = 7.2$ Hz, 3H).

Ethyl 4,7-dihydro-2-(methylthio)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13i). Following general procedure D, compound **13i** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 81%, mp = 120-121 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 8.79 (s, 1H); 4.30-4.20 (m, 4H); 2.61 (s, 3H); 1.91-1.72 (m, 2H); 1.32-1.25 (m, 7H); 0.89-0.84 (t, $J = 6.8$ Hz, 3H).

Ethyl 4,7-dihydro-2-morpholino-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13j). Following general procedure D, compound **13j** was isolated as a white solid. Eluent: ethyl acetate-methanol 9.5/0.5. Yield 82%, mp = 113-114 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 8.68 (s, 1H); 4.30-4.13 (m, 4H); 3.71-3.66 (m, 4H); 3.43-3.38 (m, 4H); 1.95-1.72 (m, 2H); 1.31-1.24 (m, 7H); 0.89-0.83 (t, $J = 6.6$ Hz, 3H).

Ethyl 4,7-dihydro-2-(4-methylpiperazin-1-yl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13k). Following general procedure D, compound **13k** was isolated as a white solid. Eluent: ethyl acetate-methanol 9/1. Yield 70%, mp = 183-184 °C. ^1H NMR (200 MHz, DMSO- d_6)

δ : 8.67 (s, 1H); 4.26-4.16 (m, 4H); 3.45-3.42 (m, 4H); 2.41-2.37 (m, 4H); 2.20 (s, 3H); 1.96-1.79 (m, 2H); 1.31-1.24 (m, 7H); 0.89-0.82 (t, $J = 6.8$ Hz, 3H).

Ethyl 2-(N-benzyl-N-methylamino)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13l). Following general procedure D, compound **13l** was isolated as a white solid. petroleum ether-ethyl acetate 1/4. Yield 82%, mp = 77-78 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 8.66 (s, 1H); 7.34-7.29 (m, 5H); 4.65 (s, 2H); 4.29-4.15 (m, 4H); 2.95 (s, 3H); 1.91-1.78 (m, 2H); 1.31-1.24 (m, 7H); 0.88-0.81 (t, $J = 6.8$ Hz, 3H).

Ethyl 2-(diallylamino)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylate (13m). Following general procedure D, compound **13m** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/4. Yield 81%, mp = 70-72 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 8.65 (s, 1H); 5.87-5.76 (m, 2H); 5.23-5.14 (m, 4H); 4.25-4.16 (m, 4H); 4.03-4.00 (d, $J = 5.8$ Hz, 2H); 1.91-1.75 (m, 2H); 1.32-1.24 (m, 7H); 0.89-0.82 (t, $J = 7$ Hz, 3H).

General procedure E for the synthesis of carboxylic acid derivatives 14a-m

A solution of appropriate carboxylic acid ethyl ester **13a-m** (1.27 mmol), methanol (20 mL), and NaOH 10% (10 mL) was stirred at room temperature for 6 h. The suspension was acidified with 10% HCl, the precipitate was collected by filtration and washed with cold water to afford the desired product.

4,7-Dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14a). Following general procedure E, compound **14a** was isolated as a white solid. Yield 72%, mp = 215 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 12.12 (br s, 1H); 8.73 (s, 1H); 8.28 (s, 1H); 4.27-4.20 (t, $J = 7$ Hz, 2H); 1.84-1.77 (m, 2H); 1.32-1.25 (m, 4H); 0.88-0.81 (t, $J = 6.6$ Hz, 3H).

4,7-Dihydro-2-methyl-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14b). Following general procedure E, compound **14b** was isolated as a white solid. Yield 85%, mp = 164-165 °C. ^1H NMR (200 MHz, DMSO- d_6) δ : 12.37 (br s, 1H); 8.68 (s, 1H); 4.23-4.16 (t, $J = 7.2$ Hz, 2H); 2.38 (s, 3H); 1.81-1.73 (m, 2H); 1.30-1.25 (m, 4H); 0.88-0.81 (t, $J = 6.8$ Hz, 3H).

4,7-Dihydro-7-oxo-4-pentyl-2-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14c).

Following general procedure E, compound **14c** was isolated as a white solid. Yield 91%, mp >300 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.24 (br s, 1H); 8.63 (s, 1H); 8.14-8.10 (m, 2H); 7.54-7.51 (m, 3H); 4.30-4.26 (t, *J* = 7 Hz, 2H); 1.98-1.81 (m, 2H); 1.39-1.27 (m, 4H); 0.89-0.82 (t, *J* = 6.6 Hz, 3H).

2-(4-Chlorophenyl)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14d).

Following general procedure E, compound **14d** was isolated as a white solid. Yield (87%), mp = 204-205 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.81 (br s, 1H); 8.92 (s, 1H); 8.16-8.12 (dd, *J* = 8.6 Hz, 2H); 7.64-7.60 (dd, *J* = 8.6 Hz, 2H); 4.37-4.30 (t, *J* = 7.2 Hz, 2H); 1.81-1.75 (m, 2H); 1.36-1.28 (m, 4H); 0.90-0.84 (t, *J* = 6.6 Hz, 3H).

4,7-Dihydro-2-(4-methoxyphenyl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14e).

Following general procedure E, compound **14e** was isolated as a white solid. Yield 86%, mp = 200-201 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.79 (br s, 1H); 8.90 (s, 1H); 8.08-8.06 (dd, *J* = 8.8 Hz, 2H); 7.11-7.08 (dd, *J* = 8.4 Hz, 2H); 4.35-4.31 (t, *J* = 7.2 Hz, 2H); 1.89-1.86 (m, 2H); 1.34-1.32 (m, 4H); 0.89-0.86 (t, *J* = 6.4 Hz, 3H).

4,7-Dihydro-7-oxo-4-pentyl-2-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14f).

Following general procedure E, compound **14f** was isolated as a pale yellow solid. Yield 87%, mp = 207-208 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.65 (br s, 1H); 8.87 (s, 1H); 8.05-8.01 (dd, *J* = 8.4 Hz, 2H); 7.37-7.33 (dd, *J* = 8 Hz, 2H); 4.34-4.31 (t, *J* = 6.8 Hz, 2H); 2.38 (s, 3H); 1.96-1.79 (m, 2H); 1.35-1.30 (m, 4H); 0.90-0.84 (t, *J* = 6.6 Hz, 3H).

2-(Furan-2-yl)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14g).

Following general procedure E, compound **14g** was isolated as a pale yellow solid. Yield 87%, mp = 187-188 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.81 (br s, 1H); 8.90 (s, 1H); 7.95-7.94 (d, *J* = 1.8 Hz, 1H); 7.21-7.19 (d, *J* = 3.4 Hz, 1H); 6.72-6.70 (dd, *J* = 3.4 Hz, 1H); 4.32-4.26 (t, *J* = 6.8 Hz, 2H); 1.87-1.81 (m, 2H); 1.34-1.27 (m, 4H); 0.90-0.83 (t, *J* = 6.6 Hz, 3H).

4,7-Dihydro-7-oxo-4-pentyl-2-(pyridin-3-yl)-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14h). Following general procedure E, compound **14h** was isolated as a white solid. Yield 86%, mp = 158-159 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.84 (br s, 1H); 9.28 (d, *J* = 1.6 Hz, 1H); 8.94 (s, 1H); 8.74-8.73 (dd, *J* = 6.4 Hz, 1H); 8.48-8.45 (dt, *J* = 8 Hz, 1H); 7.61-7.58 (dd, *J* = 8 Hz, 1H); 4.37-4.33 (t, *J* = 7.2 Hz, 2H); 1.90-1.87 (m, 2H); 1.37-1.32 (m, 4H); 0.89-0.86 (t, *J* = 6.8 Hz, 3H).

4,7-Dihydro-2-(methylthio)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14i). Following general procedure E, compound **14i** was isolated as a white solid. Yield 98%, mp = 165-166 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.51 (br s, 1H); 8.78 (s, 1H); 4.22-4.19 (t, *J* = 6.8 Hz, 2H); 2.61 (s, 3H); 1.84-1.77 (m, 2H); 1.35-1.26 (m, 4H); 0.88-0.83 (t, *J* = 6.4 Hz, 3H).

4,7-Dihydro-2-morpholino-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14j). Following general procedure E, compound **14j** was isolated as a white solid. Yield 98%, mp = 276-277 °C. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.69 (br s, 1H); 8.38 (s, 1H); 4.13-4.09 (t, *J* = 6.8 Hz, 2H); 3.70-3.66 (m, 4H); 3.41-3.36 (m, 4H); 1.78-1.73 (m, 2H); 1.30-1.25 (m, 4H); 0.88-0.81 (t, *J* = 6.6 Hz, 3H).

4,7-Dihydro-2-(4-methylpiperazin-1-yl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14k). Following general procedure E, compound **14k** was isolated as a white solid. Yield 81%, mp = 225 °C dec. ¹H NMR (200 MHz, DMSO-*d*₆) δ: 12.72 (br s, 1H); 8.73 (s, 1H); 4.21-4.17 (t, *J* = 6.6 Hz, 2H); 3.50-3.47 (m, 4H); 3.38-3.33 (m, 4H); 2.28 (s, 3H); 1.81-1.77 (m, 2H); 1.29-1.26 (m, 4H); 0.88-0.82 (t, *J* = 6.6 Hz, 3H).

2-(N-Benzyl-N-methylamino)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14l). Following general procedure E, compound **14l** was isolated as a white solid. Yield 94%, mp = 130 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.71 (br s, 1H); 8.76 (s, 1H); 7.33-7.24 (m, 5H); 4.64 (s, 2H); 4.22-4.19 (t, *J* = 7.2 Hz, 2H); 2.95 (s, 3H); 1.81-1.78 (m, 2H); 1.29-1.24 (m, 4H); 0.84-0.81 (t, *J* = 6.8 Hz, 3H).

2-(Diallylamino)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxylic acid (14m). Following general procedure E, compound **14m** was isolated as a white solid. Yield 95%,

mp = 142-143 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 12.95 (br s, 1H); 8.77 (s, 1H); 5.92-5.78 (m, 2H); 5.23-5.15 (m, 4H); 4.24-4.17 (t, *J* = 6.8 Hz, 2H); 4.05-4.02 (d, *J* = 5.6 Hz, 4H); 1.84-1.79 (m, 2H); 1.30-1.26 (m, 4H); 0.88-0.82 (t, *J* = 6.6 Hz, 3H).

General procedure F for the synthesis of adamantan-1-yl-carboxamide derivatives 16, 18, 21, 23, 25, 28-30, 32, 35, 39, 41

To a stirred solution of the appropriate carboxylic acid (0.72 mmol) in anhydrous dimethylformamide (10 mL) was added diisopropylethylamine (3.24 mmol). The resulting solution was stirred at room temperature for 10 min before addition of *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU, 1.08 mmol). The solution was stirred for another 3 h at room temperature. 1-Adamantylamine or 3,5-dimethyl-1-adamantylamine (1.08 mmol) was added and stirring was continued for 16 h. The solvent was removed under reduce pressure, the residue was dissolved in ethyl acetate (50 mL), then washed with saturated aqueous sodium bicarbonate (10 mL), water (10 mL), and brine (10 mL). The organic extract was dried (Na₂SO₄), filtered, and evaporated to dryness, and the residue was purified by flash chromatography on silica gel, eluting with ethyl acetate-petroleum ether.

*4,7-Dihydro-N-(3,5-dimethyladamantan-1-yl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (16)*. Following general procedure F, compound **16** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 7/3. Yield 60%, mp = 182 °C. MS (ESI): *m/z* 412.2 (*M*+*H*). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.82 (s, 1H); 8.67 (br s, 1H); 8.38 (s, 1H); 4.33-4.30 (t, *J* = 7 Hz, 2H); 2.18-2.04 (m, 1H); 1.90-1.65 (m, 9H); 1.38-1.24 (m, 9H); 0.89-0.82 (m, 9H). Anal. for C₂₃H₃₃N₅O₂. Calcd: C, 67.12; H, 8.08; N, 17.02. Found: C, 67.22; H, 8.18; N, 17.051.

*N-Adamantan-yl-4,7-dihydro-2-methyl-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (18)*. Following general procedure F, compound **18** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 3/2. Yield 56%, mp = 189-190 °C. MS (ESI): *m/z* 398.2 (*M*+*H*). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.78 (s, 1H); 8.68 (br s, 1H); 4.30-4.22 (t, *J* = 7 Hz, 2H); 2.41 (s,

3H); 2.05 (s, 9H); 1.93-1.81 (m, 2H); 1.66 (s, 6H); 1.34-1.24 (m, 4H); 0.89-0.82 (t, $J = 6.4$ Hz, 3H).

Anal. for $C_{22}H_{31}N_5O_2$. Calcd: C, 66.47; H, 7.86; N, 17.62. Found: C, 66.22; H, 7.73; N, 17.37.

*4,7-Dihydro-N-(3,5-dimethyladamantan-1-yl)-7-oxo-4-pentyl-2-phenyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (21)*. Following general procedure F, compound **21** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 55%, mp = 96 °C. MS (ESI): m/z 488.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 8.81 (s, 1H); 8.72 (br s, 1H); 8.16-8.13 (m, 2H); 7.57-7.54 (m, 3H); 4.39-4.35 (t, $J = 7$ Hz, 2H); 2.19-2.07 (m, 1H); 1.93-1.68 (m, 9H); 1.39-1.18 (m, 9H); 0.91-0.84 (m, 9H). Anal. for $C_{29}H_{37}N_5O_2$. Calcd: C, 71.43; H, 7.65; N, 14.36. Found: C, 71.34; H, 7.60; N, 14.29.

*N-Adamantan-1-yl-2-(4-chlorophenyl)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (23)*. Following general procedure F, compound **23** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 53%, mp = 213 °C. MS (ESI): m/z 494.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 8.85 (s, 1H); 8.69 (br s, 1H); 8.16-8.12 (dd, $J = 8.6$ Hz, 2H); 7.65-7.61 (dd, $J = 8.4$ Hz, 2H); 4.38-4.34 (t, $J = 7.2$ Hz, 2H); 2.06 (s, 9H); 1.97-1.84 (m, 2H); 1.68 (s, 6H); 1.36-1.31 (m, 4H); 0.90-0.84 (t, $J = 6.2$ Hz, 3H). Anal. for $C_{27}H_{32}ClN_5O_2$. Calcd: C, 65.64; H, 6.53; N, 14.18. Found: C, 65.59; H, 6.42; N, 14.29.

*N-Adamantan-1-yl-4,7-dihydro-2-(4-methoxyphenyl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (25)*. Following general procedure F, compound **25** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 46%, mp = 176 °C. MS (ESI): m/z 490.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 8.81 (s, 1H); 8.73 (br s, 1H); 8.09-8.05 (dd, $J = 8.8$ Hz, 2H); 7.12-7.08 (dd, $J = 9$ Hz, 2H); 4.37-4.34 (t, $J = 7.2$ Hz, 2H); 3.83 (s, 3H); 2.06 (s, 9H); 1.96-1.81 (m, 2H); 1.67 (s, 6H); 1.36-1.32 (m, 4H); 0.91-0.85 (t, $J = 6.6$ Hz, 3H). Anal. for $C_{28}H_{35}N_5O_3$. Calcd: C, 68.69; H, 7.21; N, 14.30. Found: C, 68.75; H, 7.28; N, 14.39.

*N-Adamantan-1-yl-4,7-dihydro-7-oxo-4-pentyl-2-p-tolyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (28)*. Following general procedure F, compound **28** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 44%, mp = 229 °C. MS (ESI): m/z 474.2 (M+H). 1H

NMR (200 MHz, DMSO-*d*₆) δ : 8.82 (s, 1H); 8.72 (br s, 1H); 8.05-8.01 (dd, *J* = 8 Hz, 2H); 7.38-7.34 (dd, *J* = 8.2 Hz, 2H); 4.38-4.33 (t, *J* = 7 Hz, 2H); 2.38 (s, 3H); 2.06 (s, 9H); 1.90-1.83 (m, 2H); 1.68 (s, 6H); 1.36-1.30 (m, 4H); 0.90-0.84 (t, *J* = 6.4 Hz, 3H). Anal. for C₂₈H₃₅N₅O₂. Calcd: C, 71.01; H, 7.45; N, 14.79. Found: C, 71.15; H, 7.52; N, 15.75.

N-Adamantan-1-yl-2-(furan-2-yl)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (**29**). Following general procedure F, compound **29** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 54%, mp = 175 °C. MS (ESI): *m/z* 450.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ : 8.82 (s, 1H); 8.67 (br s, 1H); 7.96-7.94 (d, *J* = 1.6 Hz, 1H); 7.22-7.19 (d, *J* = 3.4 Hz, 1H); 6.73-6.70 (dd, *J* = 3.4 Hz, 1H); 4.37-4.29 (t, *J* = 6.8 Hz, 2H); 2.06 (s, 9H); 1.96-1.82 (m, 2H); 1.67 (s, 6H); 1.37-1.30 (m, 4H); 0.90-0.84 (t, *J* = 6.2 Hz, 3H). Anal. for C₂₅H₃₁N₅O₃. Calcd: C, 66.79; H, 6.95; N, 15.58. Found: C, 66.75; H, 6.96; N, 15.61.

N-Adamantan-1-yl-4,7-dihydro-7-oxo-4-pentyl-2-(pyridin-3-yl)-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (**30**). Following general procedure F, compound **30** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 45%, mp = 160 °C. MS (ESI): *m/z* 461.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ : 9.29 (d, *J* = 1.6 Hz, 1H); 8.87 (s, 1H); 8.76-8.72 (dd, *J* = 6.4 Hz, 1H); 8.68 (br s, 1H); 8.49-8.43 (dt, *J* = 7.8 Hz, 1H); 7.63-7.57 (dd, *J* = 7.8 Hz, 1H); 4.41-4.36 (t, *J* = 6.8 Hz, 2H); 2.07 (s, 9H); 1.99-1.85 (m, 2H); 1.74-1.59 (m, 8H); 1.36-1.32 (m, 2H); 0.91-0.84 (t, *J* = 6 Hz, 3H). Anal. for C₂₆H₃₂N₆O₂. Calcd: C, 67.80; H, 7.00; N, 18.25. Found: C, 67.74; H, 7.23; N, 18.33.

N-Adamantan-1-yl-4,7-dihydro-2-(methylthio)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (**32**). Following general procedure F, compound **32** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 55%, mp = 161 °C. MS (ESI): *m/z* 430.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ : 8.75 (s, 1H); 8.67 (br s, 1H); 4.27-4.22 (t, *J* = 7 Hz, 2H); 2.61 (s, 3H); 2.05 (s, 9H); 1.91-1.74 (m, 2H); 1.67 (s, 6H); 1.33-1.25 (m, 4H); 0.89-0.83 (t, *J* = 6.4 Hz, 3H). Anal. for C₂₂H₃₁N₅O₂S. Calcd: C, 61.51; H, 7.27; N, 16.30. Found: C, 61.34; H, 7.29; N, 16.45.

N-Adamantan-1-yl-4,7-dihydro-2-morpholino-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (**35**). Following general procedure F, compound **35** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 2/3. Yield 40%, mp = 148 °C. MS (ESI): *m/z* 469.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.81 (br s, 1H); 8.64 (s, 1H); 4.08-3.99 (t, *J* = 6.8 Hz, 2H); 3.71-3.67 (m, 4H); 3.44-3.40 (m, 4H); 2.03 (s, 9H); 1.85-1.70 (m, 2H); 1.66 (s, 6H); 1.33-1.26 (m, 4H); 0.89-0.83 (t, *J* = 6.6 Hz, 3H). Anal. for C₂₅H₃₆N₆O₃. Calcd: C, 64.08; H, 7.74; N, 17.93. Found: C, 64.19; H, 7.79; N, 17.90.

N-Adamantan-1-yl-2-(*N*-benzyl-*N*-methyl-amino)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (**39**). Following general procedure F, compound **39** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 2/3. Yield 60%, mp = 139 °C. MS (ESI): *m/z* 519.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.85 (br s, 1H); 8.61 (s, 1H); 7.34-7.29 (m, 5H); 4.65 (s, 2H); 4.24-4.19 (t, *J* = 7 Hz, 2H); 2.97 (s, 3H); 2.03 (s, 9H); 1.87-1.75 (m, 2H); 1.66 (s, 6H); 1.30-1.26 (m, 4H); 0.88-0.82 (t, *J* = 6.2 Hz, 3H). Anal. for C₂₉H₃₈N₆O₂. Calcd: C, 69.29; H, 7.62; N, 16.72. Found: C, 69.32; H, 7.77; N, 16.68.

N-Adamantan-1-yl-2-(diallylamino)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide (**41**). Following general procedure F, compound **41** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 45%, mp = 110 °C. MS (ESI): *m/z* 479.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.84 (br s, 1H); 8.60 (s, 1H); 5.87-5.78 (m, 2H); 5.24-5.14 (m, 4H); 4.22-4.16 (t, *J* = 6.8 Hz, 2H); 4.04-4.01 (d, *J* = 5.6 Hz, 4H); 2.03 (s, 9H); 1.90-1.72 (m, 2H); 1.66 (s, 6H); 1.32-1.25 (m, 4H); 0.88-0.82 (t, *J* = 6.6 Hz, 3H). Anal. for C₂₇H₃₈N₆O₂. Calcd: C, 67.75; H, 8.00; N, 17.56. Found: C, 67.71; H, 7.98; N, 17.49.

General procedure G for the synthesis of N-cyclohexyl/cycloheptyl carboxamide derivatives 15, 17, 19, 20, 22, 24, 26, 27, 31, 33, 34, 36-38, 40

To a solution of the appropriate carboxylic acid (0.57 mmol) in DMF, 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide (EDCI, 0.85 mmol) and 1-hydroxybenzotriazole (HOBt, 0.85 mmol) were added and the reaction was stirred at room temperature for 10 min before adding the suitable

amine (0.85 mmol) and the stirring continued at the same temperature for 16h. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate (30 mL), washed with aqueous bicarbonate (10 mL), water (10 mL) and brine (10 mL). The organic extract was dried, filtered and concentrated and resulting products were purified by flash chromatography on silica gel, eluting with ethyl acetate and petroleum ether or ethyl acetate and methanol.

N-Cycloheptyl-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (15).

Following general procedure G, compound **15** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 2/3. Yield 46%, mp = 149 °C. MS (ESI): m/z 346.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.89 (s, 1H); 8.85-8.83 (br d, *J* = 7.8 Hz, 1H); 8.39 (s, 1H); 4.34-4.30 (t, *J* = 7 Hz, 2H); 4.17-4.01 (m, 1H); 1.96-1.75 (m, 4H); 1.70-1.42 (m, 10H); 1.35-1.24 (m, 4H); 0.88-0.83 (t, *J* = 6.4 Hz, 3H). Anal. for C₁₈H₂₇N₅O₂. Calcd: C, 62.58; H, 7.88; N, 20.27. Found: C, 62.51; H, 7.82; N, 20.39.

N-Cyclohexyl-4,7-dihydro-2-methyl-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (17). Following general procedure G, compound **17** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 2/3. Yield 41%, mp = 120 °C. MS (ESI): m/z 346.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.83 (s, 1H); 8.81-8.77 (br d, *J* = 7.8 Hz, 1H); 4.31-4.24 (t, *J* = 7 Hz, 2H); 3.91-3.78 (m, 1H); 2.41 (s, 3H); 1.94-1.54 (m, 6H); 1.43-1.24 (m, 10H); 0.89-0.82 (t, *J* = 6.6 Hz, 3H). Anal. for C₁₈H₂₇N₅O₂. Calcd: C, 62.58; H, 7.88; N, 20.27. Found: C, 62.61; H, 7.59; N, 20.32.

N-Cyclohexyl-4,7-dihydro-7-oxo-4-pentyl-2-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (19). Following general procedure G, compound **19** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 55%, mp = 161 °C. MS (ESI): m/z 408.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.90 (s, 1H); 8.85-8.81 (br d, *J* = 8 Hz, 1H); 8.17-8.12 (m, 2H); 7.57-7.54 (m, 3H); 4.40-4.35 (t, *J* = 7 Hz, 2H); 3.94-3.81 (m, 1H); 1.91-1.58 (m, 6H); 1.39-1.28 (m, 10H); 0.90-0.84 (t, *J* = 6.4 Hz, 3H). Anal. for C₂₃H₂₉N₅O₂. Calcd: C, 67.79; H, 7.17; N, 17.19. Found: C, 67.68; H, 6.94; N, 17.25.

N-Cycloheptyl-4,7-dihydro-7-oxo-4-pentyl-2-phenyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (20). Following general procedure G, compound **20** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 7/3. Yield 48%, mp = 155 °C. MS (ESI): m/z 422.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.90-8.98 (m, 2H); 8.15-8.12 (m, 2H); 7.57-7.54 (m, 3H); 4.40-4.36 (t, *J* = 7 Hz, 2H); 4.15-4.03 (m, 1H); 1.93-1.82 (m, 4H); 1.71-1.44 (m, 10H); 1.36-1.30 (m, 4H); 0.91-0.85 (t, *J* = 6.4 Hz, 3H). Anal. for C₂₄H₃₁N₅O₂. Calcd: C, 68.38; H, 7.41; N, 16.61. Found: C, 68.22; H, 7.55; N, 16.55.

2-(4-Chlorophenyl)-N-cyclohexyl-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (22). Following general procedure G, compound **22** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 4/1. Yield 53%, mp = 212 °C. MS (ESI): m/z 442.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.90 (s, 1H); 8.83-8.79 (br d, *J* = 8.2 Hz, 1H); 8.16-8.12 (dd, *J* = 8.4 Hz, 2H); 7.65-7.61 (dd, *J* = 8.4 Hz, 2H); 4.41-4.35 (t, *J* = 7.2 Hz, 2H); 3.97-3.82 (m, 1H); 2.41 (s, 3H); 1.90-1.57 (m, 6H); 1.39-1.23 (m, 10H); 0.90-0.84 (t, *J* = 6.4 Hz, 3H). Anal. for C₂₃H₂₈ClN₅O₂. Calcd: C, 62.51; H, 6.39; N, 15.85. Found: C, 62.59; H, 6.20; N, 15.72.

N-Cyclohexyl-4,7-dihydro-2-(4-methoxyphenyl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (24). Following general procedure G, compound **24** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 7/3. Yield 53%, mp = 190 °C. MS (ESI): m/z 438.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.88 (s, 1H); 8.86 (br s, 1H); 8.10-8.05 (dd, *J* = 9.2 Hz, 2H); 7.13-7.08 (dd, *J* = 8.6 Hz, 2H); 4.39-4.35 (t, *J* = 7.2 Hz, 2H); 3.42-3.84 (m, 1H); 3.83 (s, 3H); 1.98-1.61 (m, 6H); 1.38-1.30 (m, 10H); 0.90-0.85 (t, *J* = 6.6 Hz, 3H). Anal. for C₂₄H₃₁N₅O₃. Calcd: C, 65.88; H, 7.14; N, 16.01. Found: C, 65.68; H, 7.22; N, 16.12.

N-Cyclohexyl-4,7-dihydro-7-oxo-4-pentyl-2-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (26). Following general procedure G, compound **26** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 7/3. Yield 50%, mp = 200 °C. MS (ESI): m/z 422.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.88 (s, 1H); 8.87-8.86 (br d, *J* = 7.8 Hz, 1H); 8.05-8.01 (dd, *J* = 8.4 Hz, 2H); 7.38-7.34 (dd, *J* = 8 Hz, 2H); 4.39-4.36 (t, *J* = 7 Hz, 2H); 3.93-3.81 (m, 1H); 2.38 (s, 3H);

1.89-1.58 (m, 6H); 1.36-1.25 (m, 10H); 0.90-0.84(t, $J = 6.4$ Hz, 3H). Anal. for $C_{24}H_{31}N_5O_2$. Calcd: C, 68.38; H, 7.41; N, 16.61. Found: C, 68.42; H, 7.45; N, 16.69.

N-Cycloheptyl-4,7-dihydro-7-oxo-4-pentyl-2-p-tolyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (27). Following general procedure G, compound **27** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 7/3. Yield 48%, mp = 195 °C. MS (ESI): m/z 436.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 8.91-8.90 (br d, $J = 7.8$ Hz, 1H); 8.87 (s, 1H); 8.05-8.01 (dd, $J = 8$ Hz, 2H); 7.38-7.34 (dd, $J = 7.8$ Hz, 2H); 4.39-4.36 (t, $J = 7$ Hz, 2H); 4.17-4.02 (m, 1H); 2.38 (s, 3H); 1.97-1.81 (m, 4H); 1.74-1.49 (m, 10H); 1.36-1.28 (m, 4H); 0.90-0.84(t, $J = 6.4$ Hz, 3H). Anal. for $C_{25}H_{33}N_5O_2$. Calcd: C, 68.94; H, 7.64; N, 16.08. Found: C, 68.74; H, 7.68; N, 16.12.

N-Cyclohexyl-4,7-dihydro-2-(methylthio)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (31). Following general procedure G, compound **31** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 7/3. Yield 50%, mp = 192 °C. MS (ESI): m/z 378.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 8.80-8.77 (m, 2H); 4.27-4.21 (t, $J = 6.8$ Hz, 2H); 3.87-3.75 (m, 1H); 2.60 (s, 3H); 1.91-1.59 (m, 6H); 1.42-1.21 (m, 10H); 0.88-0.81 (t, $J = 6.4$ Hz, 3H). Anal. for $C_{18}H_{27}N_5O_2S$. Calcd: C, 57.27; H, 7.21; N, 18.55. Found: C, 57.21; H, 7.25; N, 18.61.

N-Cyclohexyl-4,7-dihydro-2-morpholino-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (33). Following general procedure G, compound **33** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 44%, mp = 143 °C. MS (ESI): m/z 417.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 8.95-8.91 (d, $J = 7.8$ Hz, 1H); 8.70 (s, 1H); 4.24-4.20 (t, $J = 7$ Hz, 2H); 3.89-3.72 (m, 1H); 3.71-3.67 (m, 4H); 3.45-3.40 (m, 4H); 1.81-1.47 (m, 6H); 1.39-1.20 (m, 10H); 0.88-0.82 (t, $J = 6.4$ Hz, 3H). Anal. for $C_{21}H_{32}N_6O_3$. Calcd: C, 60.56; H, 7.74; N, 20.18. Found: C, 60.61; H, 7.69; N, 20.59.

N-Cycloheptyl-4,7-dihydro-2-morpholino-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (34). Following general procedure G, compound **34** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 50%, mp = 172 °C. MS (ESI): m/z 431.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 9.00-8.96 (br d, $J = 7.8$ Hz, 1H); 8.69 (s, 1H); 4.25-4.18 (t, $J = 6.8$

Hz, 2H); 4.15-4.02 (m, 1H); 3.71-3.67 (m, 4H); 3.45-3.40 (m, 4H); 1.89-1.73 (m, 4H); 1.66-1.48 (m, 10H); 1.32-1.24 (m, 4H); 0.88-0.82 (t, $J = 6.6$ Hz, 3H). Anal. for $C_{22}H_{34}N_6O_3$. Calcd: C, 61.37; H, 7.96; N, 19.52. Found: C, 61.46; H, 7.86; N, 19.58.

N-Cyclohexyl-4,7-dihydro-2-(4-methylpiperazin-1-yl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (36). Following general procedure G, compound **36** was isolated as a white solid. Eluent: ethyl acetate-methanol 4/1. Yield 40%, mp = 168 °C. MS (ESI): m/z 430.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 8.96-8.92 (br d, $J = 8$ Hz, 1H); 8.68 (s, 1H); 4.24-4.18 (t, $J = 6.6$ Hz, 2H); 3.89-3.78 (m, 1H); 3.47-3.42 (m, 4H); 2.40-2.36 (m, 4H); 2.21 (s, 3H); 1.81-1.49 (m, 6H); 1.41-1.22 (m, 10H); 0.89-0.82 (t, $J = 6.8$ Hz, 3H). Anal. for $C_{22}H_{35}N_7O_2$. Calcd: C, 61.51; H, 8.21; N, 22.83. Found: C, 61.54; H, 8.24; N, 22.69.

N-Cycloheptyl-4,7-dihydro-2-(4-methylpiperazin-1-yl)-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (37). Following general procedure G, compound **37** was isolated as a white solid. Eluent: ethyl acetate-methanol 4/1. Yield 40%, mp = 183 °C. MS (ESI): m/z 444.2 (M+H). 1H NMR (200 MHz, DMSO- d_6) δ : 9.00-8.97 (br d, $J = 7.8$ Hz, 1H); 8.67 (s, 1H); 4.24-4.17 (t, $J = 7$ Hz, 2H); 4.12-3.99 (m, 1H); 3.47-3.42 (m, 4H); 2.40-2.36 (m, 4H); 2.21 (s, 3H); 1.87-1.72 (m, 4H); 1.64-1.46 (m, 10H); 1.35-1.24 (m, 4H); 0.88-0.82 (t, $J = 6.6$ Hz, 3H). Anal. for $C_{23}H_{37}N_7O_2$. Calcd: C, 62.28; H, 8.41; N, 22.10. Found: C, 62.39; H, 8.49; N, 22.35.

2-(N-Benzyl-N-methylamino)-N-cyclohexyl-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (38). Following general procedure G, compound **38** was isolated as a white solid. Eluent: petroleum ether-ethyl acetate 1/1. Yield 62%, mp = 157 °C. MS (ESI): m/z 451.2 (M+H). 1H NMR (400 MHz, $CDCl_3$) δ : 9.17-9.15 (br d, $J = 7.6$ Hz, 1H); 8.47 (s, 1H); 7.33-7.24 (m, 5H); 4.73 (s, 2H); 4.18-4.14 (t, $J = 7.2$ Hz, 2H); 3.06 (s, 3H); 1.98-1.73 (m, 5H); 1.56-1.30 (m, 11H); 0.91-0.87 (t, $J = 6.8$ Hz, 3H). Anal. for $C_{25}H_{34}N_6O_2$. Calcd: C, 66.64; H, 7.61; N, 18.65. Found: C, 66.51; H, 7.68; N, 18.76.

N-Cyclohexyl-2-(diallylamino)-4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-a]pyrimidine-6-carboxamide (40). Following general procedure G, compound **40** was isolated as a white solid. Elu-

ent: petroleum ether-ethyl acetate 7/3. Yield 42%, mp = 78 °C. MS (ESI): m/z 427.2 (M+H). ¹H NMR (200 MHz, DMSO-*d*₆) δ: 8.99-8.95 (br d, *J* = 7.8 Hz, 1H); 8.66 (s, 1H); 5.87-5.79 (m, 2H); 5.23-5.14 (m, 4H); 4.25-4.18 (t, *J* = 7.2 Hz, 2H); 4.04-4.01 (d, *J* = 5.6 Hz, 4H); 3.96-3.82 (m, 1H); 1.89-1.55 (m, 6H); 1.43-1.24 (m, 10H); 0.88-0.81 (t, *J* = 6.2 Hz, 3H). Anal. for C₂₃H₃₄N₆O₂. Calcd: C, 64.76; H, 8.03; N, 19.70. Found: C, 64.49; H, 7.96; N, 19.44.

Pharmacology

The affinity and the functionality of the novel compounds were studied by using human CB₁ and CB₂ receptors expressed in CHO cells (Perkin-Elmer Life and Analytical Sciences, U.S.). To calculate the affinity values, competition binding assays were performed by using [³H]CP-55,940 as radioligand (specific activity, 180 Ci/mmol; Perkin-Elmer Life and Analytical Sciences, U.S.). The effect of these compounds was also evaluated in cyclic AMP experiments that used [³H]-cAMP as radioligand (specific activity, 58 Ci/mmol; Perkin-Elmer Life and Analytical Sciences U.S.). All other reagents were of analytical grade and obtained from commercial sources.

Competition Binding Experiments on CB₁ and CB₂ Receptors. CHO cell lines expressing human CB₁ and CB₂ receptors were grown adherently and maintained in Ham's F12 containing 10% fetal bovine serum, streptomycin (100 µg/mL), penicillin (100 U/mL), and Geneticin (G418, 0.4 mg/ml) in 5% CO₂/95% air at 37 °C [8,10]. 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 at 1000g for 10 min, and the supernatant was then centrifuged at 100000g for 30 min. The membrane pellet was suspended in 50 mM Tris-HCl buffer, 0.5% BSA (pH 7.4) containing 5 mM MgCl₂, 1 mM EDTA or 2.5 mM EDTA for hCB₁ or hCB₂ receptor, respectively.

Competition binding experiments were performed using 0.5 nM [^3H]CP-55,940 along with 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 CB₁ or CB₂ receptors, respectively.

Bound and free radioactivity 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 CB₂ Receptors. CHO cells transfected with human CB₂ receptors were washed with phosphate-buffered saline, diluted trypsin and centrifuged at 200g for 10 min. The pellet containing CHO cells (1×10^6 cells/assay) was suspended in 0.5 mL of incubation mixture: KCl 2.7 mM, NaCl 150 mM, MgSO₄ 1 mM, NaH₂PO₄ 0.37 mM, CaCl₂ 1 mM, MgCl₂ 10 mM, HEPES 5 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 [11]. 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 for 10 min at 2000g 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 [^3H]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 for 10 min at 2000g. 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 following a Bio-Rad method (Bradford, 1976) with bovine albumin as reference standard [26]. Inhibitory binding constants, K_i , were calculated from the IC_{50} values according to the Cheng and Prusoff equation:[27] $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where $[C^*]$ is the concentration of the radioligand and K_D^* its dissociation constant. A weighted nonlinear least-squares curve fitting program, LIGAND [28] ,was used for computer analysis of the inhibition experiments. All the data are expressed as the mean \pm 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 with Maestro [29] and subjected to minimization into a water environment (employing the generalized Born/surface area model) by using Macromodel [30]. The minimizations were carried out by means of the conjugate gradient, the MMFFs force field and a distance-dependent dielectric constant of 1.0, until a convergence value of 0.05 kcal/(Å•mol) was attained.

The inactive CB₁ and CB₂ receptor models employed for docking studies were generated by applying a previously reported procedure [31] and then further optimized based on the recently deposited PDB structures of adenosine (PDB code 3EML) [32, PMID 18832607] and rhodopsine (PDB code 1U19) [33, PMID: 15327956] receptors. The ligands were docked using AUTODOCK4.2 [20] AUTODOCK TOOLS [34] was employed to define 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 docking sites were defined according to the previously published WIN 55212-2 docked into the CB1 and CB2 receptor models [31]. The new CB₁ and CB₂ models were aligned to the corresponding protein structures complexed with WIN 55212-2 and the ligand was considered as the central group of a grid of 54, 50, and 52 points in the x, y, and z directions. The energetic map calculations were carried out by using a grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant. The ligands were able to form an intramolecular H-bond and previous quantum mechanical studies highlighted that it was a strong interaction [31]. On this basis, this in-

teraction was supposed to be preserved in the ligands binding pose within the receptor sites and the torsions involved in this intramolecular H-bond were kept fixed during AUTODOCK calculations, so that to avoid its loss in the docking process. The ligands were subjected to 100 runs of the AUTODOCK search using the Lamarckian genetic algorithm with 5 000 000 steps of energy evaluation; an rms tolerance of 1.0 Å was used to carry out the cluster analysis of the docking solutions and all other settings were left as their defaults. For each docked ligand, the top-scored pose belonging from the cluster with the best average of estimated free energy was taken into account for energy minimization studies.

Energy Minimizations. In order to further validate the docking results and also in order to partially take into account the receptor flexibility and the possibility of small adjustments in the ligand-receptor interaction, for each docked ligand the top-scored pose was energy minimized. The energy minimizations of the receptor-ligand complexes obtained as a result of the docking protocol were carried out by using AMBER11 [21]. The complexes were embedded into a phospholipid bilayer made up of POPC molecules, which was created by means of VMD [35]. This software was also used to place the ligand-protein complexes inside the phospholipid bilayer. The systems were set in a rectangular parallelepiped water-box (TIP3P explicit solvent model) and solvated on both the “extracellular” and “intracellular” side with a 12 Å water cap. Chlorine ions were then added for the neutralization of the systems. Three steps of energy minimization were performed, each one carried out through 1000 steps of steepest descent followed by 9000 steps of conjugate gradient but with the application of different position restraints. In the first step the phospholipids and the protein were kept fixed with a restraint of 100 kcal/(mol•Å²), so that only the position of the water molecules was energy minimized. During the second step the position restraint of 100 kcal/(mol•Å²) was applied just to the receptor, thus energy minimizing the surrounding phospholipid–water environment. Finally, a third step was performed by applying no restraints and thus leaving the whole system free. All the minimized ligand-receptor complexes were very similar to that obtained after

docking calculations and the minimized ligand dispositions never differed more than 1.5 Å (RMSD) with respect to the docked poses.

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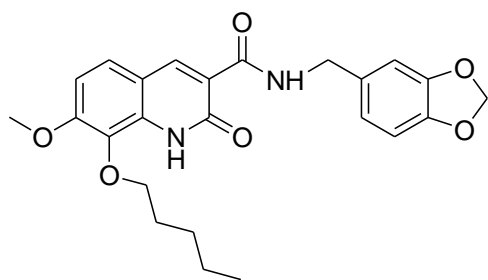
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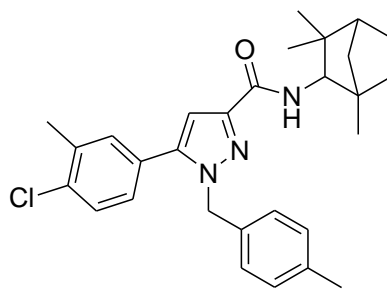
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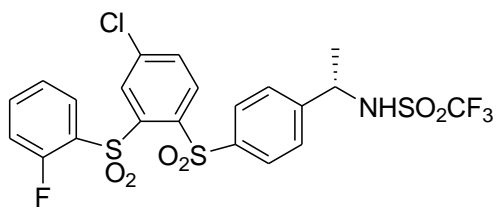
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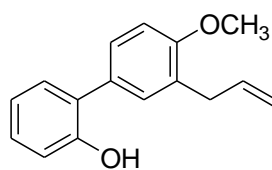
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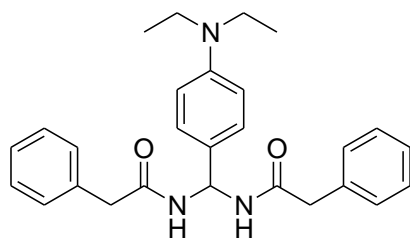
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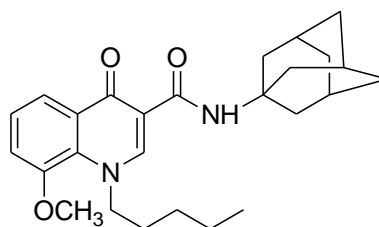
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5



6

Chart 1. Chemical structures of representative CB₂ inverse agonists

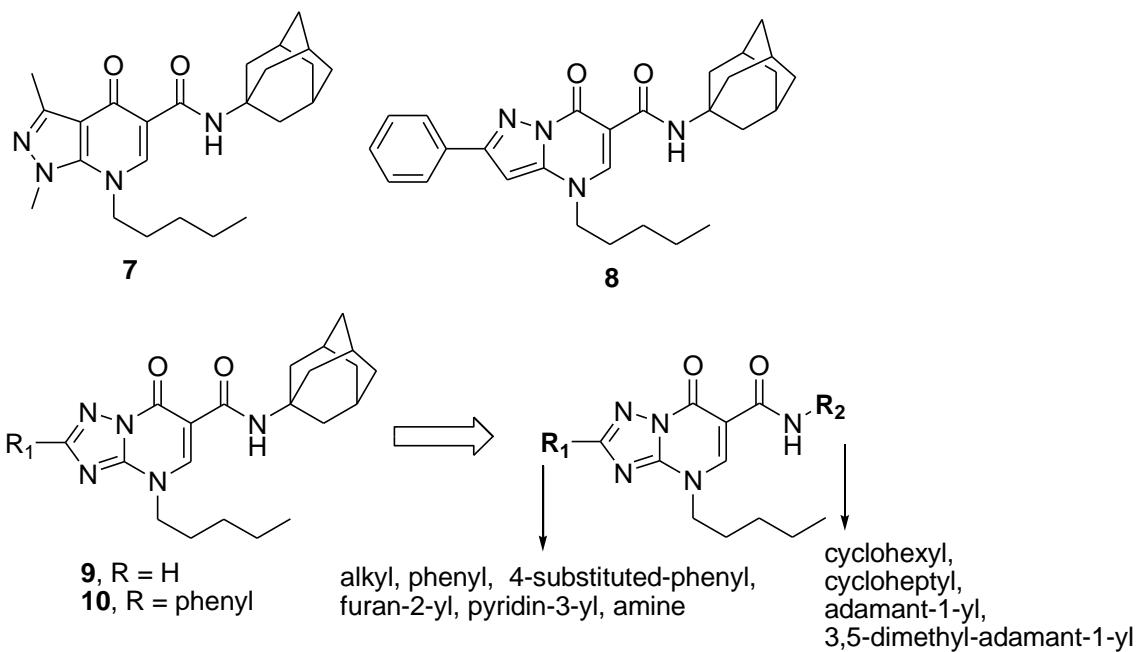
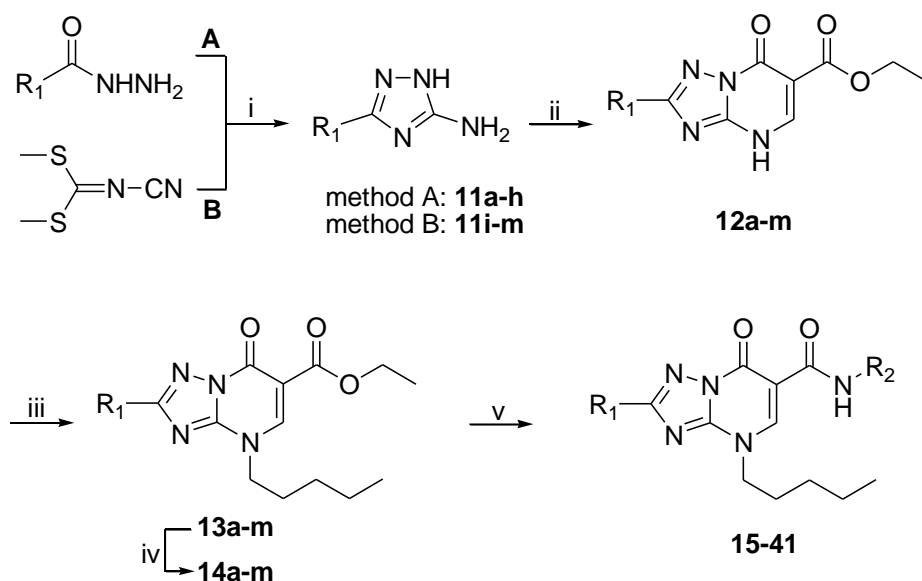
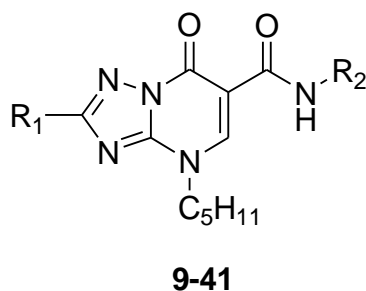


Chart 2. Structural investigations around the 4,7-dihydro-7-oxo-4-pentyl-[1,2,4]triazolo[1,5-*a*]pyrimidine-6-carboxamide scaffold



R^1 = a: H; b: CH_3 ; c: phenyl; d: 4-Cl-phenyl; e: 4- OCH_3 -phenyl;
 f: 4- CH_3 -phenyl; g: furan-2-yl; h: pyridin-3-yl; i: S- CH_3 ; j: morpholine;
 k: N-methylpiperazine; l: N-benzyl-N-methyl; m: N,N-diallylamine.

Scheme 1. Reagents and conditions: i) **A**: S-methylisothioureia hemisulfate, water, 100 °C, 6 h then NaOH 10%, 100 °C, 6 h; **B**: R_1NH_2 , hydrazine monohydrate, CH_3CN , 70 °C, 7 h; ii) DEEM, CH_3COOH , 3 h, 120 °C; iii) K_2CO_3 , DMF, 1-pentylbromide, 100 °C, 6 h; iv) 10% NaOH, MeOH, rt, 3 h; v) EDC, HOBt, DMF, amine, rt, 16 h or HBTU, DIEA, DMF, amine, rt, 16 h.

Table 1. Affinity (K_i , nM) and selectivity on human CB₁ and CB₂ receptors^a

<i>Compd</i>	<i>R₁</i>	<i>R₂</i>	<i>K_i hCB₁^b</i> (nM)	<i>K_i hCB₂^c</i> (nM)	<i>CB₁/CB₂</i>
9	H	adamantan-1-yl	712±64	20±2	36
10	ph	adamantan-1-yl	523±47	3.58±0.31	146
15	H	cycloheptyl	717 ± 66	514 ± 49	1
16	H	3,5-dimethyladamantan-1-yl	622 ± 53	25 ± 3	25
17	CH ₃	cyclohexyl	488 ± 46	674 ± 62	1
18	CH ₃	adamantan-1-yl	471 ± 42	383 ± 35	1
19	ph	cyclohexyl	3214 ± 28	43 ± 4	75
20	ph	cycloheptyl	2483 ± 237	48 ± 5	52
21	ph	3,5-dimethyladamantan-1-yl	653 ± 61	8.42 ± 0.87	77
22	4-Cl-ph	cyclohexyl	4718 ± 413	117 ± 9	40
23	4-Cl-ph	adamantan-1-yl	5216 ± 483	11.3 ± 1.9	462
24	4-OCH ₃ -ph	cyclohexyl	5849 ± 544	96 ± 8	61
25	4-OCH ₃ -ph	adamantan-1-yl	3723 ± 318	47 ± 4	79
26	4-CH ₃ -ph	cyclohexyl	>10000	31 ± 3	323
27	4-CH ₃ -ph	cycloheptyl	6352 ± 594	22 ± 2	289
28	4-CH ₃ -ph	adamantan-1-yl	1519 ± 141	6.81 ± 0.62	223
29	furan-2-yl	adamantan-1-yl	1172 ± 106	45 ± 4	26
30	pyridin-3-yl	adamantan-1-yl	>10000	228 ± 19	>44
31	S-CH ₃	cyclohexyl	>10000	716 ± 62	>14
32	S-CH ₃	adamantan-1-yl	>10000	47 ± 3	>213
33	morpholine	cyclohexyl	>10000	>10000	

34	morpholine	cycloheptyl	>10000	566 ± 52	>18
35	morpholine	adamantan-1-yl	>10000	53 ± 5	140
36	<i>N</i> -CH ₃ -piperazine	cyclohexyl	>10000	>10000	>188
37	<i>N</i> -CH ₃ -piperazine	cycloheptyl	>10000	982 ± 87	>10
38	<i>N</i> -benzyl- <i>N</i> -methyl	cyclohexyl	>10000	1332 ± 128	>7
39	<i>N</i> -benzyl- <i>N</i> -methyl	adamantan-1-yl	>10000	21 ± 2	>476
40	diallylamine	cyclohexyl	>10000	1352 ± 124	>7
41	diallylamine	adamantan-1-yl	>10000	158 ± 13	>63

^aThe data are expressed as the mean ± SEM of n = 4 independent experiments. The affinity values were calculated by using [³H]CP-55,940 as radioligand on human CB₁CHO membranes^b, and human CB₂CHO membranes^c.

Table 2. Efficacy and potency of CB₂ Ligands^a

<i>increase in cAMP production (%)</i>				<i>increase in cAMP production (%)</i>		
Compd	1μM	10μM	<i>EC</i> ₅₀ <i>hCB</i> ₂ (nM)	Compd	1μM	10μM
9	-	60 ± 5	87 ± 9	26	127 ± 12	179 ± 16
16	-	57 ± 4	88 ± 8	27	135 ± 12	182 ± 17
19	111 ± 10	173 ± 16	-	28	152 ± 14	246 ± 21
20	122 ± 13	176 ± 21	-	29	115 ± 13	185 ± 16
21	163 ± 15	243 ± 21	-	32	112 ± 11	152 ± 13
23	137 ± 12	223 ± 18	-	35	115 ± 12	174 ± 16
25	105 ± 11	176 ± 15	-	39	131 ± 12	177 ± 16

^aData are expressed as the mean ± SEM of n=4 independent experiments.

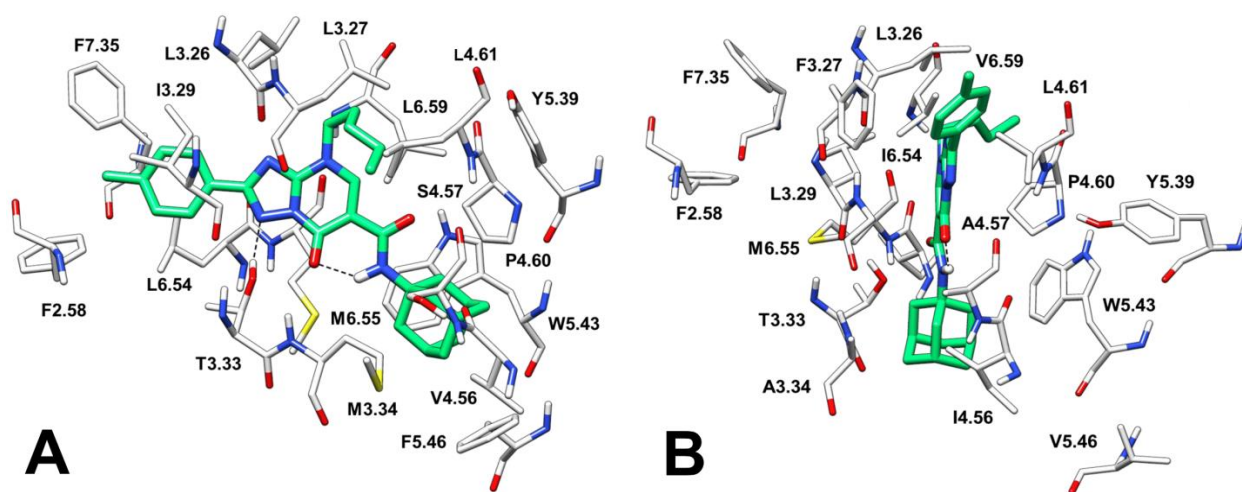


Figure1. Energy minimized complex of compound **28** docked into CB₂ (A) and CB₁ (B) receptors

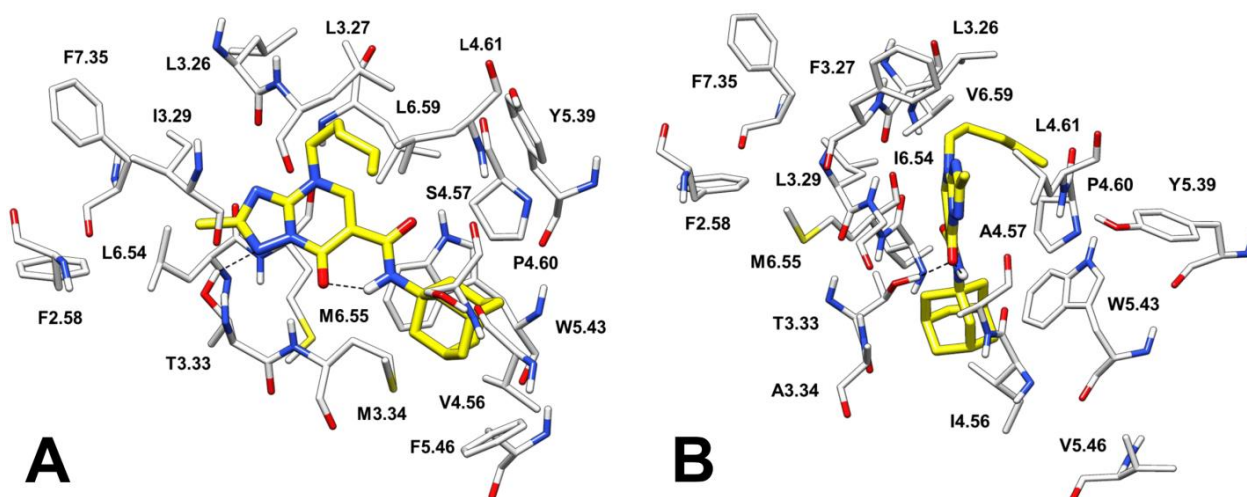


Figure 2. Energy minimized complex of compound **18** docked into CB₂ (A) and CB₁ (B) receptors

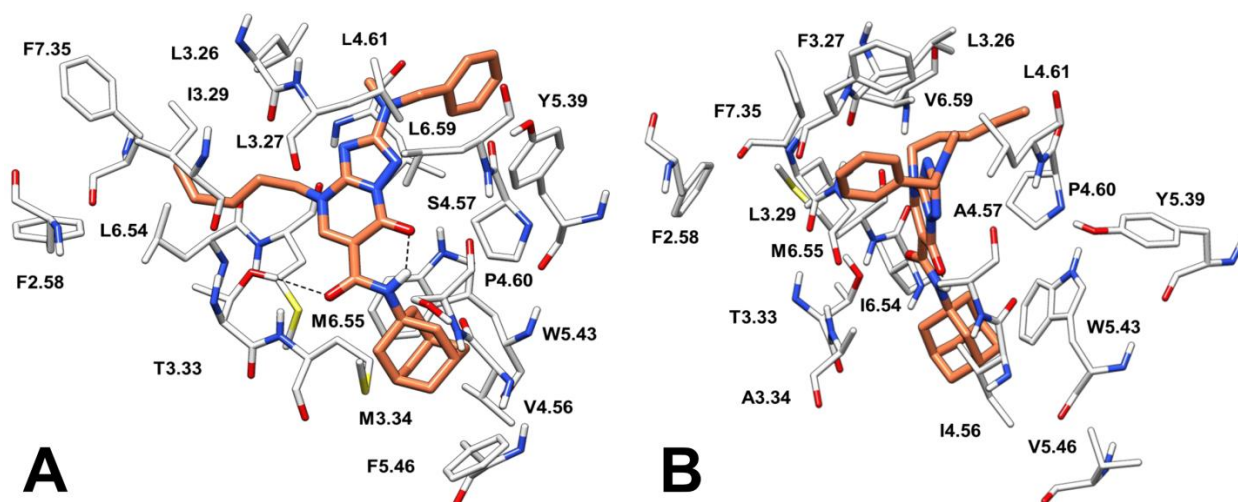


Figure 3. Energy minimized complex of compound **39** docked into CB₂ (A) and CB₁ (B) receptors