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PII: S0223-5234(19)30623-3

DOI: https://doi.org/10.1016/j.ejmech.2019.07.002

Reference: EJMECH 11499

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 19 May 2019

Revised Date: 1 July 2019

Accepted Date: 1 July 2019

Please cite this article as: A.E. Ghonim, A. Ligresti, A. Rabbito, A.M. Mahmoud, V. Di Marzo, N.A. Osman, A.H. Abadi, Structure-activity relationships of thiazole and benzothiazole derivatives as selective cannabinoid CB2 agonists with *in vivo* anti-inflammatory properties, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.07.002.

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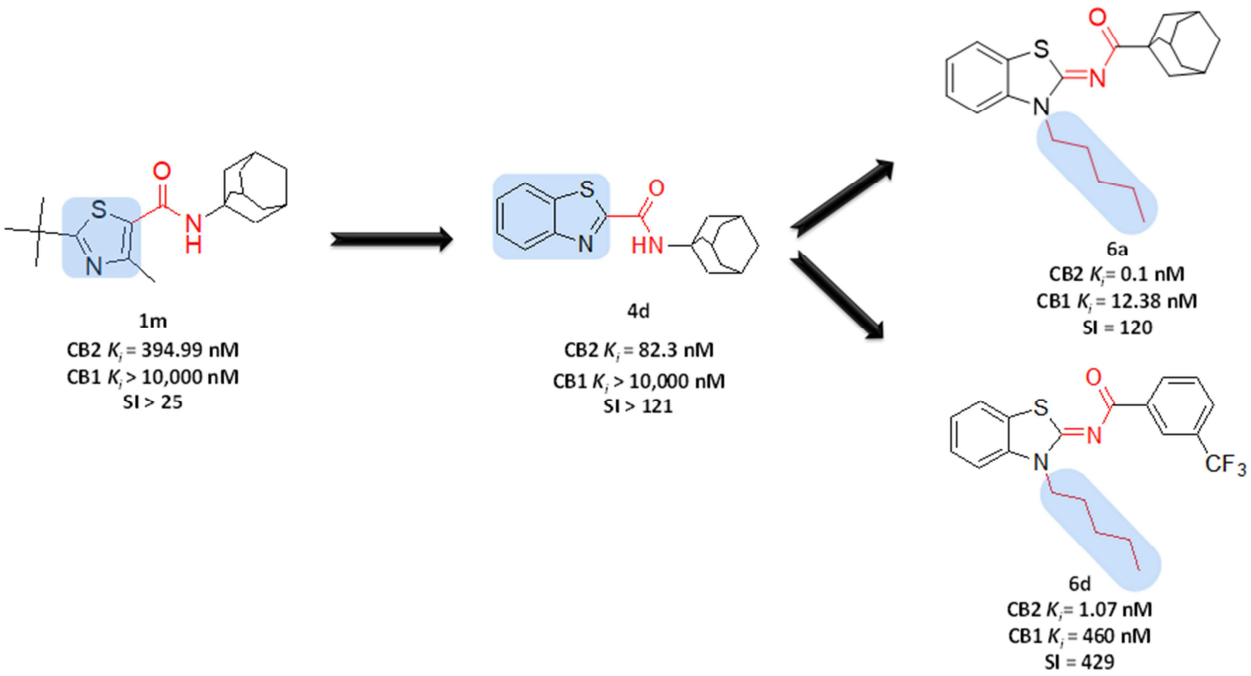

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Structure-Activity Relationships of Thiazole and Benzothiazole Derivatives as Selective Cannabinoid CB2 Agonists with In Vivo Anti-Inflammatory Properties

Aya E. Ghonim[1], Alessia Ligresti[2], Alessandro Rabbito[2], Ali Mokhtar Mahmoud[2], Vincenzo Di Marzo[2], Noha A. Osman[1*], Ashraf H. Abadi[1].

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ABSTRACT

The strong therapeutic potential of CB2 receptor agonists for use as anti-inflammatory agents that lack psychiatric side effects has attracted substantial interest. We herein describe the rational design and synthesis of novel thiazole and benzothiazole derivatives and the evaluation of their binding affinity and functional activity on CB1 and CB2 receptors. The series with the general formula *N*-(3-pentylbenzo[*d*]thiazol-2(3*H*)-ylidene) carboxamide (compounds **6a-6d)** exhibited the highest affinity and selectivity towards CB2 receptors with K_is in the picomolar or low nanomolar range, and selectivity indices (K_i hCB1/K_i hCB2) reaching up to 429 fold. Notably, these compounds also demonstrated an agonistic functional activity in cellular assays with EC_{50}s in the low nanomolar range. More interestingly, compound **6d**, the 3-(trifluoromethyl)benzamide derivative, exhibited remarkable protection against DSS-induced acute colitis in mice model.

KEYWORDS: thiazole; benzothiazole; CB2 ligands; CB2 agonists; structure-activity relationship; DSS-induced colitis.

ABBREVIATIONS

cAMP, cyclic adenosine monophosphate; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; $CDCl_3$, deuterated chloroform; Cpd, compound; DCM, dichloromethane; DMF, dimethylforamide; DMSO, dimethyl sulfoxide; DSS, dextran sulfate sodium; EtOAc, ethyl acetate; EtOH, ethanol; h, hour; HEK-293, human embryonic kidney 293; i.p, intraperitoneal; IC_{50}, 50% inhibitory concentration; IL-1β, interleukin 1beta; K_i, inhibition constant; min, minutes; MW, microwave; RLU, relative luminescence units; SAR, structure-

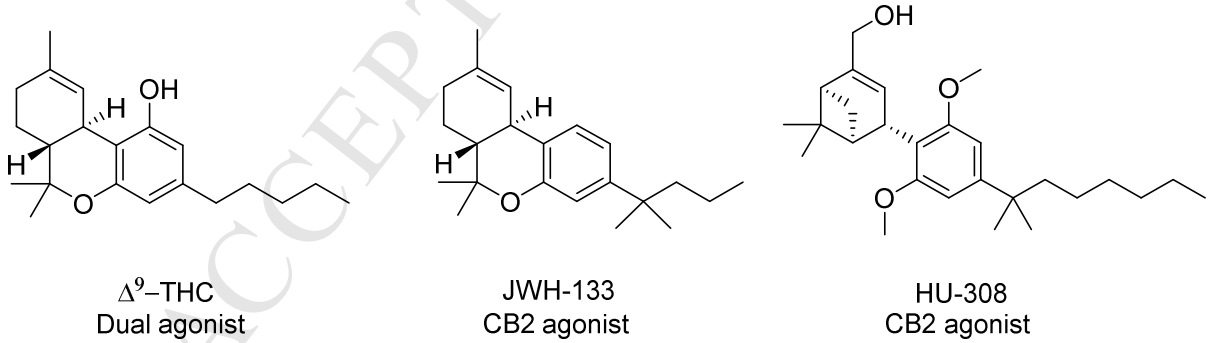
activity relationship; SEM, standard error of the mean; TFA, trifluoroacetic acid; TNF-α, tumor necrosis factor alpha; W, watt.

INTRODUCTION

Interest in the medicinal uses of phytocannabinoids sparked-off ongoing research in pursuance of a better understanding of the endocannabinoid system, the endogenous responder of such interesting molecules.[1] Characterised by a number of endogenous cannabinoids (endocannabinoids) that bind to two G-protein coupled receptors; CB1[2] and CB2,[3] in addition to biosynthetic and inactivation enzymes and transport proteins,[4] the endocannabinoid system is capable of maintaining homeostasis and balance in the body through controlling key regulatory functions including inflammation,[5] immune function,[6] cancer,[7] appetite regulation, metabolism,[8] and nociception,[9] in a rather complex neuroactive signalling fashion. Despite how all the components of this complex system present excellent therapeutic targets for a range of pathological conditions, targeting cannabinoid receptor CB2 for an anti-inflammatory psychotropic-free effect have recently gained significant momentum. By virtue of being predominantly expressed by the immune cells in addition to its inducible nature; being dramatically upregulated during inflammatory conditions in peripheral and central tissues, [10-12] CB2 receptor is thought to re-achieve a homeostatic balance following tissue injury. The latter has been reported to take place through induction of apoptosis in immune cells, inhibition of T cell proliferation, inhibition of cytokine and chemokine production by T cells and impairment of macrophage proliferation, function and release of chemokines.[13-15] This anti-inflammatory role is complemented by several recent experimental findings suggesting CB2 involvement in Δ^9-tetrahydrocannabinol (Chart 1) and cannabidiol anti-inflammatory properties.[16-17] This is in addition to the distinguishable number of synthetic selective CB2 agonists that have also shown promising central and peripheral anti-inflammatory activities in animal models of multiple sclerosis,[18] Parkinson's

disease,[19] Alzehimers,[20] uveitis,[21] autoimmune encephalitis[22] and ulcerative colitis.[23] In the gastrointestinal tract, the components of the endocannabinoid system have been detected under normal[24] and inflamed conditions, during which an upregulation of CB2 receptors, an elevation in endocannabinoids levels and a reduction in the corresponding metabolic enzymes have been witnessed, perfectly fitting a protective mechanism theory.[25-26] These events facilitate an on-target mission for selective CB2 agonists to depress the inflammation process in ulcerative colitis, thereupon, their use as a novel therapeutic tool for treating various GI inflammatory conditions has emerged. In proof of this concept, CB2 agonist JWH133 (Chart 1) was found to attenuate TNBS-induced colitis in animal models, an effect that was completely abolished by co-injecting CB2 antagonist AM630 while extensively aggravated by single administration of the latter.[25] More recently, CB2 agonist HU308 (Chart 1) mediated protective effect in colitis animal model was accredited to suppression of the NLRP3 inflammasome responsible for inflammatory process initiation, activation and amplification.[27]

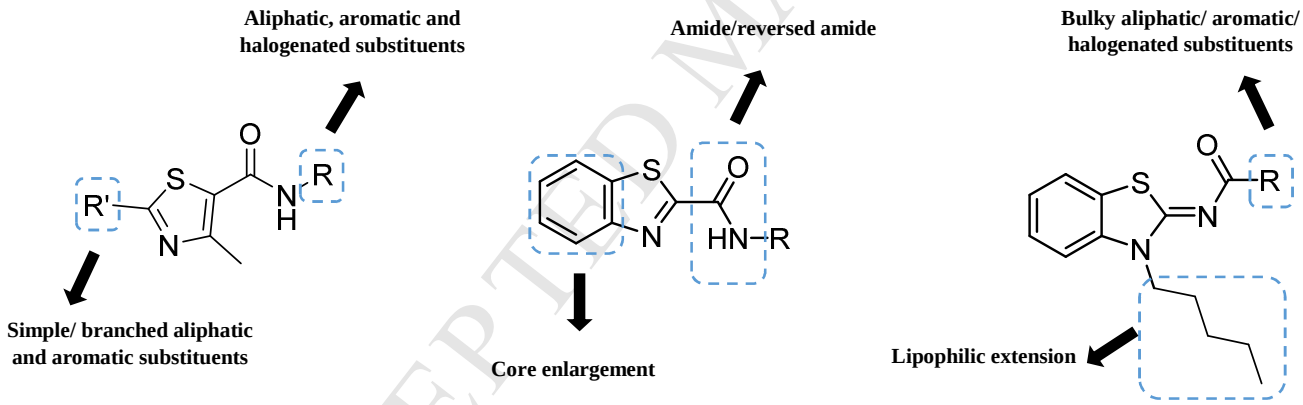
Chart 1: Chemical structure of some cannabinoid receptor agonists.



Despite the favourable preclinical outcomes of a big number of highly affine ligands specifically developed for targeting CB2 receptors, only few compounds were considered for clinical trials and none of them has currently been approved for human use, principally due to poor efficacy.[28-29] Provoked by the preclinical/clinical mismatching outcomes that are

standing in the way of harnessing the promising therapeutic utilities of CB2 selective agonists, there is an increasing interest in exploiting novel chemical scaffolds for potent and selective CB2 agonists that are clinically effective for the treatment of GI inflammatory disorders. A diverse number of chemical scaffolds have been reported to act as selective CB2 ligands. A trend of mono- or bicyclic core bearing one or more heteroatoms such as oxygen or nitrogen, a carbonyl containing linker bound to bulky aliphatic or aromatic groups is generally seen with reported selective CB2 chemotypes.[31] In this context, an interactive SAR study, composed of three phases, was carried out, thoroughly describing structural and core enhancement strategies needed to construct a novel promising series of potent CB2 agonists (Chart 2).

Chart 2: Phases of structural modifications and core enhancements employed in the interactive SAR study.



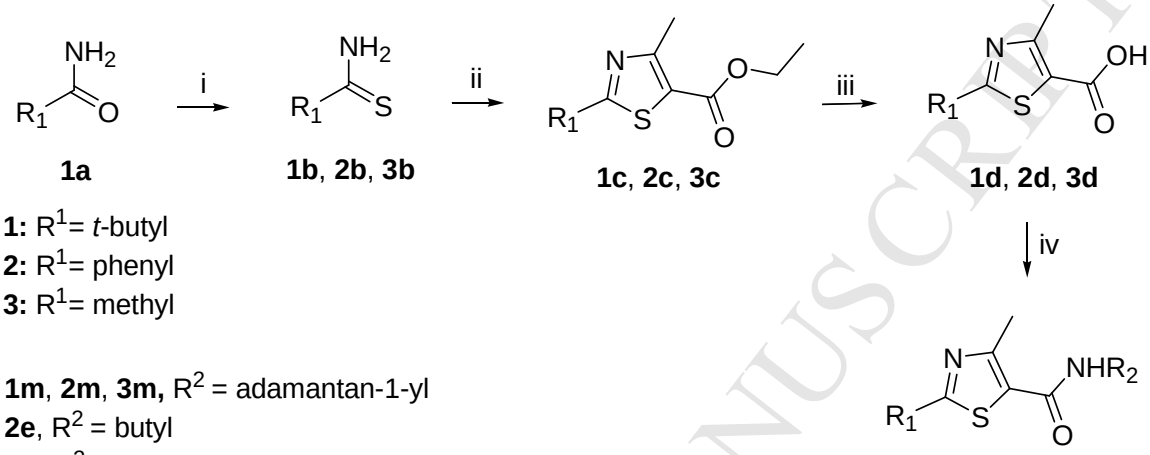
We started off by the synthesis of monocyclic **4-methyl-2-substituted thiazole-5-carboxamides** (Phase I, Chart 2) in alignment with previously reported isoxazoles,[32] imidazoles,[33] pyrazoles.[34] Next, we explored the effect of core enlargement through condensing a benzene ring to form **benzothiazole-2-carboxamides** (Phase II, Chart 2) bearing different bulky alicyclic, aromatic and halogenated amide substituents. In order to achieve chemical diversity, the 2-carboxamide linkage was reversed to form the ***N***-

(benzothiazol-2-yl) carboxamide counterparts (Phase III, Chart 2). Driven by the favourable biological data, the latter chemotype was modified by *n*-pentyl substitution on basis of other cannabinoid pharmacophores.[35-36] The newly synthesised compounds were tested in a competitive radio-ligand binding assay towards both hCB1 and hCB2 receptors overexpressed on membranes of HEK-293 cells. Affinity data (K_i values) were used to calculate the selectivity indices of these compounds. These ligands were also examined in the cAMP Hunter™ assay and the GPCR β-arrestin recruitment Tango assay,[37] with the aim of evaluation of their functional activity. To assess the *in vivo* efficacy of the newly developed chemotypes, a representative from the most potent series was selected to be tested for its anti-inflammatory action in mice model of acute DSS-induced colitis.

RESULTS AND DISCUSSION

Chemistry. A general route to the synthesis of the targeted thiazole-2-carboxamides is shown in Scheme 1. The synthesis is mainly comprised of 4 steps unless the starting thioamide was commercially available **(2b, 3b)**. In the first step, 2,2-dimethylpropyl thioamide **(1b)** was prepared in good yields from the corresponding carboxamide through thionation of the carbonyl using Lawesson's reagent in THF**.** The prepared (**1b**) or commercially available thioamides **(2b, 3b)** underwent Hantzsch thiazole synthesis by reacting with ethyl 2-chloroacetoacetate in refluxing ethanol for 24 h to give the corresponding 2-substituted-4-methyl 5-thiazole ethyl ester **(1c, 2c, 3c)**. The resulting esters **(1c, 2c, 3c)** were then subjected to base-catalyzed ester hydrolysis using 10% NaOH solution in refluxing ethanol. Subsequent direct coupling of the obtained acids **(1d, 2d, 3d)** to different amines was done using HBTU in the presence of tributylamine as a base giving rise to the final amide products **(1m, 2e-2q, 3l-3n).**

Scheme 1. Synthesis of **4-methyl-2-substituted thiazole-5-carboxamides**; **1m**, **2e-2q**, **3l-3n**[a].



1a **1b**, **2b**, **3b** **1c**, **2c**, **3c** **1d**, **2d**, **3d**

1: R^1= *t*-butyl
2: R^1= phenyl
3: R^1= methyl

1m, **2m**, **3m,** R^2 = adamantan-1-yl
2e, R^2 = butyl
2f, R^2 = hexyl
2g, R^2 = cyclopropyl
2h, R_2 = cyclopentyl
2i, R^2 = cyclohexyl
2j, R^2 = morpholin-4-yl
2k, R^2 = cyclohexylmethyl
2l, **3l,** R^2 = tetralin-1-yl
2n, **3n,** R^2 = benzyl
2o, R^2 = 2-fluorophenylmethyl
2p, R^2 = 4-fluorophenylmethyl
2q, R^2 = 2-chlorophenylmethyl

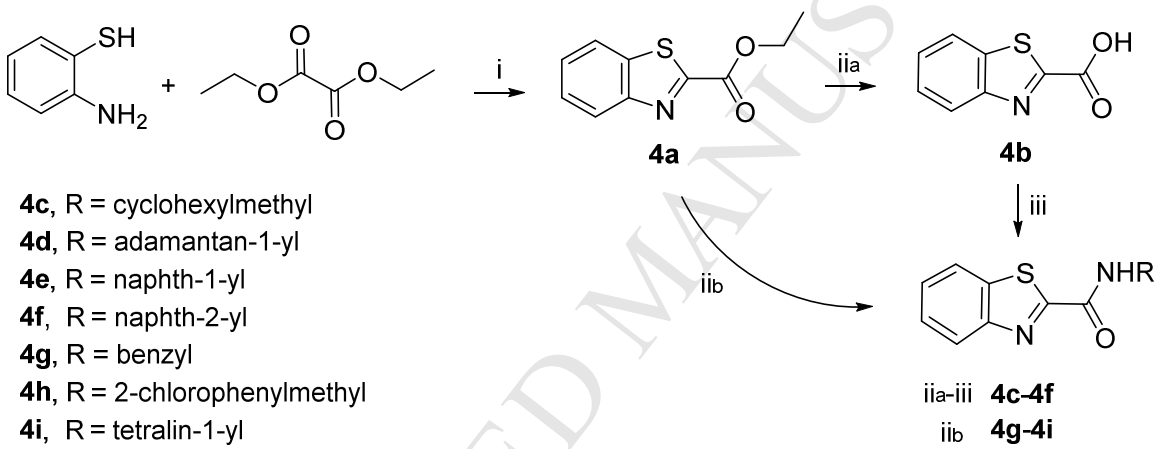
1m, **2e-2q**, **3l-3n**

[a]Reagents and conditions: (i) 1 equiv of the appropriate amide, 1 equiv 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent), THF, reflux, 4 h. (ii) 1 equiv of the thioamide, 1 equiv ethyl 2-chloroacetoacetate, EtOH, room temperature, overnight. (iii) 10 equiv 10% aqueous NaOH, ethanol, reflux, 4 h. (iv) 1 equiv of the appropriate carboxylic acid derivative, anhydrous DMF, 6 equiv tributylamine, 1.2 equiv HBTU, room temperature 30 min, 1.2 equiv of the appropriate amine, room temperature, 4-5 h.

Synthesis of benzothiazole-2-carboxamides was achieved in three steps (Scheme 2). The first step comprises refluxing 2-aminothiophenol together with diethyl oxalate at 170 ^{0}C for 2 hours then dropping the temperature to 90 ^{0}C for another 2 hours to obtain benzothiazole-2-carboxylic acid ethyl ester **4a** in average yields. Treating the resulting beznothiazole ester **4a**

with refluxing 10% NaOH and a mixture of H_2O, THF and methanol led to the formation of the acid derivative **4b** that was then coupled with different amines to provide the expected final amides of this series (**4c-4f).** In an attempt to enhance the greenness of the synthetic scheme, an alternative route was adopted to directly convert the ester **4a** to different amides in excellent yields through a direct fusion reaction at 100^0C with aliphatic amines in the presence of catalytic amounts of NH_4Cl under neat conditions, giving rise to final amide products requiring no chromatographic purification **(4g-4i).**

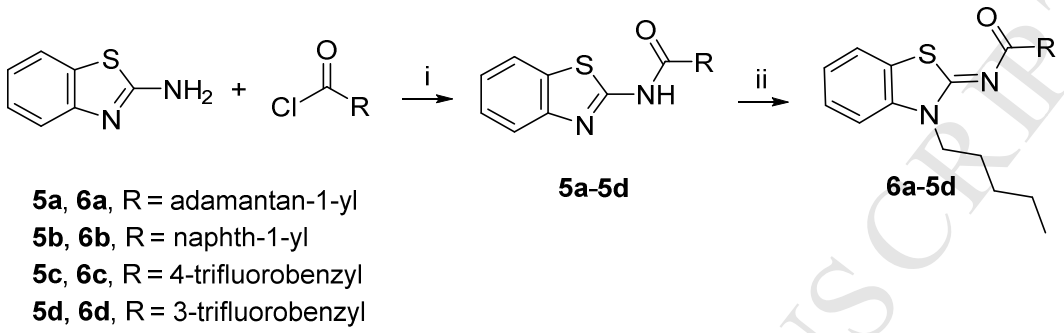
Scheme 2. Synthesis of **Benzothiazole-2-carboxamides**; **4c-4i**[a]



[a]Reagents and conditions: (i) 1 equiv 2-aminothiophenol, 2 equiv diethyl oxalate, reflux, 4 h. (iia) 10 equiv 10% aqueous NaOH methanol/THF, reflux 4 h. (iib) 1 equiv ethyl benzothiazole-2-carboxylate, 4 equiv appropriate aliphatic amine, catalytic amount of NH_4Cl, 120 ^oC, 2-3 h. (iii) 1 equiv of the carboxylic acid derivative, anhydrous DMF, 6 equiv tributylamine, 1.2 equiv HBTU, room temperature 30 min, 1.2 equiv of the appropriate amine, room temperature, 4-5 h.

As reported in Scheme 3, *N*-(benzothiazol-2-yl) carboxamides were synthesized through the reaction between the commercially available benzothiazole-2-amine and different acyl chlorides in the presence of triethylamine under inert conditions. Allowing the mixture to stir overnight gave the amides **(5a-5d)** in average yields. These, in turn, were subjected to *N*-alkylation with 1-bromopentane in the presence of NaH providing *N*-(3-pentylbenzothiazol-2(3*H*)-ylidene) carboxamides **(6a-6d)**.

Scheme 3. Synthesis of ***N*-(benzothiazol-2-yl)** and ***N*-(3-pentylbenzothiazol-2(3*H*) - ylidene) carboxamides**; **5a-5d**, **6a-6d**[a]



5a, **6a**, R = adamantan-1-yl
5b, **6b**, R = naphth-1-yl
5c, **6c**, R = 4-trifluorobenzyl
5d, **6d**, R = 3-trifluorobenzyl

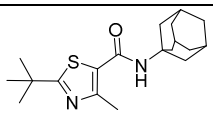
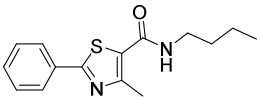
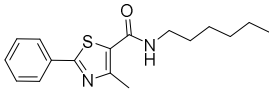
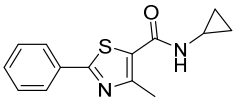
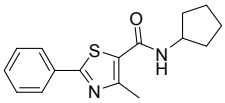
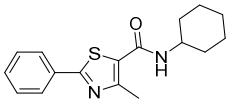
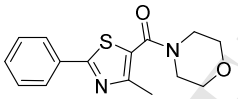
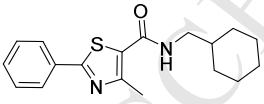
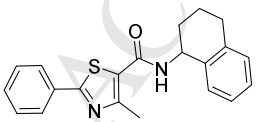
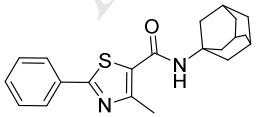
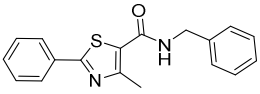
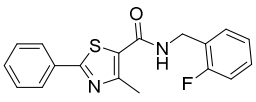
[a]Reagents and conditions: (i) 1 equiv 2-aminobenzothiazole in dry DCM, 1.5 equiv triethylamine, 1.5 equiv of the appropriate acyl chloride added at 0 ºC to room temperature, 18 h. (ii) 1.3 equiv of the appropriate *N*-(benzo[*d*]thiazol-2-yl) carboxamide in DMF, 1.9 equiv NaH, 1,4 equiv 1-bromopentane, room temperature (1 h) to 80 ^{0}C overnight.

Structure-Affinity/Activity Relationships

The newly synthesized thiazoles and benzothiazole carboxamides were evaluated in a competitive radio-ligand binding assay for their ability to displace [^{3}H]-CP-55,940 (a high affinity radio-ligand) from HEK-293 cells over-expressing human recombinant CB2 and CB1 receptors. Compounds showing greater than 50% displacement at 10 μM screening doses were tested in a dose-response curve to determine their IC_{50} values and calculate the latter into K_i values. The obtained data are shown in Table 1. *In vitro* binding data for the initial **thiazole-5-carboxamide** chemotype revealed how aliphatic/alicyclic amide substituents seem to possess a size dependant effect on the binding affinity on CB2 receptors as it was generally noticed that increasing the size of the amide substituent positively influenced the binding affinity of the compounds. This is exemplified by the proportional increase in CB2 binding affinity through replacing the cyclohexyl amide substituent in the inactive **2i** (K_i

>10,000 nM) with the bulkier tetraline in **2l** (K_i= 2300 nM) or adamantyl in **2m** (K_i= 450 nM), the latter, representing the most active compound in this series.

Table 1: Affinities (K_i values) on CB1 and CB2 of synthesized compounds towards *h*CB2 and *h*CB1 cannabinoid receptors, selectivity index (K_i hCB1/K_i hCB2), and functional activities (EC_{50} values) on CB1 and CB2 receptors. Data represent mean values for at least three separate experiments performed in duplicate and are expressed as K_i ± SD (nM).

Cpd	Structure	Binding affinity K_i (nM)		SI	CB2 EC_{50} (nM) Tango assay		CB1 EC_{50} (nM) Tango assay	
		*h*CB2	*h*CB1		Agonist	Antagonist	Agonist	Antagonist
1m		395 ± 113	>10,000	>25	3981.0	>30,000	>30,000	>30,000
2e		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2f		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2g		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2h		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2i		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2j		2860 ± 890	>10,000	>3	>30,000	>30,000	>30,000	>30,000
2k		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2l		2300 ± 190	>10,000	>4	>30,000	>30,000	>30,000	>30,000
2m		450 ± 120	>10,000	>22	>30,000	>30,000	>30,000	>30,000
2n		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2o		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000

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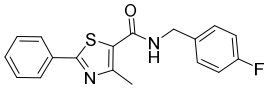
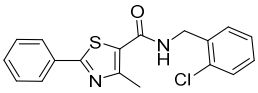
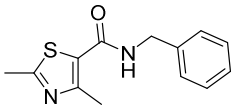
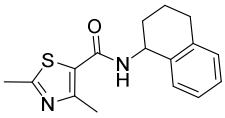
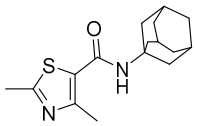
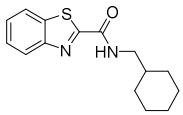
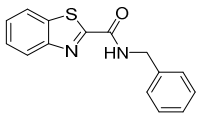
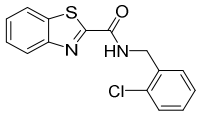
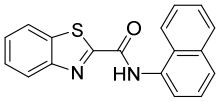
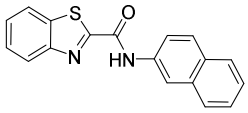
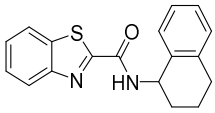
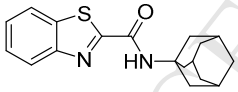
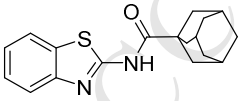
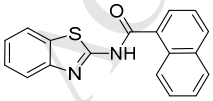
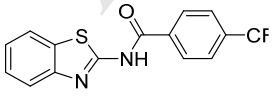
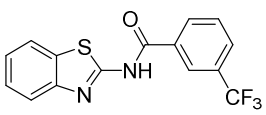
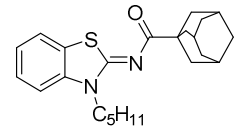
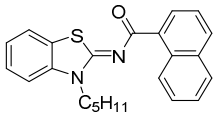
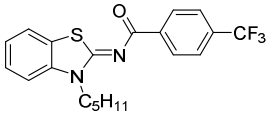
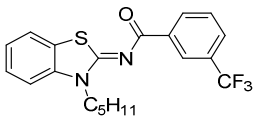
2p		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
2q		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
3n		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
3l		NT*	NT*		>30,000	436.5	>30,000	1174
3m		1530 ± 288	>10,000	>6	>30,000	>30,000	>30,000	>30,000
4c		1324 ± 193	>10,000	>7	>30,000	>30,000	>30,000	>30,000
4g		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
4h		1084 ± 345	>10,000	>9	3311	>30,000	>30,000	>30,000
4e		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
4f		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
4i		288 ± 27	1060	3	>30,000	>30,000	>30,000	>30,000
4d		82 ± 7	>10,000	>121	>30,000	>30,000	>30,000	>30,000
5a		272 ± 77	>10,000	>36	>30,000	>30,000	>30,000	>30,000
5b		1288 ± 58	>10,000	>7	>30,000	>30,000	>30,000	>30,000
5c		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000
5d		>10,000	>10,000		>30,000	>30,000	>30,000	>30,000

ACCEPTED MANUSCRIPT

6a		0.10 ± 0.002	12 ± 3	120	26.30	>30,000	1621.8	>30,000
6b		0.11 ± 0.002	2 ± 0.5	22.2	12.88	>30,000	125.89	>30,000
6c		25 ± 7	>10,000	393	2511.8	>30,000	>30,000	>30,000
6d		1 ± 0.4	460 ± 73	429	128.82	>30,000	>30,000	>30,000
SR144528		2 ± 0.5	117 ± 22	64	1360	41	-	-

*NT = not tested

The introduction of heteroatoms to the cyclohexyl amide **2i** (K_i >10,000 nM) to afford the corresponding morpholine amide **2j** (K_i= 2860 nM) was found to enhance the CB2 binding affinity. The size of the 2-substituent on the thiazole ring also appeared to deeply influence the CB2 binding affinity. A significant decrease in affinity was observed upon replacing the phenyl ring in **2m** (K_i= 450 nM) with a small methyl group in **3m** (K_i = 1530 nM). On the other hand, an aliphatic branched *tert*-butyl slightly enhanced the binding affinity **1m** (K_i= 394 nM). Compounds of this initial chemotype showed no binding affinity to CB1 receptors granting them superior CB2 selectivity despite of their modest affinity. Thus, a general preference for bulky lipophilic substituents either at position 2 or at the amide moiety was noticed for the initial set of the synthesised compounds. Subsequently, the adopted strategy during the second round of structural modifications was to increase the size of the core thiazole ring by synthesizing a set of **benzothiazole 2-carboxamides**. At this point of the SAR study, the influence of different amide substituents and of reversing the amide functionality on CB2 binding affinity were envisaged. As successfully anticipated, increasing the size of the core in the 2nd chemotype; **benzothiazole 2-carboxamides,** was generally accompanied with a marked enhancement in the CB2 binding affinity while retaining the

relatively higher CB2 over CB1 selectivity. This was demonstrated in the benzothiazole adamantly amide **4d** (K_i= 82 nM) which displayed a 5-fold increase in CB2 affinity compared to its thiazole counterpart **2m** (K_i= 450 nM). In a similar fashion, the benzothiazole tetralin amide derivative **4i** (K_i= 288.5 nM) exhibited an 8-fold rise in affinity versus its thiazole analogue **2l** (K_i= 2300 nM). As seen with the initial chemotype, a progressive enhancement in the CB2 binding affinity was observed upon increasing the bulkiness of the amide moiety from cyclohexyl methyl to tetralin then to 1-adamantyl, compounds **4c** (K_i = 1324 nM), **4i** (K_i = 288.5 nM) and **4d** (K_i= 82 nM), respectively. It was also clear that bulky aliphatic amide substituents were superior over aromatic ones where naphthyl amido derivatives **4f** and **4e** appeared to be disfavoured in terms of CB2 binding affinity. In addition, the attachment position of the naphthyl moiety to the carboxamide seemed to have no influence on the activity as both regioisomers; 1 and 2-naphthyl amides(**4e** and **4f**, respectively) were found to be inactive (K_i >10,000 nM). It has been noted that the chlorinated benzyl amide analog, **4h,** displayed a slightly higher, yet a relatively modest binding behaviour as that seen with its cyclohexyl methyl analog **4c** (K_i = 1084 nM, 1324 nM, respectively). As previously noticed, optimal CB2 affinity within this series of compounds was obtained with the aliphatic adamantly amide derivative **4d** (K_i= 82 nM).

In attempts to achieve chemical diversity other forms of the amide functionality were also assessed through reversing the amide linkage to afford the ***N*-(benzothiazol-2-yl) carboxamides**. Pharmacomodulation of the acyl substituent, through usage of different bulky lipophilic groups, was also carried out. Comparing the adamantly amide derivatives **4d** and **5a**, we can find that K_i values have raised from 82.3 nM to 217.8 nM when the carboxamide functionality in **4d** was reversed to give the respective retroamide **5a**. However, in a contradicting manner, applying the same modification to the naphthylamide derivative **4e** (K_i >10,000 nM) afforded compound **5b** (K_i= 1288 nM) with a greatly enhanced CB2 affinity. To

complete the SAR, we investigated the impact of an *n*-alkyl substituent by introducing a pentyl chain at the *N*-3 position to yield the corresponding ylidenes; ***N*-(3-pentylbenzothiazol-2(3*H*)-ylidene) carboxamides**. As obvious from the binding results, this modification induced a dramatic increase in CB2 binding affinity. This pattern was consistent with all derivatives synthesized. *N*-pentyl substitution of the *p*- and *m*- trifluoromethyl phenyl amide derivatives (**5c** and **5d**, respectively) granted these inactive derivatives CB2 binding affinities in the low nanomolar range (**6c** K_i= 25 nM, **6d** K_i= 1 nM, respectively), indicating that substitution at this position is a crucial modulator of affinity at the CB2 receptor. Analogously, K_i values of the adamantyl and 1-naphthyl amides (**5a** and **5b)** were powerfully lowered from the nanomolar to the picomolar range (**6a** K_i= 0.10 nM, **6b** K_i= 0.11 nM, respectively). This substantial enhancement in affinity implies a potential hydrophobic interaction with the binding site of the receptor at this part of the molecule. Through exploring amide substituents of different natures, elements controlling potency and CB2/CB1 subtype selectivity were deduced. The highest CB2 binding affinities were achieved by the aliphatic adamantyl **6a** and aromatic naphthyl **6b** amide derivatives, however, with a concomitant enhancement in binding affinity to the CB1 receptor (SI: **6a**= 120, **6b**= 22). In an attempt to resolve the poor selectivity issue, halogenated aromatic amide substituents were tested. Interestingly, both 3- and 4-trifluoromethylphenyl amide derivatives (**6d** and **6c**, respectively) displayed a favourable selectivity profile and a regio-dependent affinity outcome. The 4-trifluoromethyl regio-isomer **6c,** successfully eliminated the activity on CB1 receptors (K_i > 10,000 nM) at the expense of a relatively lower CB2 binding affinity (K_i = 25 nM). However, more conveniently, shifting the trifluoromethyl group to the meta position in **6d** contributed to a 25-fold enhancement in CB2 binding affinity (K_i = 1 nM) while retaining an appreciable selectivity index (SI, **6d** = 429).

***In vitro* functional characterization.**

The most potent compounds, **6a**, **6b** and **6d**, were selected for additional *in vitro* pharmacological evaluation to assess their ability to functionally activate CB2. Two orthogonal assays were employed, one evaluated their ability to inhibit cAMP accumulation, one of the primary second messengers in the G-protein-coupled effector system, and the other one tested their ability to recruit β-arrestin, an important response regulating signal transduction beyond G protein activation. To measure intracellular cAMP levels, we performed the enzyme fragment complementation (EFC) technology-based cAMP Hunter™ assay in CHO cells optimized to overexpress CB2. Compounds were tested in presence of 25µM NKH-477 solution (a water-soluble analogue of Forskolin) to stimulate adenylate cyclase and enhance basal cAMP levels. As shown in Figure 1 (left side), compounds **6a, 6b, 6d** were able to inhibit NKH-477-stimulated cAMP accumulation in cells, with EC_{50}s 57nM, 68nM, 306 nM, respectively, suggesting their action as agonists at CB2. To further confirm the efficacy of compounds **6a, 6b, 6d** as CB2 agonists, the GPCR β-arrestin recruitment Tango assay was performed Figure 1 (right side). It relies on the finding that β-arrestins are essential to stimulate agonist-induced receptor internalization.[37] Again, they showed agonistic pattern with EC_{50}s of 26.3, 12.88 and 128.82 nM, respectively. More interestingly, in both assays their observed efficacy as agonists demonstrated a similar trend to their binding data, confirming a strong correlation between both. To characterize the functionality of all other synthesised compounds, the GPCR β-arrestin recruitment Tango assay was performed.[37] For measurement of agonistic activity, the synthesized compounds were subjected to full concentration-response studies against different concentrations of the non-selective cannabinoid receptor reference agonist CP-55,940, with a maximum concentration of 30 µM.

While for measurement of the antagonistic activity, 0.3 μM (EC_{80} concentration) of the reference agonist (CP55,940) was initially added to elicit the activation of the receptors above the basal level. Subsequently, synthesized compounds were subjected to full concentration-response studies against a series of dilutions of reference antagonist SR144528; (a selective CB_2 antagonist) and/or Rimonabant ((SR141716); a selective CB_1 antagonist) with the maximum concentration used was 30 μM in order to determine the EC_{50} values of our compounds. All functional results were analyzed using Graph Pad Prism v7.0 using its built-in functions.

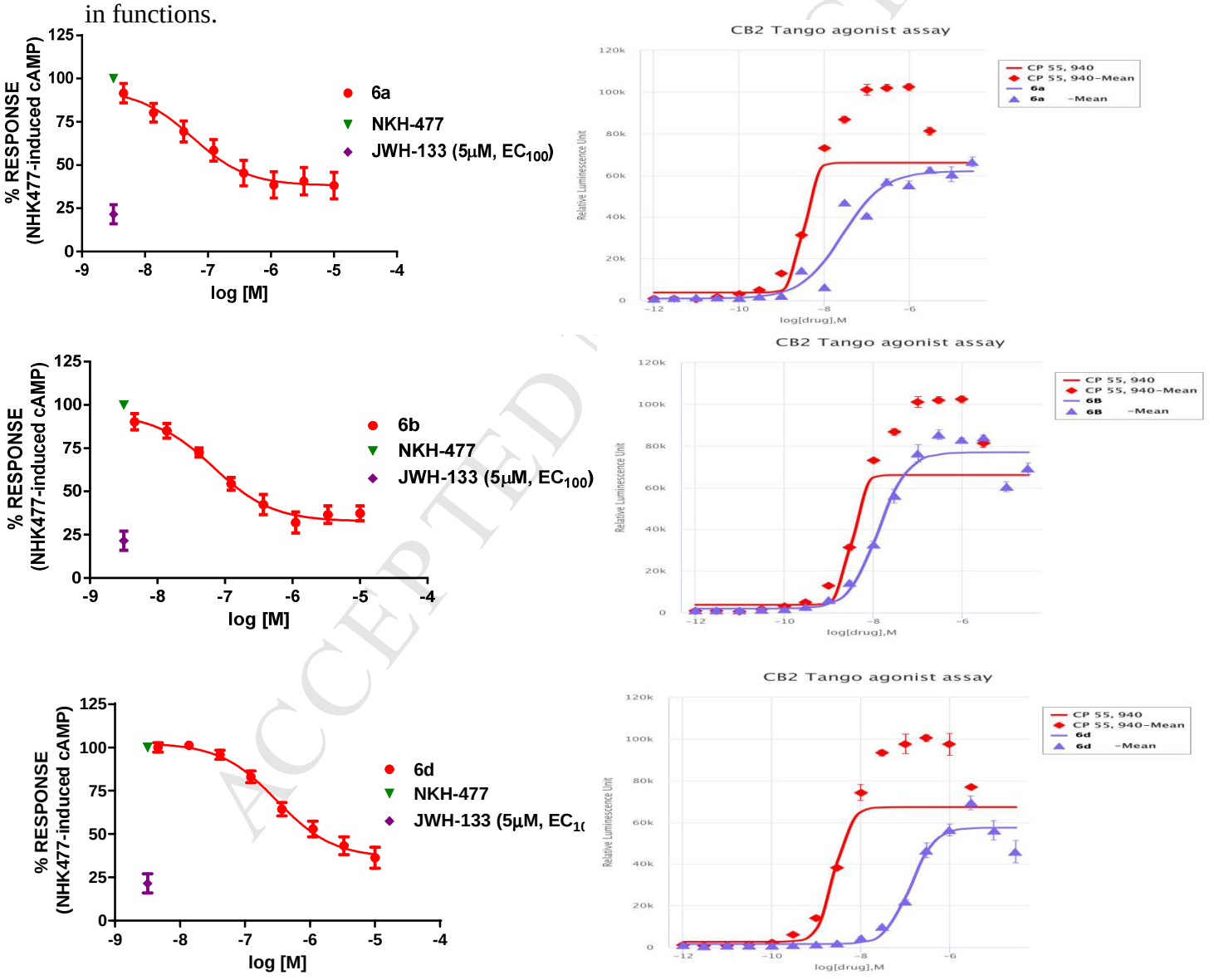


Figure 1: Left side; concentration-response curves of compounds **6a, 6b and 6d** (red) in cAMP Hunter™ assay, Effect of EC_{100} JWH-133 as reference agonist is also shown (violet). Right side; concentration-response curves

of compounds **6a, 6b and 6d** (red) in GPCR β-arrestin recruitment functional assay. Effect of CP-55,940 as a reference agonist is also shown (violet).

In Vivo Efficacy: Mouse Model of DSS-Induced Colitis

Driven by a favourable selectivity and affinity profile (*h*CB2 K_i= 1 nM, hCB1 K_i /hCB2 K_i SI > 429) and its efficacy, the CB2 agonist **6d** has been chosen for assessing the *in vivo* anti-inflammatory potential, of this novel class of compounds, in mice model of acute DSS-induced colitis. Swiss male albino mice (20-35 g) were provided with 3% DSS in distilled drinking water ad libitum for 7 days during which treated mice (n=7) recieved daily intraperitoneal injections of 10 mg/kg of compound **6d** in 4% DMSO, 2% tween in saline. Positive controls recieved vehicle and DSS water while negative controls recieved vehicle only. At day 7, the clinical severity of the colitis was assesed in the form of a disease activity index (DAI) based on weight loss, stool consistency, and the presence of blood in stool. Significantly, treatment with 10 mg/kg of compound **6d** lowered the DAI (3.286 ± 0.8081, $P < 0.05$) compared to the untreated control group (7.143 ± 1.29, $P < 0.05$), Figure 2A. After sacrificing the test subjects, colon length -being a parameter for assessing severity of colitis- was evaluated and a significant shortening of the colon was observed with the untreated control mice (6.729 ± 0.1911, P= 0.0220) relative to treated ones (7.314 ± 0.1143, P= 0.0220), Figure 2B. Histological data obtained from the distal part of the colon showed ulceration and desquamation of the lining of the mucosal epithelium and displayed oedema, haemorrhage and lymphoid hyperplasia in the sub-musoca of untreated control mice, being absent in **6d** treated mice. However, treated mice showed comparable degree of inflammatory cells infiltration in the submucosa to untreated controls (Figure 3).

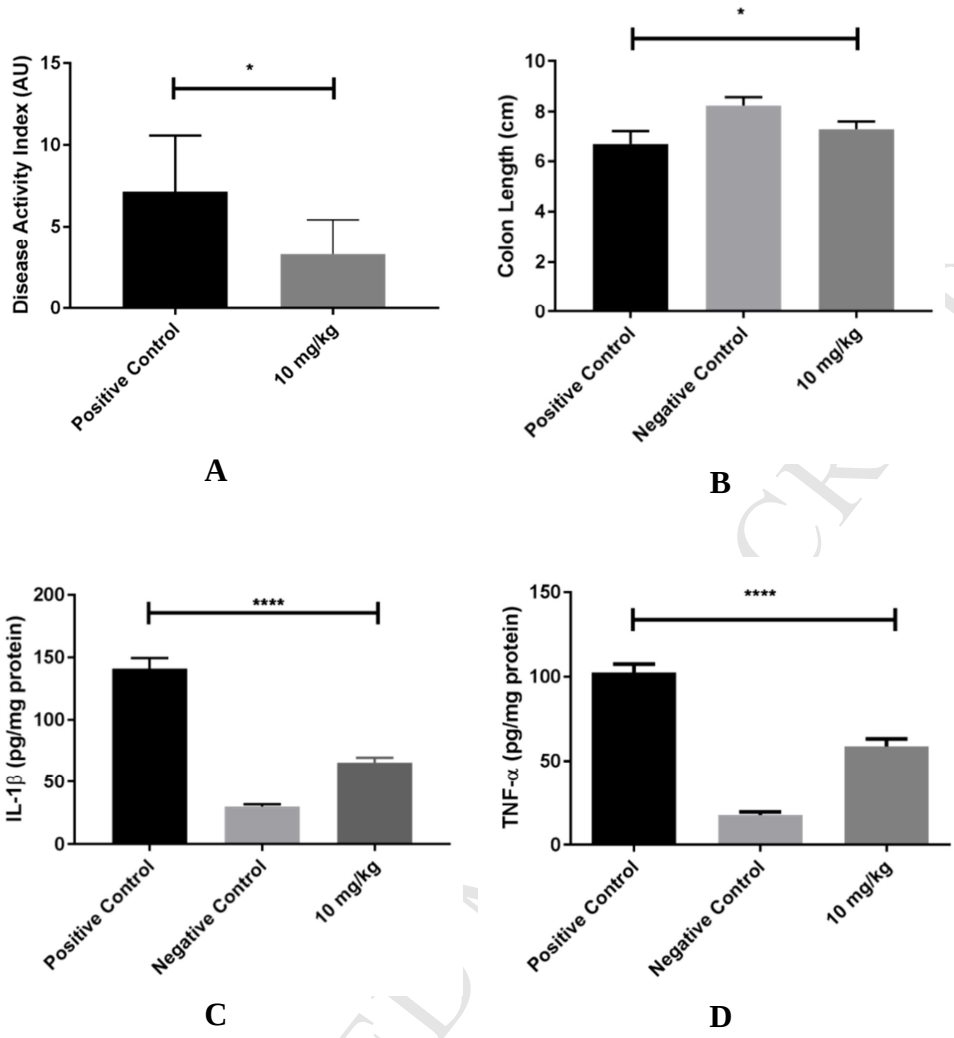


Figure 2: Effect of daily 10 mg/kg i.p treatment with compound **6d** on disease activity index DAI score (A) and colon length (B) in DSS-induced colitis mice model. Quantitative determination of inflammatory cytokines IL-1β and TNFα using ELISA in the colons of **6d**-treated mice, positive and negative controls (C, D) (values are the mean ± SEM of 8 mice per group; *$p < 0.05$; ****$p < 0.0001$ vs positive control).

To further confirm an anti-inflammatory role of **6d**, protein levels of major inflammatory cytokines such as TNF-α and IL-1β were quantified in colon tissues using ELISA. Treatment with **6d** brought about a powerful anti-inflammatory action apparent with remarkably lower levels of both TNF-α (58.71 ± 4.434, P<0.0001) and IL-1β (65.81 ± 3.694, P<0.0001) compared to untreated controls; TNF-α (102.4 ± 5.043, P<0.0001) and IL-1β (140.9 ± 8.551, P<0.0001), Figure 2C, 2D. Taken together, these data strongly indicate that compound **6d,** being a representative of our most potent series of CB2 agonists ***N*-(3-pentylbenzothiazol-**

2(3*H*)-ylidene) carboxamides, exhibits a protective effect against DSS-induced acute colitis in mice model.

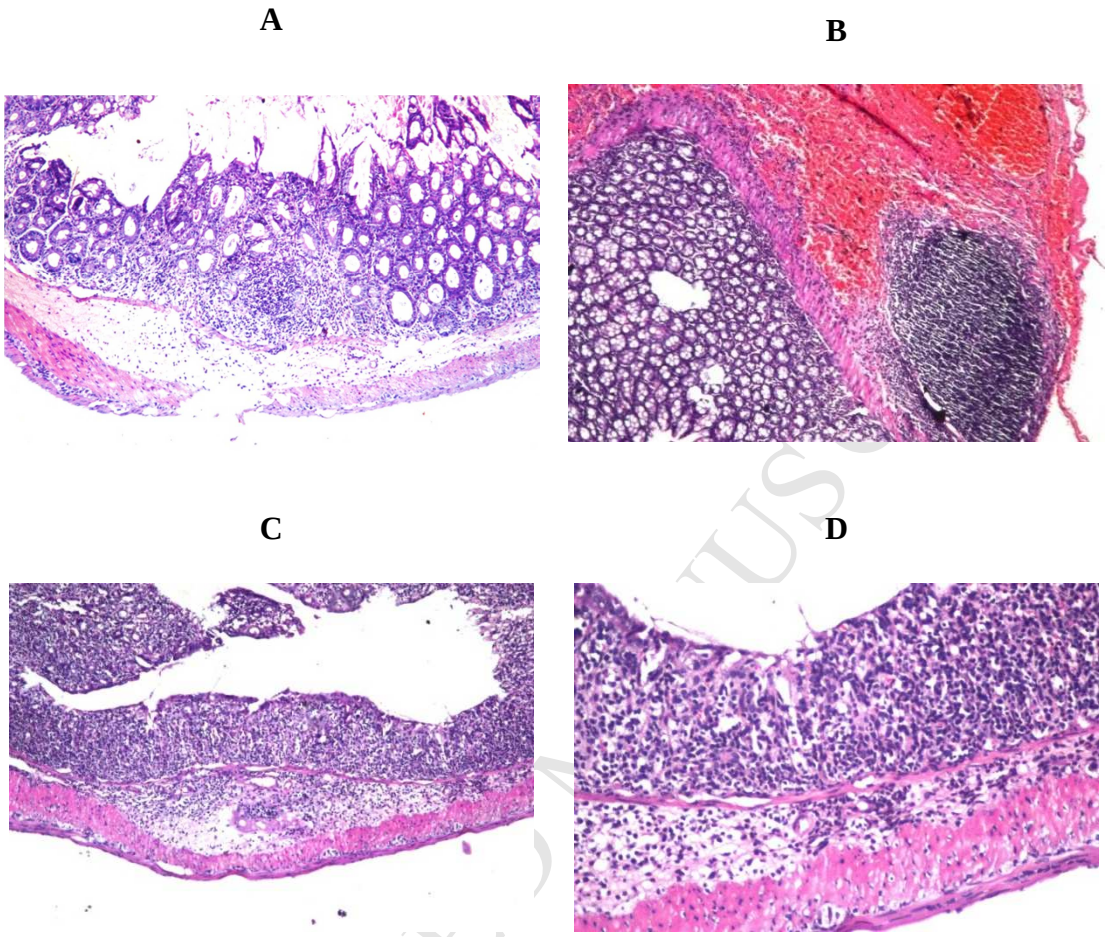


Figure 3: Representative micrographs of H&E (hematoxylin & eosin)-stained sections of distal colon from experimental groups of DSS-induced colitis model. In positive controls; massive inflammatory infiltration and aggregation in lamina propria with mucosal destruction and ulceration, as well as diffuse haemorrhage with lymphoid follicles hyperplasia was recorded (A), and (B), which is largely ameliorated in compound **6d**-treated mice that showed no histopathological alterations except for inflammatory cells infiltration in lamina propria. (C) and (D). (Magnification: A, B, C: x16, D: x40).

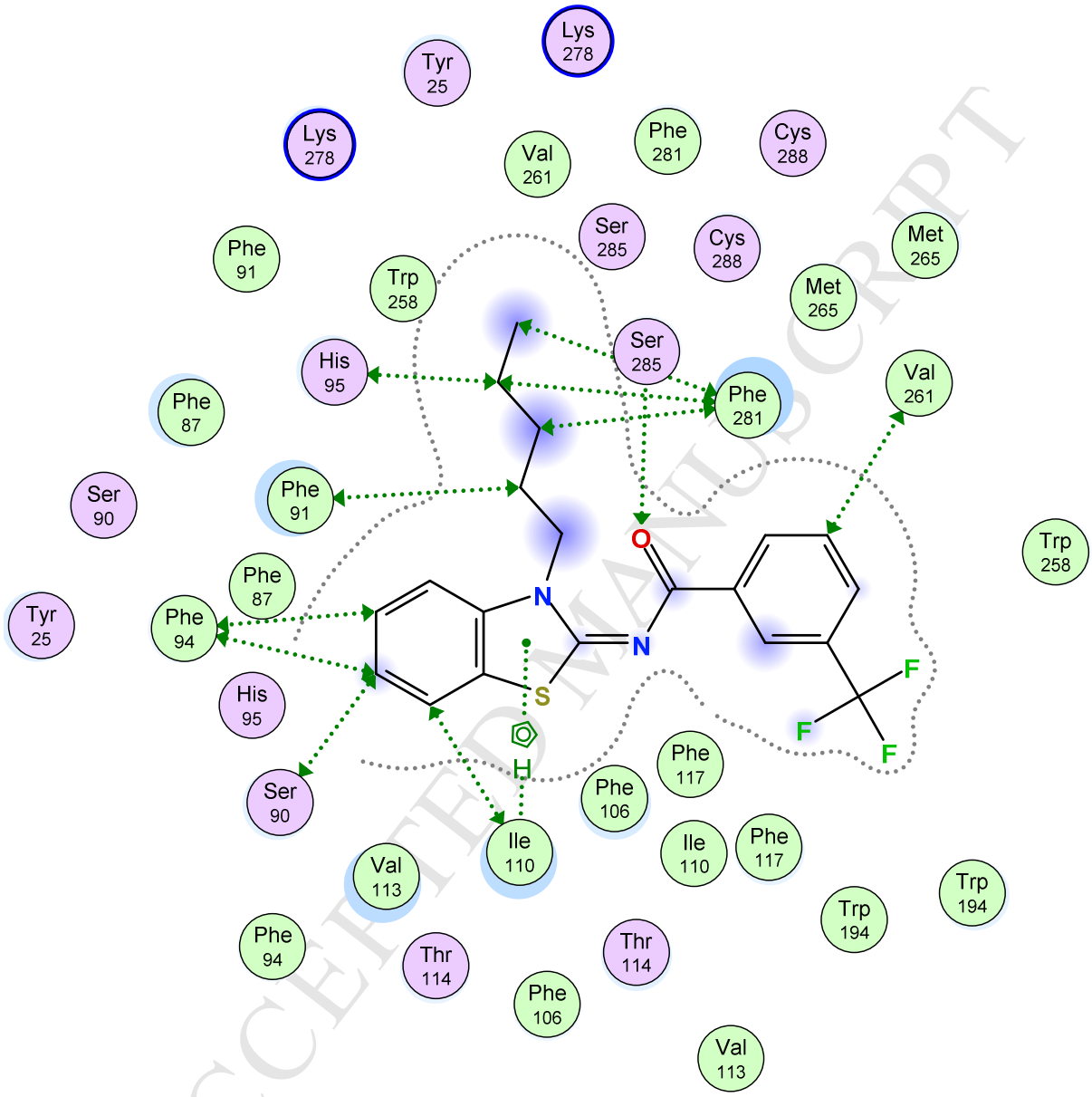
Molecular Docking

To explain the impact of different substituents on the CB2 receptors binding, compound **6d** was docked to the CB2 receptor homology model derived from the CB1 co-crystal structure PDB 5XRA. The binding site is identified from the CB1 template and from the site finder of MOE software and both identified the same binding pocket. The protein is prepared by

adding hydrogens and partial charges. Compound **6d** is subjected to conformational search and docked to the site using default conditions. The best scoring pose and close poses showed mainly hydrophobic interactions between the benzothiazole and lipophilic pocket lined with lipophilic residues like Phe106, Ile110, Val113, Phe117, the other aryl part is also embedded in a lipophilic pocket outlined with Trp194, Trp258, Val261, Met285. Moreover, the pentyl substituent is involved in hydrophobic interactions with Phe91, Phe281, this may explain the extra-potency of the disubstituted benzothiazoles (**6a-6d**) relative to their monosubstituted congeners (**5a-5d**). In addition, there is always a hydrogen bonding between the amide oxygen and Ser285, Figure 4.

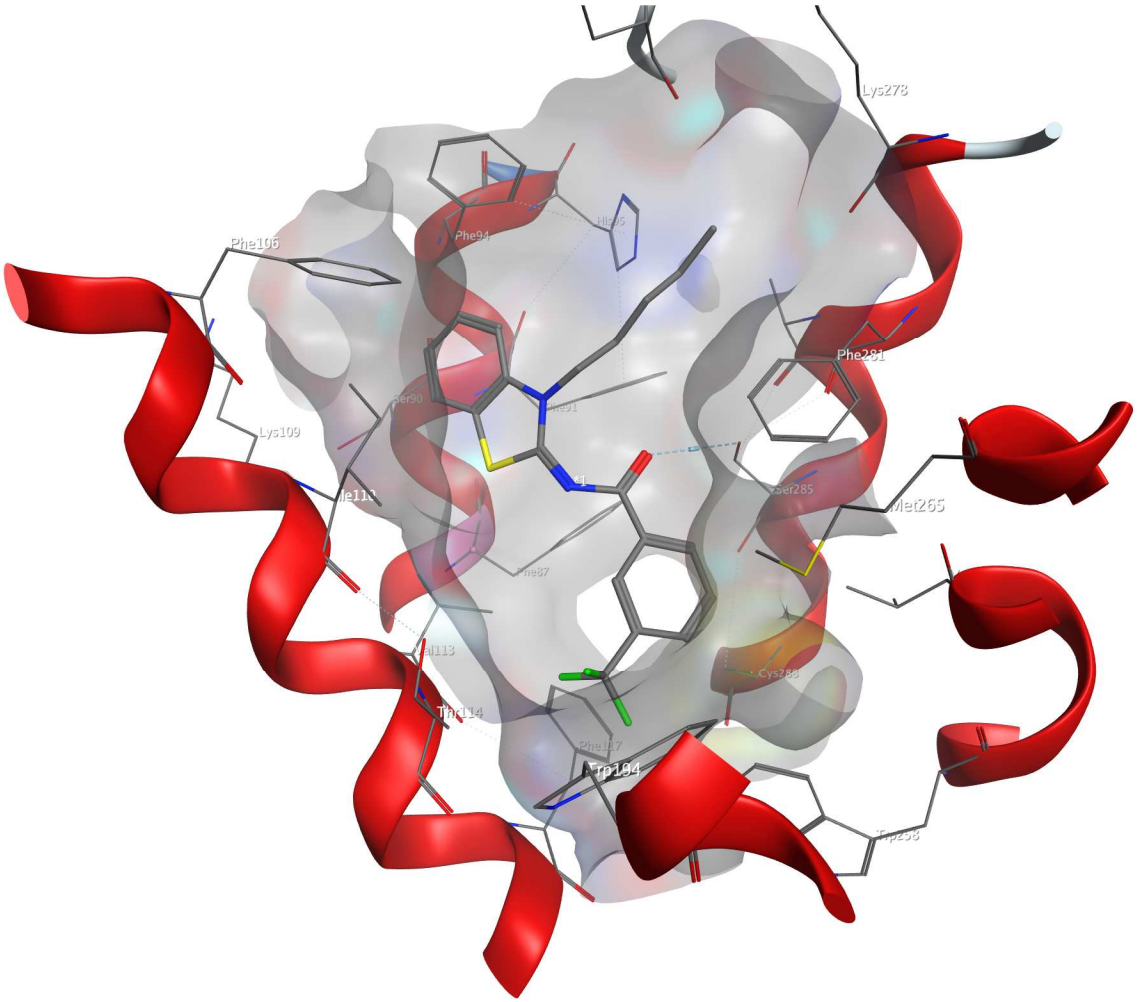
ACCEPTED MANUSCRIPT

4A



ACCEPTED MANUSCRIPT

4B



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4C

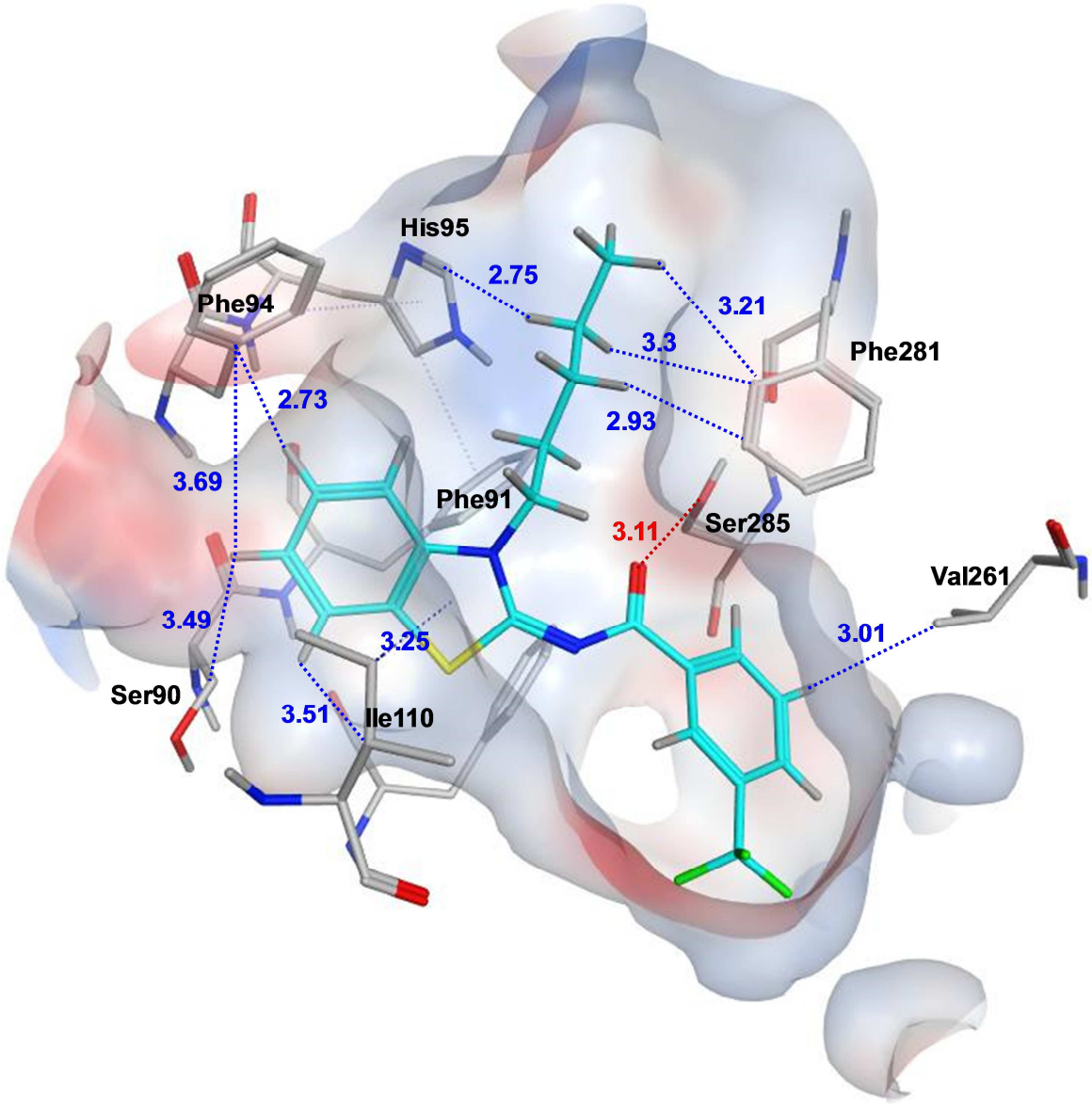

Figure 4: 2D (4A) and 3D (4B) predicted interaction of compound **6d** with the homology model of human CB2 receptor. The interactions are mainly hydrophobic and with on H-bonding with Ser 285. Figure 4C, shows the distances between key residues and corresponding groups of **6d**; Hydrogen bonds shown as red lines, hydrophobic interaction and π-π shown as blue lines

EXPERIMENTAL SECTION

Chemistry. Solvents and reagents were obtained from commercial suppliers and were used without further purification. Anhydrous reactions were carried out under argon. Reaction progress was monitored by TLC using fluorescent pre-coated silica gel plates and detection of the components was made by short UV light (λ= 254 nm). Column chromatography was carried out using silica-gel 40-60 µm mesh. ^{1}H-NMR, ^{13}C-NMR spectra were recorded at Varian 400 MHz spectrometer or at Varian 300 MHz spectrometer. ^{1}H shifts are referenced to the residual protonated solvent signal (δ 2.50 for DMSO-d_6 and δ 7.26 for $CDCl_3$) and ^{13}C shifts are referenced to the deuterated solvent signal (δ 39.5 for DMSO-d_6 and δ 77.0 for $CDCl_3$). Chemical shifts are given in parts per million (ppm), and all coupling constants (*J*) are given in Hz. The purities of the tested compounds were determined by HPLC coupled with mass spectrometry and were higher than 95% purity for all compounds. Mass spectrometric analysis (UPLC-ESI-MS) was performed using Waters ACQUITY Xevo TQD system, which consisted of an ACQUITY UPLC H-Class system and Xevo^{TM}TQD triple-quadrupole tandem mass spectrometer with an electrospray ionization (ESI) interface (Waters Corp., Milford, MA, USA). Acquity BEH C_{18}100 mm × 2.1 mm column (particle size, 1.7 µm) was used to separate analytes (Waters, Ireland). The solvent system consisted of water containing 0.1% TFA (A) and 0.1% TFA in acetonitrile (B). HPLC-method: flow rate 200 µL/min. The percentage of B started at an initial of 5% and maintained for 1 minute, then increased up to 100% during 10 min, kept at 100% for 2 min, and flushed back to 5% in 3 min. The MS scan was carried out at the following conditions: capillary voltage 3.5 kV, cone voltage 20V, radio frequency (RF) lens voltage 2.5V, source temperature 150 ◦C, and desolvation gas temperature 500 ◦C. Nitrogen was used as the desolvation and cone gas at a flow rate of 1000 and 20 L/h, respectively. System operation and data acquisition were controlled using Mass Lynx 4.1 software (Waters). Melting points were determined on BuchiB-540 Melting Point apparatus and are uncorrected.

General synthetic methods and experimental details for some key compounds.

Procedure for the synthesis of 2,2-dimethyl propanthioamide (1b).

A solution of 2,2-dimethylpropanamide **1a** (1 g, 9.8 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent) (1.98 g, 4.9 mmol) in THF (15 mL) was heated to reflux for 4 h. The reaction mixture was then cooled to room temperature and poured into a saturated aqueous solution of NaHCO3 (50 mL). The mixture was extracted with ether (35 mL) and the organic layer was dried over anhydrous $MgSO_4$ then evaporated under reduced pressure giving the desired white powder **1b** that was used without further purification in a yield of 78.9%; mp: 117-119 ^{o}C.[39]

General procedure for the synthesis of ethyl 2-substituted-4-methyl 5-thiazole carboxylates (1c, 2c, 3c).

To a suspension of different thioamides **1b, 2b, 3b** (7.28 mmol) in ethyl alcohol (15 mL) was added at room temperature ethyl 2-chloroacetoacetate (1 equiv.). The solution was refluxed for 24 h, and then the solvent was removed under reduced pressure. The solid material was washed with cooled hexane (2 x 10 mL) to give the corresponding pure ethyl carboxylate derivative**s 1c, 2c, 3c** in different yields.

Ethyl 2-(*tert*-butyl)-4-methylthiazole-5-carboxylate (1c). Prepared from 2,2-dimethyl propanthioamide (1b). Yellow solid 75.3%; mp: 43-47 ^{o}C.[40]

Ethyl 4-methyl-2-phenylthiazole-5-carboxylate (2c). Prepared from commercially available thiobenzamide **2b**. White solid 78%; mp: 42-43 ^{o}C; ^{1}H-NMR (δ): (300 MHz, DMSO) δ 8.01 – 7.92 (m, 2H), 7.59 – 7.45 (m, 3H), 4.28 (q, *J* = 7.1 Hz, 2H), 2.67 (s, 3H),

1.29 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (δ): (75 MHz, DMSO) δ 169.28, 161.68, 160.57, 132.39, 131.74, 129.66, 126.84, 121.58, 61.51, 17.54, 14.44.[41]

Ethyl 2,4-dimethylthiazole-5-carboxylate (3c). Prepared from commercially available methylthioamide **2c**. Red solid 91.3%; mp: 48-50 ^{o}C; MS (ESI): *m/z* 186.08 $[M + H]^{+}$.[42]

Procedure for the synthesis of 4-methyl 2-substituted thiazole-5-carboxylic acid (1d, 3d).

The appropriate ethyl ester **(1c, 3c)** (5.8 mmol) was refluxed for 4 h in a mixture of equal volumes of aqueous 10% NaOH (20 mL) and Ethanol (20 mL). After completion of the reaction, ethanol was evaporated under reduced pressure and the remaining solution was then extracted with ethyl acetate. The organic layer was discarded while the aqueous layer was acidified to pH 3 with aqueous 10% hydrochloric acid. The mixture was then evaporated under reduced pressure and the solid obtained was then dissolved in methanol while the remaining insoluble NaCl was discarded. Evaporating the methanol afforded the desired pure acids **1d** and **3d**.

2-(*tert*-butyl)-4-methylthiazole-5-carboxylic acid (1d). Prepared from ethyl 2-(*tert*-butyl)-4-methylthiazole-5-carboxylate **1c**. White solid 95%; mp: 154-157 ^{o}C.[43]

2,4-Dimethylthiazole-5-carboxylic acid (3d). Prepared from ethyl 2,4-dimethylthiazole-5-carboxylate **3c**. Yellow solid 90%; mp: 233-235 ^{o}C.[44]

Procedure for the synthesis of 4-methyl-2-phenylthiazole-5-carboxylic acid (2d).

Ethyl 4-methyl-2-phenylthiazole-5-carboxylate **2c** (5.8 mmol) was refluxed for 4 h in a mixture of equal volumes of aqueous 10% NaOH (20 mL) and ethanol (20 mL). After cooling in ice, the solution was acidified to pH 3 with 10% aqueous hydrochloric acid. The resulting precipitate **2d** was collected by filtration, washed with H_2O, dried under vacuum and used without further purification. White solid 97%; mp: 210-212 ^{o}C; ^{1}H-NMR (δ): (300

MHz, DMSO) δ 7.96 (dd, *J* = 5.7, 1.7 Hz, 2H), 7.51 (d, *J* = 5.6 Hz, 3H), 2.66 (s, 3H); ^{13}C-NMR (δ): (75 MHz, DMSO) δ 168.72, 163.21, 159.89, 132.59, 131.51, 129.60, 126.75, 123.14, 17.40.[42]

Procedure for the synthesis of 4-methyl 2-substituted thiazole-5-carboxamides (1m, 3l-3n):

To a magnetically stirred suspension of the carboxylic acid derivative **1d** or **3d** (1.6 mmol) in anhydrous DMF were successively added tributylamine (Hunig's base) (6 equiv.) and N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl) uronium hexafluorophosphate (HBTU) (1.2 equiv.). The mixture was allowed to stir at room temperature for 30 minutes. The appropriate amine (1.2 equiv.) was then added, and the solution was stirred at room temperature for 4-5 h. The mixture was diluted with EtOAc, washed with aqueous NaHCO3 solution, 1N HCl and brine, dried over anhydrous MgSO4 and concentrated to give a crude resin. The crude product was further purified using column chromatography.

***N*-(adamantan-1-yl)-2-(*tert*-butyl)-4-methylthiazole-5-carboxamide (1m).** Prepared from 1-adamantyl-amine. Column chromatography on silica gel (Hexane:Acetone, 9:1) afforded **1m** as a white solid (58.2%); mp: 138-139 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 5.40 (s, 1H), 2.61 (s, 3H), 2.10 (s, 3H), 2.08 (s, 6H), 1.70 (s, 6H), 1.40 (s, 9H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 180.26, 161.45, 154.11, 125.83, 53.01, 41.92, 37.83, 36.45, 30.80, 29.60, 17.35; MS (ESI): *m/z* 333.24 $[M + H]^+$.

***N*-benzyl-2,4-dimethylthiazole-5-carboxamide (3n)**. Prepared from benzylamine. Column chromatography on silica gel (DCM:Methanol, 98.5:1.5) afforded **3n** as a yellow solid (75.6%); mp: 90-91 ºC; ^{1}H-NMR (δ): (300 MHz, $CDCl_3$) δ 7.40 – 7.26 (m, 5H), 6.03 (s, 1H), 4.57 (d, *J* = 5.7 Hz, 2H), 2.64 (s, 6H); ^{13}C-NMR (δ): (75 MHz, $CDCl_3$) δ 166.01, 161.87, 155.36, 137.95, 128.96, 127.93, 127.85, 125.32, 44.25, 19.44, 17.34; MS (ESI): *m/z* 247.073 $[M + H]^+$.

2,4-Dimethyl-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)thiazole-5-carboxamide (3l). Prepared from 1,2,3,4-tetrahydronaphth-1-yl amine. Column chromatography on silica gel (Hexane:Acetone, 7.5:2.5) afforded **3l** as a yellow solid (71.4%); mp: 123-125 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.35 – 7.29 (m, 1H), 7.23 – 7.15 (m, 2H), 7.15 – 7.10 (m, 1H), 5.92 (d, *J* = 7.8 Hz, 1H), 5.37 – 5.28 (m, 1H), 2.90 – 2.74 (m, 2H), 2.65 (s, 6H), 2.19 – 2.08 (m, 1H), 1.94 – 1.83 (m, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 165.95, 161.28, 155.03, 137.83, 136.38, 129.44, 128.72, 127.63, 126.55, 125.73, 48.32, 30.37, 29.35, 20.19, 19.44, 17.37; MS (ESI): *m/z* 287.22 $[M + H]^+$.

***N*-(adamantan-1-yl)-2,4-dimethylthiazole-5-carboxamide (3m)**. Prepared from 1-adamantyl-amine. Column chromatography on silica gel (Heptane:Acetone, 7.5:2.5) afforded **3m** as a white solid (75.2%); mp: 76-79 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 5.38 (s, 1H), 2.63 (s, 3H), 2.60 (s, 3H), 2.10 (s, 3H), 2.08 (s, 6H), 1.70 (s, 6H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 165.38, 161.07, 153.86, 127.21, 53.11, 41.90, 36.43, 29.59, 19.37, 17.20; MS (ESI): *m/z* 291.06 $[M + H]^+$.

Procedure for the synthesis of 4-methyl 2-substituted thiazole-5-carboxamides (2e-2q): To a magnetically stirred suspension of the carboxylic acid derivative **2d** (1.6 mmol) in anhydrous DCM were successively added tributylamine (Hunig's base) (6 equiv.) and *N*,*N*,*N*′,*N*′-Tetramethyl-O-(1*H*-benzotriazol-1-yl) uronium hexafluorophosphate (HBTU) (1.2 equiv.) and the mixture was allowed to stir at room temperature for 30 minutes. The appropriate amine (1.2 equiv.) was then added, and the solution was stirred at room temperature for 4-5 h. The solvent was then evaporated under reduced pressure and the resulting crude product was dissolved in EtOAc, washed with aqueous $NaHCO_3$ solution, 1N HCl and brine, dried over anhydrous $MgSO_4$ and concentrated to give a crude resin. The crude product was further purified using column chromatography.

***N*-butyl-4-methyl-2-phenylthiazole-5-carboxamide (2e)**. Prepared from butylamine. Column chromatography on silica gel (Hexane:Acetone, 8.5:1.5) afforded **2e** as a yellow solid (79.9%); mp: 95-96 ºC; ^{1}H-NMR (δ): (300 MHz, $CDCl_3$) δ 7.93 – 7.85 (m, 2H), 7.46 – 7.37 (m, 3H), 5.87 (s, 1H), 3.47 – 3.37 (m, 2H), 2.72 (s, 3H), 1.65 – 1.54 (m, 2H), 1.48 – 1.34 (m, 2H), 0.96 (t, *J* = 7.3 Hz, 3H); ^{13}C-NMR (δ): (75 MHz, $CDCl_3$) δ 167.10, 161.98, 155.88, 133.04, 130.83, 129.13, 126.80, 126.06, 40.07, 31.81, 20.27, 17.53, 13.88; MS (ESI): *m/z* 275.09 $[M + H]^+$.

***N*-hexyl-4-methyl-2-phenylthiazole-5-carboxamide (2f)**. Prepared from hexylamine. Column chromatography on silica gel (Hexane:Acetone, 8.8:1.2) afforded **2f** as a yellow solid (87.2%); mp: 97-98 ºC; ^{1}H-NMR (δ): (300 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 6.7, 2.9 Hz, 2H), 7.47 – 7.39 (m, 3H), 5.88 (s, 1H), 3.40 – 3.34 (m, 2H), 2.72 (s, 3H), 1.65 – 1.54 (m, 2H), 1.44 – 1.26 (m, 6H), 0.89 (t, *J* = 6.6 Hz, 3H); ^{13}C-NMR (δ): (75 MHz, $CDCl_3$) δ 167.10, 161.96, 155.86, 133.04, 130.83, 129.13, 126.80, 126.08, 40.38, 31.58, 29.71, 26.76, 22.67, 17.52, 14.13; MS (ESI): *m/z* 303.113 $[M + H]^+$.

***N*-cyclopropyl-4-methyl-2-phenylthiazole-5-carboxamide (2g)**. Prepared from cyclopropylamine. Column chromatography on silica gel (Hexane:Acetone, 8.8:1.2) afforded **2g** as a white solid (91.5%); mp: 143-145 ºC; ^{1}H-NMR (δ): (300 MHz, $CDCl_3$) δ 7.95 – 7.87 (m, 2H), 7.49 – 7.38 (m, 3H), 5.96 (s, 1H), 2.91 – 2.82 (m, 1H), 2.73 (s, 3H), 0.92 – 0.83 (m, 2H), 0.67 – 0.61 (m, 2H); ^{13}C-NMR (δ): (75 MHz, $CDCl_3$) δ 167.16, 163.39, 156.74, 132.99, 130.93, 129.18, 126.86, 126.43, 23.42, 17.58, 7.11; MS (ESI): *m/z* 259.08 $[M + H]^+$.

***N*-cyclopentyl-4-methyl-2-phenylthiazole-5-carboxamide (2h)**. Prepared from cyclopentylamine. Column chromatography on silica gel (Hexane:Acetone, 8.8:1.2) afforded **2h** as a white solid (93%); mp: 170-171 ºC; ^{1}H-NMR (δ): (300 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 6.4, 2.5 Hz, 2H), 7.48 – 7.39 (m, 3H), 5.79 (d, *J* = 6.0 Hz, 1H), 4.43 – 4.28 (m, 1H), 2.72 (s,

3H), 2.16 – 2.01 (m, 2H), 1.81 – 1.59 (m, 4H), 1.57 – 1.43 (m, 2H); ^{13}C-NMR (δ): (75 MHz, $CDCl_3$) δ 167.48, 162.02, 156.14, 133.46, 131.24, 129.56, 127.22, 126.62, 52.53, 33.80, 24.32, 17.93; MS (ESI): *m/z* 287.129 $[M + H]^+$.

***N*-cyclohexyl-4-methyl-2-phenylthiazole-5-carboxamide (2i)**. Prepared from cyclohexylamine. Column chromatography on silica gel (Hexane:EtOAc, 8:2) afforded **2i** as a white solid (78.7%); mp: 111-112 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.94 – 7.90 (m, 2H), 7.46 – 7.42 (m, 3H), 5.64 (d, *J* = 7.5 Hz, 1H), 4.00 – 3.89 (m, 1H), 2.73 (s, 3H), 2.07 – 1.99 (m, 2H), 1.80 – 1.70 (m, 2H), 1.70 – 1.56 (m, 4H), 1.50 – 1.36 (m, 2H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 167.08, 161.09, 155.74, 133.09, 130.84, 129.17, 126.84, 126.35, 49.12, 33.32, 25.66, 24.95, 17.56.; MS (ESI): *m/z* 301.08 $[M + H]^+$.

(4-Methyl-2-phenylthiazol-5-yl)(morpholin-4-yl)methanone (2j). Prepared from morpholine. Column chromatography on silica gel (Hexane:EtOAc, 6:4) afforded **2j** as yellowish oil (91.4%); ^{1}H-NMR (δ): (300 MHz, $CDCl_3$) δ 7.94 – 7.86 (m, 2H), 7.47 – 7.40 (m, 3H), 3.77 – 3.70 (m, 4H), 3.70 – 3.60 (m, 4H), 2.50 (s, 3H); ^{13}C-NMR (δ): (75 MHz, $CDCl_3$) δ 168.18, 162.90, 152.99, 132.90, 130.81, 129.20, 126.78, 123.97, 67.00, 16.70; MS (ESI): *m/z* 289.05 $[M + H]^+$.

***N*-(cyclohexylmethyl)-4-methyl-2-phenylthiazole-5-carboxamide (2k)**. Prepared from cyclohexylmethylamine. Column chromatography on silica gel (Hexane:Acetone, 9:1) afforded **2k** as a white powder (74.4%); mp: 147-148 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.94 – 7.87 (m, 2H), 7.46 – 7.38 (m, 3H), 5.88 (s, 1H), 3.28 (t, *J* = 5.4 Hz, 2H), 2.74 (s, 3H), 1.81 – 1.64 (m, 5H), 1.64 – 1.52 (m, 1H), 1.33 – 1.11 (m, 3H), 1.05 – 0.91 (m, 2H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 167.12, 162.02, 155.85, 133.08, 130.85, 129.16, 126.83, 126.16, 46.50, 38.11, 31.05, 26.50, 25.94, 17.59; MS (ESI): *m/z* 315.05 $[M + H]^+$.

4-Methyl-2-phenyl-*N*-(1,2,3,4-tetrahydronaphthalen-1-yl)thiazole-5-carboxamide (2l). Prepared from 1,2,3,4-tetrahydronaphth-1-yl amine. Column chromatography on silica gel (DCM:Methanol, 9.9:0.1) afforded **2l** as a white powder (84 %); mp: 139-140 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.97 – 7.87 (m, 2H), 7.47 – 7.41 (m, 3H), 7.38 – 7.33 (m, 1H), 7.24 – 7.19 (m, 2H), 7.17 – 7.12 (m, 1H), 6.01 (d, *J* = 8.2 Hz, 1H), 5.43 – 5.31 (m, 1H), 2.92 – 2.78 (m, 2H), 2.76 (s, 3H), 2.22 – 2.11 (m, 1H), 1.99 – 1.85 (m, 3H); MS (ESI): *m/z* 349.17 $[M + H]^+$.

***N*-(adamantan-1-yl)-4-methyl-2-phenylthiazole-5-carboxamide (2m).** Prepared from 1-adamantylamine. Column chromatography on silica gel (Hexane:EtOAc, 8.6:1.4) afforded **2m** as a white powder (95.2%); mp: 142-143 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.93 – 7.89 (m, 2H), 7.46 – 7.41 (m, 3H), 5.49 (s, 1H), 2.70 (s, 3H), 2.12 (s, 9H), 1.72 (s, 6H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 166.68, 161.06, 155.25, 133.14, 130.74, 129.13, 127.38, 126.80, 53.21, 41.92, 36.45, 29.62, 17.51; MS (ESI): *m/z* 353.55 $[M + H]^+$.

***N*-benzyl-4-methyl-2-phenylthiazole-5-carboxamide (2n).** Prepared from benzylamine. Column chromatography on silica gel (Hexane:EtOAc, 8:2) afforded **2n** as a white powder (86.3%); mp: 133-134 ºC; ^{1}H-NMR (δ): (300 MHz, $CDCl_3$) δ 7.96 – 7.86 (m, 2H), 7.48 – 7.39 (m, 3H), 7.39 – 7.26 (m, 5H), 6.15 (s, 1H), 4.62 (d, *J* = 5.7 Hz, 2H), 2.75 (s, 3H); ^{13}C-NMR (δ): (75 MHz, $CDCl_3$) δ 167.37, 161.90, 156.59, 137.92, 132.97, 130.94, 129.17, 128.99, 127.98, 127.89, 126.85, 125.50, 44.35, 17.65; MS (ESI): *m/z* 309.04 $[M + H]^+$.

***N*-(2-fluorobenzyl)-4-methyl-2-phenylthiazole-5-carboxamide (2o).** Prepared from 2-fluorobenzylamine. Column chromatography on silica gel (Heptane:EtOAc, 8.1:1.9) afforded **2o** as a white powder (93.8%); mp: 128-129 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.93 – 7.88 (m, 2H), 7.46 – 7.39 (m, 4H), 7.33 – 7.26 (m, 1H), 7.17 – 7.04 (m, 2H), 6.24 (s, 1H), 4.67 (d, *J* = 5.9 Hz, 2H), 2.74 (s, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 167.52, 161.27 (d,

$^{1}J_{CF}$ = 245.9 Hz), 161.88, 156.45, 132.94, 130.98, 130.57 (d, $^{3}J_{CF}$ = 4.2 Hz), 129.74 (d, $^{3}J_{CF}$ = 8.2 Hz), 129.18, 126.88, 125.59, 124.94 (d, $^{2}J_{CF}$ = 14.6 Hz), 124.61 (d, $^{4}J_{CF}$ = 3.6 Hz), 115.64 (d, $^{2}J_{CF}$ = 21.2 Hz), 38.47 (d, $^{3}J_{CF}$ = 3.6 Hz), 19.92; MS (ESI): *m/z* 327.47 $[M + H]^{+}$.

***N*-(4-fluorobenzyl)-4-methyl-2-phenylthiazole-5-carboxamide (2p).** Prepared from 4-fluorobenzylamine. Column chromatography on silica gel (Heptane:EtOAc, 8.1:1.9) afforded **2p** as a white powder (72.4%); mp: 149-150 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.94 – 7.86 (m, 2H), 7.48 – 7.39 (m, 3H), 7.35 – 7.28 (m, 2H), 7.08 – 6.98 (m, 2H), 6.17 (s, 1H), 4.57 (d, *J* = 5.8 Hz, 2H), 2.74 (s, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 167.44, 162.44 (d, $^{1}J_{CF}$ = 246.2 Hz), 161.93, 156.75, 133.79 (d, $^{4}J_{CF}$ = 3.3 Hz), 132.94, 130.99, 129.68 (d, $^{3}J_{CF}$ = 8.2 Hz), 129.18, 126.86, 125.32, 115.84 (d, $^{2}J_{CF}$ = 21.5 Hz), 43.60, 17.66; MS (ESI): *m/z* 326.87 $[M + H]^{+}$.

***N*-(2-chlorobenzyl)-4-methyl-2-phenylthiazole-5-carboxamide (2q).** Prepared from 2chlorobenzylamine. Column chromatography on silica gel (Heptane:Acetone, 8.5:1.5) afforded **2q** as a white powder (94.2%); mp: 137-138 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.94 – 7.86 (m, 2H), 7.51 – 7.37 (m, 5H), 7.31 – 7.17 (m, 2H), 6.38 (s, 1H), 4.70 (d, *J* = 6.0 Hz, 2H), 2.74 (s, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 167.54, 161.84, 156.40, 135.38, 133.84, 132.99, 130.93, 130.71, 129.77, 129.37, 129.16, 127.38, 126.86, 125.71, 42.34, 17.65; MS (ESI): *m/z* 343.47 $[M + H]^{+}$, *m/z* 345.07 $[M + 3H]^{+}$.

Procedure for the synthesis of ethyl benzo[*d*]thiazole-2-carboxylate (4a).

A mixture of 2-aminothiophenol (7.98 mmol) and diethyl oxalate (2 equiv.) was heated at mild reflux for 4 h, during the first two hours, the temperature was decreased from 147 to 93^{0} C. After cooling, the mixture was poured into a solution consisting of (25 mL) of conc. hydrochloric acid, (75 mL) of water and (35 mL) of ethanol with vigorous stirring. A precipitate was formed, filtered and dried under vacuum to give the titled compound. Yellow

solid (55%); mp: 68-72 °C; ^{1}H-NMR (δ): (400 MHz, CDCl3) δ 8.26 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.98 (dd, *J* = 7.3, 1.5 Hz, 1H), 7.61 – 7.51 (m, 2H), 4.56 (q, *J* = 7.1 Hz, 2H), 1.49 (t, *J* = 7.1 Hz, 3H).[45]

Procedure for the synthesis of benzo[*d*]thiazole-2-carboxylic acid (4b).

Ethyl benzothiazole-2-carboxylate **4a** (4.8 mmol) was refluxed for 4 h along with 1 g NaOH in a mixture of methanol (22 mL), water (9 mL) and THF (11 mL). After completion of the ester hydrolysis, the organic solvents were evaporated under reduced pressure and the aqueous solution was extracted with DCM. The organic phase was discarded while the aqueous phase was acidified to pH 3 using 1 N HCl, extracted with EtOAc twice and the combined organic phases were passed over anhydrous $MgSO_4$. Evaporating the organic solvent under reduced pressure afforded the pure desired acid **4b**. Yellow solid (81.4%); mp: 103-104; ^{1}H-NMR (δ): (400 MHz, CDCl3) δ 9.09 (s, 1H), 8.20 (d, *J* = 8.0 Hz, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 7.60 – 7.46 (m, 2H).[45]

Procedure for the synthesis of benzo[*d*]thiazole-2-carboxamides (4c-4h)

To a magnetically stirred suspension of the carboxylic acid **4b** (1.6 mmol) in anhydrous DCM were successively added tributylamine (Hunig's base) (6 equiv.) and *N*,*N*,*N*′,*N*′-Tetramethyl-O-(1*H*-benzotriazol-1-yl)uroniumhexafluorophosphate (HBTU) (1.2 equiv.) and the mixture was allowed to stir at room temperature for 30 minutes. The appropriate amine (1.2 equiv.) was then added, and the solution was stirred at room temperature for 4-5 hrs. The solvent was then evaporated under reduced pressure and the resulting crude product was dissolved in EtOAc, washed with aqueous $NaHCO_3$ solution, 1N HCl and brine, dried over anhydrous $MgSO_4$ and concentrated to give a crude resin. The crude product was further purified using column chromatography.

***N*-(cyclohexylmethyl)benzo[*d*]thiazole-2-carboxamide (4c).** Prepared from cyclohexylmethyl amine. Column chromatography on silica gel (DCM:Hexane, 6.5:3.5) afforded **4c** as a white solid (91.5%); mp: 106–107 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 7.6, 0.7 Hz, 1H), 7.99 – 7.94 (m, 1H), 7.57 – 7.45 (m, 3H), 3.35 (t, *J* = 6.6 Hz, 2H), 1.89 – 1.71 (m, 4H), 1.72 – 1.58 (m, 2H), 1.34 – 1.18 (m, 3H), 1.09 – 0.97 (m, 2H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 164.43, 160.03, 153.04, 137.28, 126.88, 126.73, 124.33, 122.55, 46.17, 38.20, 31.00, 26.48, 25.94; MS (ESI): *m/z* 275.07 $[M + H]^+$

***N*-(adamantan-1-yl)benzo[*d*]thiazole-2-carboxamide (4d).** Prepared from 1-adamantylamine. Column chromatography on silica gel (DCM:Heptane, 1:1) afforded **4d** as a white solid (92.6%); mp: 156-157 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 8.06 – 8.01 (m, 1H), 7.97 – 7.92 (m, 1H), 7.57 – 7.49 (m, 1H), 7.49 – 7.41 (m, 1H), 7.20 (s, 1H), 2.20 – 2.12 (m, 9H), 1.79 – 1.69 (m, 6H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 165.90, 158.82, 153.02, 137.36, 126.78, 126.59, 124.19, 122.52, 52.85, 41.61, 36.42, 29.58; MS (ESI): *m/z* 313.09 $[M + H]^+$

***N*-(naphthalen-1-yl)benzo[*d*]thiazole-2-carboxamide (4e).** Prepared from 1-naphthylamine. Column chromatography on silica gel (Heptane:DCM, 6.5:3.5) afforded **4e** as yellow crystals (55.3%); mp: 198-201 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 9.85 (s, 1H), 8.31 (d, *J* = 7.5 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 1H), 8.08 (d, *J* = 8.2 Hz, 1H), 8.01 (d, *J* = 7.9 Hz, 1H), 7.90 (d, *J* = 8.0 Hz, 1H), 7.74 (d, *J* = 8.3 Hz, 1H), 7.65 – 7.57 (m, 2H), 7.57 – 7.48 (m, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 164.31, 158.17, 152.91, 137.66, 134.26, 131.63, 129.06, 127.18, 127.13, 126.73, 126.48, 126.35, 126.04, 126.02, 124.69, 122.68, 120.43, 119.55; MS (ESI): *m/z* 305.13 $[M + H]^+$

***N*-(naphthalen-2-yl)benzo[*d*]thiazole-2-carboxamide (4f)**. Prepared from 2-naphthylamine. Column chromatography on silica gel (Heptane:DCM, 6.5:3.5) afforded **4f** as a yellow solid

(65.78%); mp: 192-193 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 8.46 (d, *J* = 2.2 Hz, 1H), 8.17 – 8.12 (m, 1H), 8.03 – 7.99 (m, 1H), 7.90 – 7.80 (m, 3H), 7.72 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.63 – 7.57 (m, 1H), 7.56 – 7.41 (m, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 164.22, 157.85, 152.84, 137.61, 134.56, 133.95, 131.08, 129.21, 127.95, 127.77, 127.18, 127.12, 126.85, 125.52, 124.53, 122.65, 119.67, 117.03; MS (ESI): *m/z* 305.23 $[M + H]^+$

Procedure for the synthesis of benzothiazole-2-carboxamides (4g-4i)

Ethyl benzothiazole-2-carboxylate **4a** (1 mmol) was taken in a round bottom flask (10 mL). To it the aliphatic amine (4 equiv.) was added in excess along with (50 mg) of NH_4Cl. The reaction mixture was heated at 150 ^{0}C for 2-3 h under magnetic stirring. After completion of reaction (TLC), the mixture was diluted using EtOAc, washed with1N HCl and brine, dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to furnish the final product that was then washed with cooled hexane.

***N*-benzylbenzo[*d*]thiazole-2-carboxamide (4g).** Prepared from benzylamine. Beige solid (91%); mp: 109-110 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 8.05 – 8.01 (m, 1H), 7.99 – 7.95 (m, 1H), 7.78 (s, 1H), 7.57 – 7.45 (m, 2H), 7.42 – 7.34 (m, 4H), 7.33 – 7.28 (m, 1H), 4.70 (d, *J* = 6.1 Hz, 2H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 163.87, 159.92, 152.97, 137.49, 137.25, 128.95, 128.14, 127.92, 126.93, 126.85, 124.38, 122.53, 44.01; MS (ESI): *m/z* 269.16 $[M + H]^+$

***N*-(2-chlorobenzyl) benzo[*d*]thiazole-2-carboxamide (4h)**. Prepared from 2-chlorobenzylamine. White solid (94.2%); mp: 134-135 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 8.06 (dd, *J* = 8.1, 0.5 Hz, 1H), 7.96 (dd, *J* = 7.9, 0.6 Hz, 1H), 7.88 (s, 1H), 7.60 – 7.44 (m, 3H), 7.43 – 7.34 (m, 1H), 7.30 – 7.17 (m, 2H), 4.78 (d, *J* = 8.6 Hz, 2H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 163.66, 160.02, 152.99, 135.00, 133.93, 130.45, 129.78, 129.38, 127.33,

126.95, 126.89, 124.47, 122.52, 41.87; MS (ESI): *m/z* 303.23 $[M + H]^+$, *m/z* 305.09 $[M + 3H]^+$

***N*-(1,2,3,4-tetrahydronaphthalen-1-yl)benzo[*d*]thiazole-2-carboxamide (4i)**. Prepared from 1,2,3,4-tetrahydronaphth-1-yl amine. White solid (93%); mp: 152-153 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 8.03 – 8.00 (m, 1H), 8.00 – 7.96 (m, 1H), 7.70 (d, *J* = 8.9 Hz, 1H), 7.57 – 7.45 (m, 2H), 7.40 – 7.35 (m, 1H), 7.25 – 7.13 (m, 3H), 5.41-5.36 (m, 1H), 2.97 – 2.72 (m, 2H), 2.32 – 2.14 (m, 1H), 2.07 – 1.86 (m, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 164.22, 159.34, 153.02, 137.81, 137.29, 135.99, 129.43, 129.08, 127.72, 126.91, 126.80, 126.55, 124.39, 122.53, 48.24, 30.25, 29.35, 20.12; MS (ESI): *m/z* 331.55 $[M + Na]^+$

Procedure for the synthesis of *N*-(benzo[d]thiazol-2-yl) carboxamides (5a-5d)

The appropriate acyl chloride (1.5 mmol) was slowly added under inert atmosphere to a cooled (0 °C) solution of 2-aminobenzothiazole (1 mmol) and TEA (1.5 mmol) in dry DCM (10 mL). After being stirred at room temperature for 18 h, the solution was washed with 1 N HCl, saturated solution of NaHCO3 and brine, then dried over anhydrous MgSO4 and evaporated to dryness. The crude product was then purified using column chromatography.

***N*-(benzo[d]thiazol-2-yl)adamantane-1-carboxamide (5a)** Prepared from 1-adamantanecarbonyl chloride. Column chromatography on silica gel (Hexane:Acetone, 9.5:0.5) afforded **5a** as a white solid (48%); mp: 167–169 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.81 (d, *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 8.1 Hz, 1H), 7.43 (t, *J* = 7.5 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 2.00 (s, 9H), 1.76 (s, 6H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 176.63, 159.38, 147.85, 131.96, 126.36, 123.99, 121.54, 120.54, 41.49, 38.73, 36.39, 28.05; MS (ESI): *m/z* 313.07 $[M + H]^+$

***N*-(benzo[*d*]thiazol-2-yl)-1-naphthamide (5b)** Prepared from 1-naphthoyl chloride. Column chromatography on silica gel (DCM:Hexane, 8:2) afforded **5b** as a white solid (40.5%); mp: 203–206 ºC; ^{1}H-NMR (δ): (400 MHz, DMSO) δ 12.99 (s, 1H), 8.30 – 8.24 (m, 2H), 8.14 (d, *J* = 8.3 Hz, 2H), 8.06 – 8.01 (m, 4H), 7.92 (dd, *J* = 7.1, 1.1 Hz, 2H), 7.81 – 7.77 (m, 2H), 7.66 – 7.58 (m, 6H), 7.49 – 7.43 (m, 2H), 7.37 – 7.32 (m, 2H); ^{13}C-NMR (δ): (101 MHz, DMSO) δ 168.21, 158.75, 148.87, 133.58, 132.03, 131.95, 131.50, 130.15, 128.93, 127.90, 127.61, 126.97, 126.63, 125.33, 125.30, 124.18, 122.20, 121.00; MS (ESI): *m/z* 305.23 $[M + H]^+$

***N*-(benzo[*d*]thiazol-2-yl)-4-(trifluoromethyl)benzamide (5c)** Prepared from 4-(trifluoromethyl)benzoyl chloride. Column chromatography on silica gel (DCM:Hexane, 9.5:0.5) afforded **5c** as a white solid (42.8%); mp: 235–237 ºC; ^{1}H-NMR (δ): (400 MHz, DMSO) δ 13.15 (s, 1H), 8.31 (d, *J* = 8.1 Hz, 2H), 8.02 (dd, *J* = 7.9, 0.6 Hz, 1H), 7.93 (d, *J* = 8.2 Hz, 2H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.47 (ddd, *J* = 8.2, 6.0, 1.3 Hz, 1H), 7.35 (ddd, *J* = 8.3, 6.0, 1.1 Hz, 1H); ^{13}C-NMR (δ): (101 MHz, DMSO) δ 165.37, 152.56, 135.70, 133.64, 131.90 (q, $^{2}J_{CF}$ = 32.2 Hz), 130.36, 129.43, 126.73, 126.53, 125.88 (q, $^{3}J_{CF}$ = 3.5 Hz), 124.05 (q, $^{1}J_{CF}$ = 272.2 Hz), 123.64, 122.12, 111.43; MS (ESI): *m/z* 322.99 $[M + H]^+$

***N*-(benzo[*d*]thiazol-2-yl)-3-(trifluoromethyl)benzamide (5d)** Prepared from 3-(trifluoromethyl)benzoyl chloride. Column chromatography on silica gel (DCM:Hexane, 9.5:0.5) afforded **5d** as a white solid (43.5%); mp: 213–214 ºC; ^{1}H-NMR (δ): (400 MHz, DMSO) δ 13.18 (s, 1H), 8.52 (s, 1H), 8.41 (d, *J* = 7.9 Hz, 1H), 8.02 (dd, *J* = 7.9, 0.6 Hz, 2H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 7.6 Hz, 1H), 7.47 (ddd, *J* = 8.2, 6.1, 1.3 Hz, 1H), 7.34 (ddd, *J* = 8.3, 6.2, 1.1 Hz, 1H); ^{13}C-NMR (δ): (101 MHz, DMSO) δ 165.48, 152.96, 135.70, 133.64, 130.36, 129.52 (q, $^{3}J_{CF}$ = 3.2 Hz), 129.32 (q, $^{2}J_{CF}$= 32.3 Hz), 128.37, 126.73, 125.47 (q, $^{3}J_{CF}$ = 3.8 Hz), 124.30 (q, $^{1}J_{CF}$ = 272.5 Hz), 124.25, 122.28, 120.49, 113.15; MS (ESI): *m/z* 323.03 $[M + H]^+$

Procedure for the synthesis of *N*-(3-pentylbenzo[*d*]thiazol-2(3*H*)-ylidene)carboxamide (6a-6d)

To a solution of the appropriate *N*-(benzo[*d*]thiazol-2-yl) carboxamide (1.28 mmol) in DMF (4.0 mL) was added sodium hydride (1.92 mmol) and 1-bromopentane (1.41 mmol). The reaction mixture was stirred at room temperature for 1 h and then heated at 80 ^{0}C overnight. Afterwards, the mixture was cooled to room temperature, quenched with iced water and extracted with ethyl acetate. The organic layer was dried over anhydrous $MgSO_4$, filtered, and then evaporated. The resulting residue was then washed with cooled hexane to give the pure final product.

***N*-(3-pentylbenzo[d]thiazol-2(3*H*)-ylidene)adamantane-1-carboxamide (6a).** Prepared from **5a** and 1-bromopentane. white solid (96%); mp: 167–169 ºC; ^{1}H-NMR (δ): (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 7.8 Hz, 1H), 7.45 – 7.37 (m, 1H), 7.32 – 7.21 (m, 2H), 4.38 (t, *J* = 7.3 Hz, 2H), 2.06 (s, 3H), 2.01 (s, 6H), 1.91 – 1.81 (m, 2H), 1.76 (s, 6H), 1.45 – 1.35 (m, 4H), 0.92 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (δ): (101 MHz, $CDCl_3$) δ 188.83, 166.94, 136.85, 127.17, 126.71, 123.38, 123.01, 111.23, 45.71, 43.08, 39.53, 37.06, 29.18, 28.56, 27.26, 22.48, 14.11; MS (ESI): *m/z* 383.15 $[M + H]^+$

***N*-(3-pentylbenzo[*d*]thiazol-2(3*H*)-ylidene)-1-naphthamide (6b)**. Prepared from **5b** and 1-bromopentane. White solid (95.2%); mp: 191–193 ºC; ^{1}H-NMR (δ): (400 MHz, DMSO) δ 9.15 (dd, *J* = 8.4, 0.9 Hz, 1H), 8.47 (dd, *J* = 7.2, 1.3 Hz, 1H), 8.11 (d, *J* = 8.1 Hz, 1H), 8.01 (dd, *J* = 8.3, 1.2 Hz, 1H), 7.95 (dd, *J* = 7.9, 1.0 Hz, 1H), 7.74 (d, *J* = 8.2 Hz, 1H), 7.65 – 7.52 (m, 4H), 7.42 – 7.34 (m, 1H), 4.54 (t, *J* = 7.3 Hz, 2H), 1.83 (p, J = 7.0 Hz, 2H), 1.40 – 1.31 (m, 4H), 0.82 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (δ): (101 MHz, DMSO) δ 176.24, 166.54, 136.81, 134.03, 133.93, 132.51, 131.35, 130.12, 128.91, 127.70, 127.44, 126.57, 126.35,

126.17, 125.37, 124.37, 123.56, 112.88, 45.85, 28.77, 27.32, 22.19, 14.20; MS (ESI): *m/z* 375.19 $[M + H]^+$

***N*-(3-pentylbenzo[*d*]thiazol-2(3*H*)-ylidene)-4-(trifluoromethyl)benzamide (6c)**. Prepared from **5c** and 1-bromopentane. White solid (91.2%); mp: 107–109 ºC; ^{1}H-NMR (δ): (400 MHz, DMSO) δ 8.34 (d, *J* = 8.2 Hz, 2H), 8.12 (dd, *J* = 7.7, 0.6 Hz, 1H), 7.97 (d, *J* = 8.3 Hz, 2H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.57 – 7.35 (m, 1H), 7.3 – 7.32 (m, 1H), 4.51 (t, *J* = 7.3 Hz, 2H), 1.81 (p, *J* = 7.1 Hz, 2H), 1.41 – 1.34 (m, 4H), 0.83 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (δ): (101 MHz, DMSO) δ 173.56, 167.69, 139.88, 136.60, 133.04 (q, $^2J_{CF}$ = 32.1 Hz), 129.67, 127.03, 126.86, 125.04 (q, $^3J_{CF}$ = 3.6 Hz), 124.05 (q, $^1J_{CF}$ = 272.5 Hz), 123.94, 122.98, 111.62, 45.93, 29.02, 27.29, 22.31, 13.90; MS (ESI): *m/z* 393.07 $[M + H]^+$

***N*-(3-pentylbenzo[*d*]thiazol-2(3*H*)-ylidene)-3-(trifluoromethyl)benzamide (6d)**. Prepared from **5d** and 1-bromopentane. White crystals (92.3%); mp: 101–103 ºC; ^{1}H-NMR (δ): (400 MHz, DMSO) δ 8.53 (s, 1H), 8.49 (d, *J* = 7.8 Hz, 1H), 7.97 – 7.91 (m, 2H), 7.77 (dd, *J* = 7.9, 6.1 Hz, 2H), 7.56 (ddd, *J* = 8.4, 7.4, 1.2 Hz, 1H), 7.41 – 7.36 (m, 1H), 4.56 (t, *J* = 7.3 Hz, 2H), 1.83 (p, *J* = 7.0 Hz, 2H), 1.44 – 1.36 (m, 4H), 0.85 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (δ): (101 MHz, DMSO) δ 172.43, 167.47, 137.67, 136.75, 133.15, 130.30, 129.80 (q, $^2J_{CF}$ = 32.3 Hz), 129.64 (q, $^3J_{CF}$ = 3.4 Hz), 127.94, 126.86, 125.49 (q, $^3J_{CF}$ = 3.9 Hz), 124.30 (q, $^1J_{CF}$ = 272.5 Hz), 123.67, 122.51, 113.15. 45.86, 28.72, 27.15, 22.06, 14.17; MS (ESI): *m/z* 393.18 $[M + H]^+$

Biological assays

Competitive radioligand binding assay. Membranes of HEK-293 cells transfected with the human recombinant CB1 receptor (B_{max}= 2.5 pmol/mg protein) and human recombinant CB2 receptor (B_{max}= 4.7 pmol/mg protein) were incubated with [^{3}H]-CP-55,940 (0.14 nM/k_d=

0.18 nM and 0.084 nM/k_d= 0.31 nM, for CB1 and CB2 receptors, respectively) as the high affinity ligand and displaced with 0.1, 1, 10, or 25 µM of the newly synthesized compounds. IC_{50} values were determined for compounds showing >50% displacement at 10 µM. Nonspecific binding was defined by 10 mM of WIN55,212-2 as the heterologous competitor (K_i values 9.2 nM and 2.1 nM respectively for CB1 and CB2 receptors). All compounds were tested following the procedure described by the manufacturer (Perkin-Elmer, Italy). Displacement curves were generated by incubating drugs with [^{3}H]-CP-55,940 for 90 minutes at 30°C. K_i values were calculated by applying the Cheng-Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds.[46]

Functional Activity at CB2 *in vitro*. The cAMP Hunter™ assay enzyme fragment complementation chemiluminescent detection kit was used to characterize the functional activity in CB2 receptor-expressing cell lines. Gi-coupled cAMP modulation was measured following the manufacturer's protocol (DiscoveRx, Fremont, CA). Briefly, CHO-K1 cells overexpressing the human CB2R were plated into a 96 well plate (30,000 cells/well), and incubated overnight at 37 °C, 5% CO_2. Cells were incubated 30 min at 37 °C with 3x dose-response solutions of samples prepared in presence of cell assay buffer containing a 3x of 25 µM NKH-477 solution (a water soluble analogue of Forskolin) to stimulate adenylate cyclase and enhance basal cAMP levels. Luminescence measurements were measured using a GloMax Multi Detection System (Promega, Italy). Data are reported as mean ± SEM of three independent experiments conducted in triplicate and were normalized considering the NKH-477 stimulus alone as 100% of the response. The percentage of response was calculated using the following formula: % RESPONSE = 100% x (1– (RLU of test sample – RLU of NKH-477 positive control) / (RLU of vehicle – RLU of NKH-477 positive control). The data were analyzed using PRISM software (GraphPad Software Inc, San Diego, CA).[46]

GPCR β-arrestin recruitment assay[37]. To measure GPCR mediated ß-arrestin translocation activity, the Tango assay system originally developed by Richard Axel was adopted.[37] GPCR tango constructs were codon-optimized for better expression in mammalian cell lines, synthesized by Blue Heron Biotech (Bothell, WA), and finally confirmed by sequencing. The HTLA cells (an HEK293 cell line stably expressing a tTA-dependent luciferase reporter and a ß-arrestin2-TEV fusion gene) were gifted from Richard Axel's lab, and are maintained in DMEM supplemented with 10% FBS and 2 µg/ml Puromycin and 100 µg/ml Hygromycin B. An N-terminal Flag epitope tag was designed into the GPCR tango constructs for confirmation of surface expression and comparison of expression levels. HTLA cells were transfected with GPCR tango constructs overnight and were plated in Poly-L-Lys (PLL) coated 384-well white clear-bottom cell culture plates at a density of 15,000 cells in 40 µl per well of DMEM with 1% dFBS. The cells are incubated for at least 6 hours (usually overnight) for them to recover before receiving drug stimulation. Drug stimulation solutions are prepared in filtered Tango assay buffer (20 mM HEPES, 1x HBSS, pH 7.40) at 5x and added to cells (10 µl per well) for overnight. To measure antagonist activity, drug solutions are made at 6x of the final concentration and are preincubated with cells for 30 min before addition of 10 µl of EC_{80} concentration of CP-55,940, a reference agonist. The EC_{80} concentration is determined in separate preliminary dose-response assays. On the day of measurement, medium and drug solutions are removed and 20 µl per well of BrightGlo reagent from Promega (diluted by 20-fold with Tango assay buffer) is added. The plate is incubated for 20 minutes at room temperature in the dark before being counted on a luminescence counter. All functional results were analyzed using GraphPad Prism v5.0.

Colitis Mouse Model. All experiments were approved and conducted in accordance with the guidelines of the German University in Cairo and The National Research Centre ethics committee's guidelines following the recommendations of The National Institutes of Health

Guide for the Care and Use of Laboratory Animals. All mice were maintained in pathogen-free conditions with standard laboratory chow and water ad libitum. Swiss male albino mice (20-35 g) of body weight were purchased from the animal house colony of The National Research Centre (Cairo, Egypt). Acute colitis was induced using 3%w/v DSS (40 kD molecular weight, Alfa Aesar) in distilled drinking water made fresh every 2 days provided ad libitum for 7 days. Body weight was determined daily. At day 7, disease activity index (DAI) was assessed according to body weight variation indicated as percentage loss of baseline body weight (0= none, 1= 0-10%, 2=11-15%, 3=16-20%, 4= >20%), stool consistency (0= normal, 2=loose stool, 4= diarrhea) and bleeding (0= normal, 1=hemoccult positive, 2= hemoccult positive and visual pellet bleeding, 4= gross bleeding, blood around anus). The presence of fecal occult blood was assessed using ABONTM Biopharm (Hangzhou) FOB kit. The statistical significance of the colitis clinical scores between treatments was analyzed with an unpaired t test using GraphPad Prism 7.04 software. A p value of <0.05 was considered statistically significant.

Histopathological analysis. For histopathological analysis, mice were euthanized by cervical dislocation and the colons were flushed with saline. Autopsy samples were taken from the distal part of the colon of mice in different groups, washed with saline and fixed in 10% formol saline for 24 h. Washing was done in tap water then serial dilutions of methyl, ethyl and absolute ethyl alcohols were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 ˚C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtrome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin (H&E) stain for examination through light electric microscope. The severity of the histopathological alteration in the colons of different mice groups were assessed by examining ulceration and desquamation of the lining mucosal epithelium, inflammatory cells

infiltration in mucosa with lamina propria, inflammatory cells infiltration in submucosa, oedema in submucosa, haemorrhage in submucosa and lymphoid hyperplasia in submucosa.

Enzyme-linked immunosorbent assay (ELISA)

The levels of IL-1β and TNF-α were quantified using commercial Mouse IL-1 Beta PicoKine™ ELISA Kit (Catalog Number:EK0394) and RayBio® Mouse TNF-alpha ELISA Kit (Catalog #: ELM-TNFa), respectively.

Molecular docking.

The homology protein cnr2_human Active 5XRA was downloaded from the PDB, opened in MOE, subjected to quick preparation, and hydrogens and partial charges were added**. The**

The binding site is identified from the CB1template and from the site finder of MOE software and both identified the same binding pocket and extended by 4.5 ^{o}A. Compound 6d is subjected to conformational search and docked to the site using default conditions and the resulted database was docked to using triangle matcher as the placement method, scorning London delta G and refinement by induced fit.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

Acknowledgment

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The authors gratefully acknowledge the National Institute of Mental Health - Psychoactive Drug Screening Program (NIMH-PDSP) team and Prof. Bryan L. Roth, University of North Carolina, for carrying out the functional assays. The contribute of AMM is a part of his INCIPIT PhD programme, co-funded by COFUND scheme Marie Skłodowska - Curie Actions. The contribute of AEG is a part of her Master thesis partially funded by the Faculty of Postgraduate Studies, GUC.

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References

1. Gaoni, Y.; Mechoulam, R., Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J Am Chem Soc.* **1964,** *86* (8), 1646-1647.

2. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I., Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **1990,** *346* (6284), 561-4.

3. Munro, S.; Thomas, K. L.; Abu-Shaar, M., Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993,** *365* (6441), 61-5.

4. Pertwee, R. G.; Howlett, A. C.; Abood, M. E.; Alexander, S. P.; Di Marzo, V.; Elphick, M. R.; Greasley, P. J.; Hansen, H. S.; Kunos, G.; Mackie, K.; Mechoulam, R.; Ross, R. A., International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacol Rev* **2010,** *62* (4), 588-631.

ACCEPTED MANUSCRIPT

5. Alhouayek, M.; Muccioli, G. G., The endocannabinoid system in inflammatory bowel diseases: from pathophysiology to therapeutic opportunity. *Trends Mol Med* **2012,** *18* (10), 615-25.

6. Klein, T. W.; Newton, C.; Larsen, K.; Lu, L.; Perkins, I.; Nong, L.; Friedman, H., The cannabinoid system and immune modulation. *J Leukoc Biol* **2003,** *74* (4), 486-96.

7. Caffarel, M. M.; Andradas, C.; Mira, E.; Perez-Gomez, E.; Cerutti, C.; Moreno-Bueno, G.; Flores, J. M.; Garcia-Real, I.; Palacios, J.; Manes, S.; Guzman, M.; Sanchez, C., Cannabinoids reduce ErbB2-driven breast cancer progression through Akt inhibition. *Mol Cancer* **2010,** *9*, 196.

8. Engeli, S.; Bohnke, J.; Feldpausch, M.; Gorzelniak, K.; Janke, J.; Batkai, S.; Pacher, P.; Harvey-White, J.; Luft, F. C.; Sharma, A. M.; Jordan, J., Activation of the peripheral endocannabinoid system in human obesity. *Diabetes* **2005,** *54* (10), 2838-43.

9. Ibrahim, M. M.; Deng, H.; Zvonok, A.; Cockayne, D. A.; Kwan, J.; Mata, H. P.; Vanderah, T. W.; Lai, J.; Porreca, F.; Makriyannis, A.; Malan, T. P., Jr., Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc Natl Acad Sci U S A* **2003,** *100* (18), 10529-33.

10. Wright, K.; Rooney, N.; Feeney, M.; Tate, J.; Robertson, D.; Welham, M.; Ward, S., Differential expression of cannabinoid receptors in the human colon: cannabinoids promote epithelial wound healing. *Gastroenterology* **2005,** *129* (2), 437-53.

11. Michler, T.; Storr, M.; Kramer, J.; Ochs, S.; Malo, A.; Reu, S.; Goke, B.; Schafer, C., Activation of cannabinoid receptor 2 reduces inflammation in acute experimental pancreatitis via intra-acinar activation of p38 and MK2-dependent mechanisms. *Am J Physiol Gastrointest Liver Physiol* **2013,** *304* (2), G181-92.

12. Fernandez-Ruiz, J.; Romero, J.; Velasco, G.; Tolon, R. M.; Ramos, J. A.; Guzman, M., Cannabinoid CB2 receptor: a new target for controlling neural cell survival? *Trends Pharmacol Sci* **2007,** *28* (1), 39-45.

13. Lombard, C.; Nagarkatti, M.; Nagarkatti, P., CB2 cannabinoid receptor agonist, JWH-015, triggers apoptosis in immune cells: potential role for CB2-selective ligands as immunosuppressive agents. *Clin Immunol* **2007,** *122* (3), 259-70.

14. Klein, T. W.; Newton, C.; Friedman, H., Cannabinoid receptors and immunity. *Immunol Today* **1998,** *19* (8), 373-81.

15. Rieder, S. A.; Chauhan, A.; Singh, U.; Nagarkatti, M.; Nagarkatti, P., Cannabinoid-induced apoptosis in immune cells as a pathway to immunosuppression. *Immunobiology* **2010,** *215* (8), 598-605.

16. Yang, L.; Li, F. F.; Han, Y. C.; Jia, B.; Ding, Y., Cannabinoid receptor CB2 is involved in tetrahydrocannabinol-induced anti-inflammation against lipopolysaccharide in MG-63 cells. *Mediators Inflamm* **2015,** *2015*, 362126.

17. Petrosino, S.; Verde, R.; Vaia, M.; Allara, M.; Iuvone, T.; Di Marzo, V., Anti-inflammatory Properties of Cannabidiol, a Nonpsychotropic Cannabinoid, in Experimental Allergic Contact Dermatitis. *J Pharmacol Exp Ther* **2018,** *365* (3), 652-663.

18. Velayudhan, L.; Van Diepen, E.; Marudkar, M.; Hands, O.; Suribhatla, S.; Prettyman, R.; Murray, J.; Baillon, S.; Bhattacharyya, S., Therapeutic potential of cannabinoids in neurodegenerative disorders: a selective review. *Curr Pharm Des* **2014,** *20* (13), 2218-30.

19. Gomez-Galvez, Y.; Palomo-Garo, C.; Fernandez-Ruiz, J.; Garcia, C., Potential of the cannabinoid CB(2) receptor as a pharmacological target against inflammation in Parkinson's disease. *Prog Neuropsychopharmacol Biol Psychiatry* **2016,** *64*, 200-8.

20. Croxford, J. L.; Yamamura, T., Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? *J Neuroimmunol* **2005,** *166* (1-2), 3-18.

ACCEPTED MANUSCRIPT

21. Toguri, J. T.; Lehmann, C.; Laprairie, R. B.; Szczesniak, A. M.; Zhou, J.; Denovan-Wright, E. M.; Kelly, M. E., Anti-inflammatory effects of cannabinoid CB(2) receptor activation in endotoxin-induced uveitis. *Br J Pharmacol* **2014,** *171* (6), 1448-61.

22. Shao, B. Z.; Wei, W.; Ke, P.; Xu, Z. Q.; Zhou, J. X.; Liu, C., Activating cannabinoid receptor 2 alleviates pathogenesis of experimental autoimmune encephalomyelitis via activation of autophagy and inhibiting NLRP3 inflammasome. *CNS Neurosci Ther* **2014,** *20* (12), 1021-8.

23. Kimball, E. S.; Schneider, C. R.; Wallace, N. H.; Hornby, P. J., Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. *Am J Physiol Gastrointest Liver Physiol* **2006,** *291* (2), G364-71.

24. Massa, F.; Storr, M.; Lutz, B., The endocannabinoid system in the physiology and pathophysiology of the gastrointestinal tract. *J Mol Med (Berl)* **2005,** *83* (12), 944-54.

25. Storr, M. A.; Keenan, C. M.; Zhang, H.; Patel, K. D.; Makriyannis, A.; Sharkey, K. A., Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. *Inflamm Bowel Dis* **2009,** *15* (11), 1678-85.

26. D'Argenio, G.; Valenti, M.; Scaglione, G.; Cosenza, V.; Sorrentini, I.; Di Marzo, V., Up-regulation of anandamide levels as an endogenous mechanism and a pharmacological strategy to limit colon inflammation. *FASEB J* **2006,** *20* (3), 568-70.

27. Ke, P.; Shao, B. Z.; Xu, Z. Q.; Wei, W.; Han, B. Z.; Chen, X. W.; Su, D. F.; Liu, C., Activation of Cannabinoid Receptor 2 Ameliorates DSS-Induced Colitis through Inhibiting NLRP3 Inflammasome in Macrophages. *PLoS One* **2016,** *11* (9), e0155076.

28. Morales, P.; Hernandez-Folgado, L.; Goya, P.; Jagerovic, N., Cannabinoid receptor 2 (CB2) agonists and antagonists: a patent update. *Expert Opin Ther Pat* **2016,** *26* (7), 843-56.

29. Dhopeshwarkar, A.; Mackie, K., CB2 Cannabinoid receptors as a therapeutic target-what does the future hold? *Mol Pharmacol* **2014,** *86* (4), 430-7.

30. Hua, T.; Vemuri, K.; Nikas, S. P.; Laprairie, R. B.; Wu, Y.; Qu, L.; Pu, M.; Korde, A.; Jiang, S.; Ho, J. H.; Han, G. W.; Ding, K.; Li, X.; Liu, H.; Hanson, M. A.; Zhao, S.; Bohn, L. M.; Makriyannis, A.; Stevens, R. C.; Liu, Z. J., Crystal structures of agonist-bound human cannabinoid receptor CB1. *Nature* **2017,** *547* (7664), 468-471.

31. Spinelli, F.; Capparelli, E.; Abate, C.; Colabufo, N. A.; Contino, M., Perspectives of Cannabinoid Type 2 Receptor (CB2R) Ligands in Neurodegenerative Disorders: Structure-Affinity Relationship (SAfiR) and Structure-Activity Relationship (SAR) Studies. *J Med Chem* **2017,** *60* (24), 9913-9931.

32. Tourteau, A.; Andrzejak, V.; Body-Malapel, M.; Lemaire, L.; Lemoine, A.; Mansouri, R.; Djouina, M.; Renault, N.; El Bakali, J.; Desreumaux, P.; Muccioli, G. G.; Lambert, D. M.; Chavatte, P.; Rigo, B.; Leleu-Chavain, N.; Millet, R., 3-Carboxamido-5-aryl-isoxazoles as new CB2 agonists for the treatment of colitis. *Bioorg Med Chem* **2013,** *21* (17), 5383-94.

33. Nanda, K. K.; Henze, D. A.; Della Penna, K.; Desai, R.; Leitl, M.; Lemaire, W.; White, R. B.; Yeh, S.; Brouillette, J. N.; Hartman, G. D.; Bilodeau, M. T.; Trotter, B. W., Benzimidazole CB2 agonists: design, synthesis and SAR. *Bioorg Med Chem Lett* **2014,** *24* (4), 1218-21.

34. Ohta, H.; Ishizaka, T.; Tatsuzuki, M.; Yoshinaga, M.; Iida, I.; Yamaguchi, T.; Tomishima, Y.; Futaki, N.; Toda, Y.; Saito, S., Imine derivatives as new potent and selective CB2 cannabinoid receptor agonists with an analgesic action. *Bioorg Med Chem* **2008,** *16* (3), 1111-24.

35. Lucchesi, V.; Hurst, D. P.; Shore, D. M.; Bertini, S.; Ehrmann, B. M.; Allara, M.; Lawrence, L.; Ligresti, A.; Minutolo, F.; Saccomanni, G.; Sharir, H.; Macchia, M.; Di Marzo, V.; Abood, M. E.; Reggio, P. H.; Manera, C., CB2-selective cannabinoid receptor ligands: synthesis, pharmacological evaluation, and molecular modeling investigation of 1,8-Naphthyridin-2(1H)-one-3-carboxamides. *J Med Chem* **2014,** *57* (21), 8777-91.

36. Ohta, H.; Ishizaka, T.; Tatsuzuki, M.; Yoshinaga, M.; Iida, I.; Tomishima, Y.; Toda, Y.; Saito, S., N-Alkylidenearylcarboxamides as new potent and selective CB(2) cannabinoid receptor agonists with good oral bioavailability. *Bioorg Med Chem Lett* **2007,** *17* (22), 6299-304.

37. Kroeze, W. K.; Sassano, M. F,; Huang, X. P.; Lansu, K.; McCorvy, J. D.; Giguère, P. M.; Sciaky, N.; Roth, B. L. PRESTO-Tango as an open-source resource for interrogation of the druggable human GPCRome. *Nat Struct Mol Biol.,* **2015**, 22(5), 362-9.

38. McGuinness, D.; Malikzay, A.; Visconti, R.; Lin, K.; Bayne, M.; Monsma, F.; Lunn, C. A., Characterizing cannabinoid CB2 receptor ligands using DiscoveRx PathHunter beta-arrestin assay. *J Biomol Screen* **2009,** *14* (1), 49-58.

39. Battula, S. R. K.; Rama Kishore Putta, V. P.; Subbareddy, G. V.; Chakravarthy, I. E.; Saravanan, V., A divergent and metal free synthesis of sulfoximine tethered imidazoles, imidazopyridines, imidazothiazoles, imidazobenzothiazines, thiazoles and selenazoles. *Org Biomol Chem* **2017,** *15* (17), 3742-3755.

40. Deng, S. W., M.; Turtle, E. D.; Ho, W.-B.; Arend, M. P.; Cheng, H.; Flippin, L. A. Preparation of Pyrrolopyridines and Thiazolopyridines, Particularly N-[(4-Hydroxy-1H-pyrrolo[2,3-c]- pyridin-5-yl)carbonyl]glycine and N-(7-Hydroxythiazolo[4,5-c]- pyridin-6-yl)carbonyl]glycine Derivatives, as Hypoxia Inducible Factor Hydroxylase Modulators. WO2007115315A2, 2007; CAplus AN 2007:1145598.

41. Li, Z.; Qiu, Q.; Xu, X.; Wang, X.; Jiao, L.; Su, X.; Pan, M.; Huang, W.; Qian, H., Design, synthesis and Structure-activity relationship studies of new thiazole-based free fatty acid receptor 1 agonists for the treatment of type 2 diabetes. *Eur J Med Chem* **2016,** *113*, 246-57.

ACCEPTED MANUSCRIPT

42. Pravin M., S. S., Hemant R., Rahul N., Synthesis and Biological Activity of (2-Substituted-4-methylthiazol-5-yl)(4-substituted piperazin-1-yl)methanone Derivatives. . *J Kor Chem Soc* **2014,** *58* (1), 62-67.

43. Shiga, Y.; Okada, I.; Fukuchi, T., Synthesis and Acaricidal Activity of (1,3,4-Thiadiazol-2-yl)pyrazole-5-carboxamides and (1,3,4-Thiadiazol-2-yl)thiazole-5-carboxamides. *J Pestic Sci* **2003,** *28* (3), 310-312.

44. Scott, W. J.; Hentemann, M. F.; Rowley, R. B.; Bull, C. O.; Jenkins, S.; Bullion, A. M.; Johnson, J.; Redman, A.; Robbins, A. H.; Esler, W.; Fracasso, R. P.; Garrison, T.; Hamilton, M.; Michels, M.; Wood, J. E.; Wilkie, D. P.; Xiao, H.; Levy, J.; Stasik, E.; Liu, N.; Schaefer, M.; Brands, M.; Lefranc, J., Discovery and SAR of Novel 2,3-Dihydroimidazo[1,2-c]quinazoline PI3K Inhibitors: Identification of Copanlisib (BAY 80-6946). *ChemMedChem* **2016,** *11* (14), 1517-30.

45. Bhat, M. B., S. L.; Hemanth Kumar, N. K.; Mahadeva Kumar, S., Synthesis and characterization of novel benzothiazole amide derivatives and screening as possible antimitotic and antimicrobial agents. *Res Chem Intermed* **2017,** *43* (1), 361-378.

46. Osman, N. A.; Ligresti, A.; Klein, C. D.; Allara, M.; Rabbito, A.; Di Marzo, V.; Abouzid, K. A.; Abadi, A. H., Discovery of novel Tetrahydrobenzo[b]thiophene and pyrrole based scaffolds as potent and selective CB2 receptor ligands: The structural elements controlling binding affinity, selectivity and functionality. *Eur J Med Chem* **2016,** *122*, 619-634.

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Highlights

- Novel thiazole and benzothiazole derivatives have been synthesized and evaluated for their CB1 and CB2 receptor binding affinity and functional activity.
- Potent and selective CB2 receptor agonists were characterized and structure–activity relationships were concluded.
- Compounds **6a**-**6d** displayed CB2 K_i values in the picomolar or low nanomolar range and CB2 selectivity up to 429 fold over CB1.
- Compound **6d** exhibited a remarkable anti-inflammatory effect against DSS-induced acute colitis in mice model.

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