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Original article

Tricyclic pyrazoles part 7. Discovery of potent and selective dihydrothienocyclopentapyrazole derived CB2 ligands

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ABSTRACT

A series of dihydrothienocyclopentapyrazole-based derivatives was synthesized and evaluated for the affinity at CB1 and CB2 receptors. The major term, the 6-methyl-1-(1,4-dichlorophenyl)-*N*-piperidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2-*c*]pyrazole-3-carboxamide (**6a**), displayed a high affinity and good selectivity for CB2 receptors (Ki values of 2.30 nM for CB2 receptor and 440 nM for CB1 receptors respectively). Subsequent analogue preparation resulted in the identification of compounds such as **6b**, **6d**, **6e**, **6k**, **6l**, **6m**, **6s** and **6t** that showed 1.3–485 fold selectivity for CB2 receptors with potencies in the 1.1–7.2 nM range. These compounds profiled as full agonists at CB2 receptor in an inhibition assay of P-ERK 1/2 up regulation in HL-60 cells.

1. Introduction

The cannabinoid subtype 2 receptor (CB2), member of the superfamily of G protein-coupled receptors, is expressed at high density on immune cells [1]. CB2 receptors are also expressed in peripheral tissues and organs, i.e. spleen, tonsils, and pancreas [2]. Moreover, the presence of this cannabinoid receptor subtype has also been evidenced in rat microglia cells, human perivascular microglial cells, and neuritic plaques-associated microglial cells in brain humans of patients affected by Alzheimer's disease [3].

The CB2 receptor together with CB1 receptor [4–6], endogenous ligands (anandamide-AEA [7] and 2-arachidonoylglycerol-2AG [8,9]), anabolic and catabolic enzymes [10–12] and endocannabinoid-binding protein and transporters [13,14] are components of the physiological endocannabinoid system.

This signaling system plays a crucial role in numerous physiological and pathological functions and it is involved in maintaining the physiological steady state and homeostasis [15].

In recent years drug discovery organizations have taken an increased interest in CB2 ligands to develop potential drugs for the treatment of multiple diseases such as cancer [16,17], multiple sclerosis [18–20], Alzheimer's disease [3], neuropathic and inflammatory pain [3,18–22], diabetes [23] and autoimmune uveoretinitis [24].

As already described in preceding publications [25–27], our search for CB2 receptor ligands started by screening condensed tricyclic pyrazole compounds as conformational restricted analogues of SR144528 (**1**) [28], the CB2 selective congener of the CB1 antagonist/inverse agonist compound SR141716A (Rimonabant, **2**) [29] (See Fig. 1).

These efforts provided three main lead derivatives: 6-methyl-1-(2′,4′-dichlorophenyl)-*N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (**3**) [25], the corresponding 6-chlorine derivative (**4**) [25], and 6-chloro-7-methyl-1-(2′,4′-dichlorophenyl)-*N*-fenchyl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide (**5**) [27]. Particularly, compounds **3** and **4** exhibited excellent affinity and selectivity to CB2 receptors (affinity expressed as Ki in the order of 0.037–0.34 nM; selectivity towards CB1 receptors up to 9810) (See Fig. 2).

According to widest structure activity relationship (SAR) studies carried out in our laboratories based on three main series

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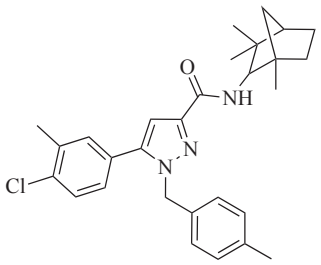
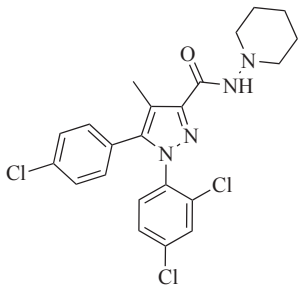


1 SR144528
Ki CB2 = 0.6 nM
Ki CB1 = 400 nM
Ki CB1 / Ki CB2 (selectivity ratio) = 666:1



2 SR141716A (Rimonabant)
Ki CB2 = 514 nM
Ki CB1 = 1.8 nM
Ki CB1 / Ki CB2 (selectivity ratio) = 0.0035:1

Fig. 1. Structures and cannabinoid receptor affinities of reference compounds **1** and **2**.

characterized respectively by five, six, and seven carbon atoms in the central carbocyclic ring of the condensed tricyclic pyrazole compounds, the planar dihydroindenopyrazole ring system appeared as important feature to assure CB2 affinity. In contrast, the enlargement of the size of the central ring in the condensed tricyclic scaffold determined a shifting to CB1 affinity and selectivity [30–32]. This behavior was further supported by the results of other groups, although with marked differences in absolute values of Ki CB1 for some of the lead compounds [33,34].

To further investigate the relevance of these condensed tricyclic compounds in the development of novel CB2 ligands with improved pharmacological profiles, we have recently oriented our focus on the replacing the benzene ring of the tricyclic indenopyrazole scaffold in compounds **3**–**5** with a ring-fused thiophene moiety to obtain a novel dihydrothienocyclopentapyrazole scaffold as illustrated in Fig. 3.

It was postulated that bioisosteric approach used in this case could be a rational and efficient strategy to develop novel thienocyclopentapyrazole CB2 selective ligands and probably a more detailed insight into CB2 subtype-specific receptor pharmacology might by expected.

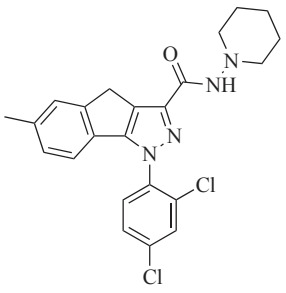
In this paper we report the synthesis of thiophene based analogues of **3**–**5** with the general structure **6** (Fig. 4), the affinity values to cannabinoid receptors, and preliminary data concerning their intrinsic activity.

2. Chemistry

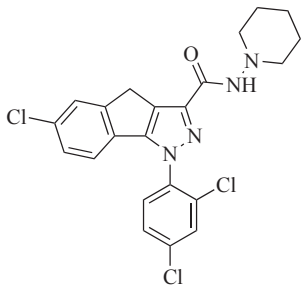
According to the retrosynthetic analysis in Scheme 1, the target dihydrothienocyclopentapyrazoles **6a**–**t** could be prepared using a multistep route starting from substituted thienoindanones **7** [35] and **8**. Thus, methylthienoindanones were transformed to ethyl thienoindanonoxoacetate intermediates **9**, **10** using a Claisen reaction and subsequent condensation with an aromatic/methylarylhydrazine afforded the tricyclic dihydrocyclopentapyrazoles **11**–**14**. Final elaboration to the designed compounds **6** was accomplished by functional interconversion of the esters **11**–**14** via acids **15**–**18** to the amides/hydrazides.

Preparation of the 3-methyl-thienoindan-4-one **8** started with 3-methyl-2-thiophen-carbaldehyde **19** and malonic acid that gave the propenoic acid **20** [36], that was converted to the corresponding propanoic acid **21** [36] and cyclized to the desired ketone under poliphosphoric acid conditions in 40% yield (Scheme 2).

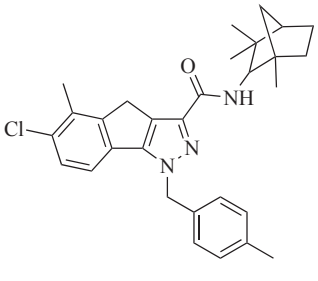
Claisen reaction of ketones **7**,**8** with diethyl oxalate and sodium ethoxide provided the diketoesters **9**,**10** in good yields. Subsequent cyclization with 2,4-dichlorophenylhydrazine hydrochloride or *p*-



3
Ki CB2 = 0.037 nM
Ki CB1 = 363 nM
Ki CB1 / Ki CB2 = 9810:1

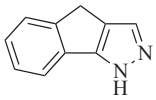


4
Ki CB2 = 0.34 nM
Ki CB1 = 2050 nM
Ki CB1 / Ki CB2 = 6029:1

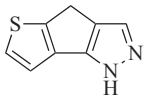


5
Ki CB2 = 23 nM
Ki CB1 = 4500 nM
Ki CB1 / Ki CB2 = 195.6:1

Fig. 2. Structures and cannabinoid receptor affinities of reference compounds **3**, **4**, and **5**.

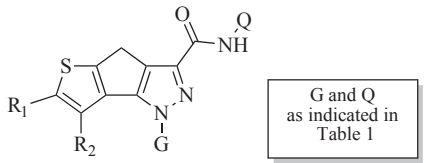


Dihydroindenopyrazole scaffold



Dihydrothienocyclopentapyrazole scaffold

Fig. 3. Comparison of dihydroindenopyrazole scaffold with the bioisosteric dihydrothienocyclopentapyrazole one.



6

Fig. 4. General structures of novel thienocyclopentapyrazole compounds.

methylbenzylhydrazine hydrochloride provided the dihydrothienocyclopentapyrazole esters **11**–**14** in 53–64% overall yield. Hydrolisis of tricyclic esters **11**–**14** provided the acids **15**–**18**.

The acids **15**–**18** were directly converted to the corresponding acid chlorides which were treated with appropriate amines/hydrazines to furnish target compounds **6a**–**t** (Scheme 3).

3. Biology

3.1. Radioreceptor binding assays

The affinity of the novel compounds for CB1 and CB2 receptors were determined according to the previously reported procedures [26]. Assays were performed by using mouse brain (minus cerebellum) and mouse spleen homogenates for CB1 and CB2 receptors respectively. [^{3}H]-(1*R*,3*R*,4*R*)-3-[2-hydroxy-4-(1,1-dimethylheptyl) phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol ([^{3}H]CP-55,940) was employed as radio-labeled ligand. The experimental data (IC_{50} values) were converted into Ki values [37].

3.2. CB2 intrinsic activity by in vitro assays

Intrinsic activity of the novel derivatives towards CB2 receptors was evaluated through *in vitro* tests based on the determination of phosphorylated ERK 1/2 (P-ERK 1/2) expression in human promyelocytic leukemia HL-60 (HL-60) cell line, following the treatment of the cells with the cannabinoid compounds. As previously reported, this cell line expresses CB2 but not CB1 receptors, and it represents a reliable platform for the evaluation of intrinsic activity of CB2 ligands [38]. In fact, the exposure of HL-60 cells to the cannabinoid agonists CP-55,940, as well as to other synthetic selective CB2 agonists, or to the endogenous cannabinoid ligand 2-AG, elicited rapid phosphorylation and activation of the ERK 1/2 [26,39]. This up-regulation of P-ERK 1/2 was counteracted by a pretreatment of HL-60 cells with the reference CB2 selective antagonist SR144528, with the subsequent evidence of CB2 mediated effect of the cannabinoid agonist on the adopted cells.

4. Results and discussion

4.1. Cannabinoid receptor affinities

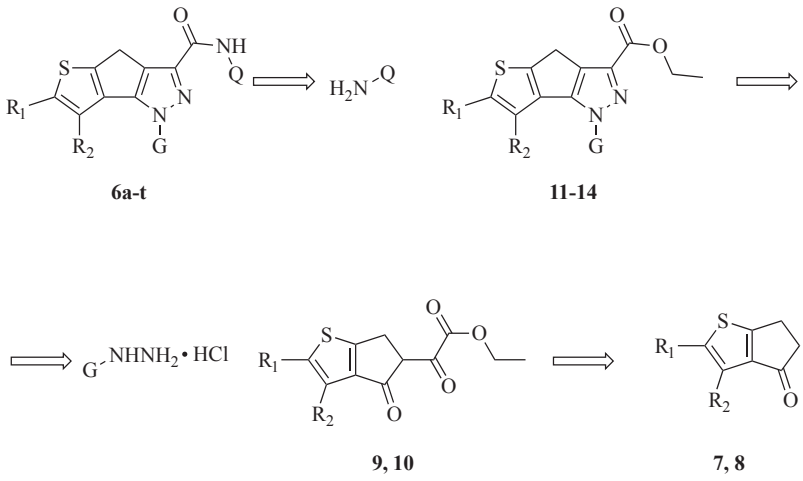
The goal of this work was to prepare a series of 1,4-dihydrocyclopentapyrazole carboxamide analogues of CB2 ligands **3**, **4** and **5** and to determine their affinities at CB1 and CB2 receptors. According to the obtained binding data of the novel compounds **6a**–**t**, the replacement of the indenopyrazole tricyclic system of **3**–**5** induced a significant impact on cannabinoid receptor affinity (Table 1).

In general, all the investigated thienocyclopentapyrazole derivatives evidenced poor binding affinity for CB1 receptors, with the exception of compound **6e** which showed Ki CB1 lower than 10 nM.

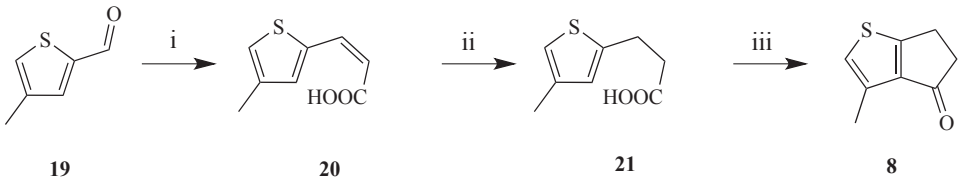
Comparison of **6a** respect to **3** suggested that CH=CH/S bioisosteric replacement elicited a marked effect on potency at CB2 receptor subtype leading to 62-fold decrease in CB2 binding affinity.

Compound **6a**, however, was chosen as a convenient benchmark for all of the others, in terms of presenting structure binding affinity relationship.

Initially, the effect of changing the size of the carboxamide moiety was examined. The affinity for the CB2 receptor of the compound **6b** having a seven-membered ring was only slightly increased (compare **6a** vs **6b**) but with good improvement in CB2 selectivity (Ki CB1/Ki CB2 = 401). On the contrary the compound having a five membered ring (**6c**) had markedly decreased CB2 affinity (16.6 fold reduction: compare **6c** vs **6a**). Replacement of the piperidine nitrogen with a methylene group also led to a three-fold decrease in binding at CB2 receptor subtype (compare **6a** vs **6d**).



Scheme 1. Retrosynthetic pathways to the target dihydrothienocyclopentapyrazoles **6a**–**t**.



Scheme 2. Synthesis of compound **8**. *Reagents and conditions*: (i) $CH(COOH)_2$, pyridine, piperidine, 115 °C, overnight, 65%; (ii) H_2, $Pd(OH)_2$, EtOH, 15 psi, 25 °C, 18 h, 89%; (iii) PPA, 75 °C, 1 h, 32%.

Interestingly, introduction of a bulky alkyl group at the carboxamide nitrogen afforded compound **6e** which showed a 2.2-fold decrease in Ki CB2 value and as well as increased CB1 affinity (Ki = 6.5 nM), thereby producing reduced selectivity. In contrast to the bioisosteric *N*-piperidine derivative **6a**, compound **6e** represents an unselective cannabinoid ligand.

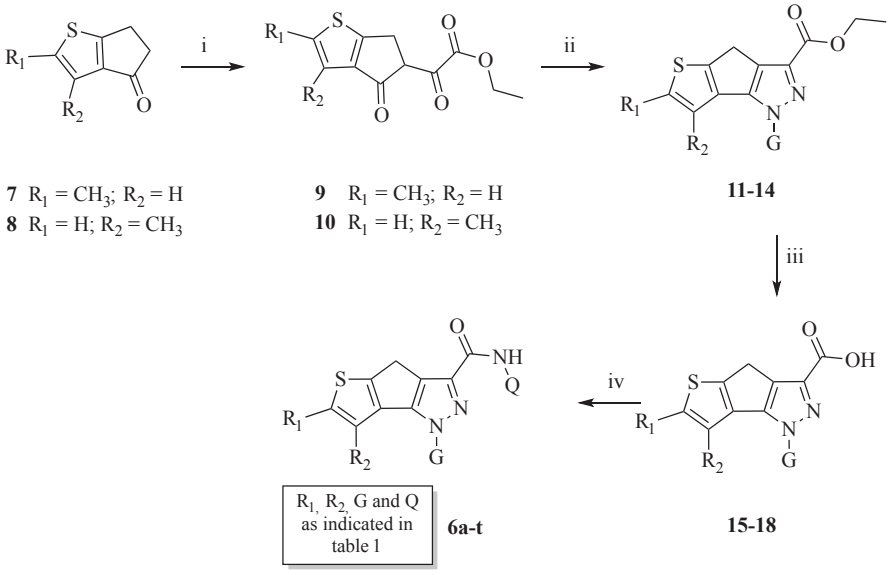
In order to investigate the effect of methyl shifting from C_6 to C_7 of the tricyclic platform on CB receptor affinities, compounds **6f**–**l** with simple or branched rings on C_3-carboxamide function were examined.

Thus compound **6e** represents an unselective CB ligand in contrast to the bioisosteric *N*-piperidin derivative **6a**. Very high Ki values indicating low CB1 affinity for CB1 receptors were determined (Ki > 2000) and much lower affinity for CB2 receptor were observed compared to **6a** with the exception of derivatives **6k**, **l**. Compounds **6k**, **l** contain in the carboxamide function an alkyl bulky residue such as menthyl or fenchyl group in place of a simple ring as in **6f**–**j**. This led to a moderate decrease in binding affinities respect to **6a**, with Ki values of 6.7 and 5.3 nM at CB2, respectively. As a result, the menthyl analogue **6k** had the highest CB1–CB2 selectivity (CB1:CB2 ratio = 485).

We also explored the effect related to the replacement of the N_1-dichlorophenyl group with *p*-methylbenzyl moiety on cannabinoid receptor affinity (compounds **6m**–**t**). Compound **6m**, which contain a 6-methyl group in the tricyclic system and a menthyl motif in the C3 carbamoyl function showed CB2 affinity similar to that of the homologue derivative **6e**. In the mean time, the replacement of substituent at 1 position of pyrazole ring showed a 27-fold reduction of CB1 affinity (**6m** Ki CB1 = 180 nM vs **6e** Ki CB1 = 6.5 nM) with a consequent improvement of CB2 selectivity. According to the 1-(2,4-dichlorophenyl) analogue derivatives (compounds **6f**–**l**), novel derivatives **6n**–**t** bearing a 7-methyl group evidenced low affinity to CB1 receptor as well as reduced CB2 receptor affinity. However, as for the reference homologue subclass, menthyl and fenchyl groups on C3-carboxamide function determined significant affinity for CB2 receptor subtype.

When the simple carboxamide substitution pattern of **6n**–**r** was replaced with a menthyl moiety, the resulting compound, **6s**, had 2.75 fold increase in CB1 receptor affinity with Ki CB1 from 440 nM for **6a** to 160 nM for **6s**.

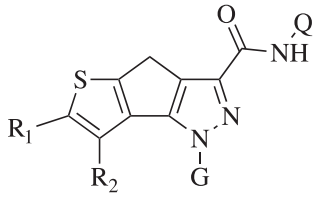
On the other hand, this structural modification resulted in a similar potency toward CB2 affinity with a Ki CB2 value of 2.3 nM for **6a** and 2.7 nM for **6s**. This result may indicate a sensitivity of both receptors to steric bulk of the carboxamide moiety for congeners possessing a methylbenzyl motif at N_1 position of the thienocyclopentapyrazole template.



11, 15 $R_1 = CH_3$; $R_2 = H$; G = 2,4-$Cl_2C_6H_3$
12, 16 $R_1 = H$; $R_2 = CH_3$; G = 2,4-$Cl_2C_6H_3$
13, 17 $R_1 = CH_3$; $R_2 = H$; G = $CH_2C_6H_4$-*p*-CH_3
14, 18 $R_1 = H$; $R_2 = CH_3$; G = $CH_2C_6H_4$-*p*-CH_3

Scheme 3. Synthesis of compounds **6a**–**t**. *Reagents and conditions*: (i) Na, EtOH, $(COOEt)_2$, r.t., 5 h; (ii) 2,4-dichlorophenylhydrazine hydrochloride or *p*-methylbenzylhydrazine hydrochloride, AcOH, 110 °C, 8 h; (iii); EtOH/H_2O 1:1, NaOH, 80 °C 5 h; (iv) $SOCl_2$, CH_2Cl_2, 40 °C, 5 h, then NH_2-Q, CH_2Cl_2, 40 °C, 2 h.

Table 1
Structures and binding data of 1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2-*c*]pyrazole derivatives **6**.



6a-t

Compd	R_1	R_2	G	Q	Receptor affinity[a,b] (nM)		CB2 ratio Ki CB1/Ki CB2
					Ki CB1[a,b]	Ki CB2[a,c]	
6a	CH_3	H			440 ± 25	2.3 ± 0.3	191.3:1
6b	CH_3	H			442 ± 38	1.1 ± 0.1	401.8:1
6c	CH_3	H			1386 ± 330	38.3 ± 3.2	36.2:1
6d	CH_3	H			447 ± 63	7.2 ± 1.3	62.1:1
6e	CH_3	H			6.5 ± 13	5.1 ± 0.6	1.3:1
6f	H	CH_3			3918 ± 107	578 ± 83	6.8:1
6g	H	CH_3			3798 ± 709	321 ± 75	11.8:1
6h	H	CH_3			4731 ± 161	817 ± 136	5.8:1
6i	H	CH_3			3076 ± 594	501 ± 76	6.1:1
6j	H	CH_3			4705 ± 294	747 ± 200	6.3:1
6k	H	CH_3			3255 ± 274	6.7 ± 0.9	485.8:1
6l	H	CH_3			2245 ± 71	5.3 ± 1.0	423.6:1
6m	CH_3	H			180 ± 32	4.8 ± 0.8	37.5:1
6n	H	CH_3			377 ± 37	125 ± 31	3.0:1
6o	H	CH_3			1276 ± 55	69 ± 14	18.5:1
6p	H	CH_3			4500 ± 500	515 ± 96	8.7:1
6q	H	CH_3			1977 ± 474	191 ± 44	10.4:1
6r	H	CH_3			4500 ± 250	1758 ± 474	2.6:1

(*continued on next page*)

Table 1 (*continued*)

Compd	R_1	R_2	G	Q	Receptor affinity[a,b] (nM)		CB2 ratio Ki CB1/Ki CB2
					Ki CB1[a,b]	Ki CB2[a,c]	
6s	H	CH_3			160 ± 37	2.7 ± 0.2	59.3:1
6t	H	CH_3			420 ± 64	2.4 ± 0.1	175.0:1

[a] Ki values were obtained from five independent experiments run in triplicate and are expressed as the mean of the five values ± standard error.
[b] Affinity of compounds was assayed using mouse brain (minus cerebellum) homogenate and [^{3}H]-CP-55,940.
[c] Affinity of compounds was evaluated using mouse spleen homogenate and [^{3}H]-CP-55,940.

However the replacement of the C_3 carboxamide menthyl substituent with a fenchyl group to give **6t** elicited a similar binding profile for CB receptor if compared to the major term **6a**.

4.2. Intrinsic activity by in vitro assays

To evaluate intrinsic activity of the novel CB2 ligands, all the novel compounds showing Ki values for CB2 receptors lower than 10 nM were assayed by *in vitro* test based on HL-60 cells. According to the profile determined with the reference cannabinoid agonist WIN55,212-2, significant increasing of P-ERK 1/2 expression in HL-60 cells has been evidenced for all the tested compounds (Table 1; P-ERK 1/2 values in absence of AM630). CB2 agonist of the novel derivatives has been confirmed through the inhibition of the induced P-ERK 1/2 up-regulation by the pre-treatment of HL-60 cells with the reference selective CB2 antagonist AM630 (Table 1; P-ERK 1/2 values with AM630 at the concentration of 75 nM).

5. Conclusions

In this report we have identified a novel series of dihydrothienocyclopentapyrazole-based cannabinoid receptor ligands that are selective for CB2 receptor subtype.

The more potent and selective analogues in this series have binding affinity of 1.1–7.2 nM at CB2 receptor and selectivity over CB1 of up to 485-fold. According to *in vitro* assays based on the evaluation of P-ERK 1/2 expression in HL-60 cells, the novel compounds act as CB2 agonists.

6. Experimental protocols

6.1. Chemistry

6.1.1. General procedures

All reagents and solvents were purchased from Aldrich and used as received. Low boiling petroleum ether was the fraction collected between 40 and 60 °C. Melting points were determined on a Büchi 510 capillary apparatus and are uncorrected. The NMR spectra were obtained with a Varian VXR-200 spectrometer at 200 for ^{1}H and 50.0 MHz for ^{13}C. Chemical shifts are reported in ppm downfield from internal Me_4Si in $CDCl_3$. IR spectra were recorded on a JASCO FT/IR-460 plus equipment. The following abbreviations were used to describe peak patterns where appropriate: singlet (s), doublet (d), triplet (t), multiplet (m) and broad (br). Elemental analyses were performed on a Perkin–Elmer 240 B analyser. TLC was performed on Merck silica gel 60 TLC plates F254 and visualized using UV or phosphomolibdic acid. Flash chromatography was carried out on silica gel (230–400 mesh).

6.1.1.1. General procedure I: synthesis of carboxamides and hydrazides. A mixture of the appropriate 1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2*c*]pyrazole-3-carboxylic acid **15**–**18** (0.6 mmol) and thionyl chloride (3.0 equiv) in CH_2Cl_2 (10 mL) was refluxed for 5 h. The solvent and the excess of $SOCl_2$ were removed under reduced pressure. The resulting dark solid in CH_2Cl_2 (10 mL) was dropwise added to a solution of requisite amine or hydrazine (1.5 equiv) in CH_2Cl_2 (15 mL) at 0 °C. The mixture was warmed to room temperature and refluxed for 2 h. The mixture was then poured into a separatory funnel and brine was added. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic layer were washed with water, dried over Na_2SO_4 and concentrated under reduced pressure.

The analytically pure product was isolated by an appropriate method of purification as below indicated.

*6.1.1.1.1. 6-Methyl-1-(2,4-dichlorophenyl)-N-(piperidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6a**).* General procedure **I** was used to convert **15** and *N*-aminopiperidine into the title product. Purification by flash chromatography afforded **6a** (0.217 g, 82%) as a yellow solid. Rf = 0.32 ($CHCl_3$/MeOH 99:1); mp 177–178 °C; IR (ν/cm^{-1}): 3089, 1662; ^{1}H NMR ($CDCl_3$, ppm/δ): 1.38–1.60 (m, 2H), 1.64–1.90 (m, 4H), 2.49 (s, 3H), 2.80–2.98 (m, 4H), 3.85 (s, 2H), 6.46 (s, 1H), 7.35–7.40 (m, 1H), 7.42 (dd, *J* = 8.4 Hz, 2.2 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.9, 23.2, 25.3, 28.9, 57.0, 115.4, 128.0, 129.2, 130.1, 130.3, 130.7, 134.6, 135.3, 135.8, 141.1, 143.6, 148.6, 149.2, 159.0; Anal. Calcd for $C_{21}H_{20}Cl_2N_4OS$: C, 56.38; H, 4.51; N, 12.52. Found: C, 56.41; H, 4.55; N, 12.45.

*6.1.1.1.2. 6-Methyl-1-(2,4-dichlorophenyl)-N-(homopiperidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6b**).* General procedure **I** was used to convert **15** and *N*-aminohomopiperidine into the title product. Purification by flash chromatography afforded **6b** (0.218 g, 79%) as a yellow solid. Rf = 0.36 ($CHCl_3$/MeOH 99:1); mp 148–151 °C; IR (ν/cm^{-1}): 3401, 1672; ^{1}H NMR ($CDCl_3$, ppm/δ): 1.55–1.90 (m, 8H), 2.49 (s, 3H), 3.18 (t, *J* = 5 Hz, 4H), 3.84 (s, 2H), 6.46 (s, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.63 (s, 1H), 8.00–8.10 (bs, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.9, 23.2, 25.3, 28.9, 58.2, 115.4, 128.0, 129.2, 130.1, 130.4, 130.8, 134.6, 135.4, 135.9, 141.2, 143.7, 148.6, 149.2, 159.4; Anal. Calcd for $C_{22}H_{22}Cl_2N_4OS$: C, 57.27; H, 4.81; N, 12.14. Found: C, 57.32; H, 4.80; N, 12.17.

*6.1.1.1.3. 6-Methyl-1-(2,4-dichlorophenyl)-N-(pyrrolidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6c**).* General procedure **I** was used to convert **15** and *N*-aminopyrrolidine hydrochloride into the title product adding 1.5 equiv of triethylamine to the mixture. Purification by flash chromatography afforded **6c** (0.192 g, 74%) as a yellow solid. Rf = 0.25 ($CHCl_3$/MeOH 99:1); mp 100–103 °C; IR (ν/cm^{-1}): 3434, 1670; ^{1}H NMR ($CDCl_3$, ppm/δ): 1.80–2.00 (m, 4H), 2.49 (s, 3H), 2.90–3.15 (m, 4H), 3.86 (s, 2H), 6.46 (s, 1H), 7.41 (dd, *J* = 9.6 Hz, 1.2 Hz, 1H), 7.53 (d, *J* = 9.6 Hz, 1H), 7.63 (d, *J* = 1.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 16.0, 22.3, 29.0, 55.4, 115.4, 128.1, 129.2, 130.1, 130.4, 130.8, 134.6, 135.4, 135.9, 141.2, 143.7, 148.7, 149.2, 159.2; Anal. Calcd for $C_{20}H_{18}Cl_2N_4OS$: C, 55.43; H, 4.19; N, 12.93; Found: C, 55.40; H, 4.24; N, 13.00.

*6.1.1.1.4. 6-Methyl-1-(2,4-dichlorophenyl)-N-cyclohexyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6d**).* General procedure **I** was used to convert **15** and cyclohexylamine into the title product. Purification by flash chromatography afforded **6d** (0.244 g, 91%) as a yellow solid. Rf = 0.64 ($CHCl_3$/MeOH 99:1); mp 103–105 °C; IR (ν/cm^{-1}): 3405, 3301, 3100, 1662; ^1H NMR ($CDCl_3$, ppm/δ): 1.20–1.43 (m, 4H), 1.66–1.73 (m, 4H), 1.95–2.10 (m, 2H), 2.49 (s, 3H), 3.84 (s, 2H), 3.84–4.00 (m, 1H), 6.46 (s, 1H), 6.80 (d, *J* = 7.5 Hz, 1H, NH exch. D_2O), 7.42 (dd, *J* = 8.7 Hz, 2.1 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.63 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 16.0, 24.9, 25.5, 28.9, 33.1, 48.0, 115.4, 128.8, 129.3, 129.7, 130.4, 130.8, 134.8, 135.4, 136.0, 142.0, 143.6, 148.5, 149.4, 160.9; Anal. Calcd for $C_{22}H_{21}Cl_2N_3OS$: C, 59.20; H, 4.74; N, 9.41. Found: C, 59.27; H, 4.73; N, 9.48.

*6.1.1.1.5. 6-Methyl-1-(2,4-dichlorophenyl)-N-menthyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6e**).* General procedure **I** was used to convert **15** and L-menthylamine into the title product. Purification by flash chromatography afforded **6e** (0.175 g, 58%) as a yellow solid. Rf = 0.75 ($CHCl_3$/MeOH 99:1); mp 170–172 °C; IR (ν/cm^{-1}): 3399, 1658; ^1H NMR ($CDCl_3$, ppm/δ): 0.75–1.00 (m, 9H), 1.10–1.30 (m, 2H), 1.43–1.80 (m, 5H), 1.90–2.10 (m, 2H), 2.49 (s, 3H), 3.85 (s, 2H), 3.90–4.00 (m, 1H), 6.46 (s, 1H), 6.65 (d, *J* = 7.3 Hz, 1H, NH exch. D_2O), 7.42 (dd, *J* = 8.4 Hz, 2.1 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 2.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 16.0, 16.2, 21.1, 22.1, 23.8, 26.7, 28.9, 31.9, 34.5, 43.1, 47.8, 49.7, 115.4, 128.0, 129.2, 129.7, 130.4, 130.7, 134.7, 135.3, 135.9, 142.0, 143.6, 148.5, 149.4, 161.0; $[\alpha]_D^{20}$: −2.18° ($CHCl_3$, *c* = 0.14); Anal. Calcd for $C_{26}H_{29}Cl_2N_4OS$: C, 62.15; H, 5.82; N, 8.36. Found: C, 62.19; H, 5.77; N, 8.32.

*6.1.1.1.6. 7-Methyl-1-(2,4-dichlorophenyl)-N-(piperidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6f**).* General procedure **I** was used to convert **16** and *N*-aminopiperidine into the title product. Purification by flash chromatography afforded **6f** (0.220 g, 82%) as a yellow solid. Rf = 0.27 ($CHCl_3$/MeOH 99:1); mp 165–167 °C; IR (ν/cm^{-1}): 3390, 3315, 3247, 3091, 1675; ^1H NMR ($CDCl_3$, ppm/δ): 1.38–1.68 (m, 2H), 1.60–1.90 (m, 7H), 2.88–3.00 (m, 4H), 3.86 (s, 2H), 6.81 (s, 1H), 7.32–7.40 (m, 1H), 7.42 (dd, *J* = 8.4 Hz, 2.2 Hz, 1H), 7.52 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 2.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 14.4, 23.3, 25.3, 28.6, 57.0, 124.3, 127.9, 128.1, 130.0, 130.4, 130.6, 133.7, 135.7, 136.5, 136.9, 140.9, 149.9, 151.7, 159.0. Anal. Calcd for $C_{21}H_{20}Cl_2N_4OS$: C, 56.38; H, 4.51; N, 12.52. Found: C, 56.40; H, 4.57; N, 12.57.

*6.1.1.1.7. 7-Methyl-1-(2,4-dichlorophenyl)-N-(homopiperidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6g**).* General procedure **I** was used to convert **16** and *N*-aminohomopiperidine into the title product. Purification by flash chromatography afforded **6g** (0.213 g, 77%) as a yellow solid. Rf = 0.34 ($CHCl_3$/MeOH 99:1); mp 150–152 °C; IR (ν/cm^{-1}): 3401, 3300, 3247, 3077, 1673; ^1H NMR ($CDCl_3$, ppm/δ): 1.51–1.91 (m, 11H), 3.15–3.21 (m, 4H), 3.79–4.00 (m, 2H), 6.81 (s, 1H), 7.40–7.47 (m, 1H), 7.50–7.58 (m, 1H), 7.61 (s, 1H), 8.02 (bs, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 14.4, 26.3, 26.9, 28.5 29.6, 58.2, 124.3, 127.9, 128.1, 130.0, 130.4, 130.5, 133.6, 135.6, 136.5, 136.9, 140.9, 149.8, 151.6, 159.3. Anal Calcd for $C_{22}H_{22}Cl_2N_4OS$: C, 57.27; H, 4.81; N, 12.14. Found: C, 57.30; H, 4.86; N, 12.14.

*6.1.1.1.8. 7-Methyl-1-(2,4-dichlorophenyl)-N-(pyrrolidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6h**).* General procedure **I** was used to convert **16** and *N*-aminopyrrolidine hydrochloride into the title product adding 1.5 equiv of triethylamine to the mixture. Purification by flash chromatography afforded **6h** (0.171 g, 66%) as a yellow solid. Rf = 0.30 ($CHCl_3$/MeOH 99:1); mp 170–180 °C; IR (ν/cm^{-1}): 3397, 3309, 3234, 1666; ^1H NMR ($CDCl_3$, ppm/δ): 1.71 (s, 3H), 1.85–1.98 (m, 4H), 2.95–3.15 (m, 4H), 3.87 (s, 2H), 6.81 (s, 1H), 7.44 (dd, *J* = 8.6 Hz, 2.2 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 1H), 7.61 (d, *J* = 2.2 Hz, 1H), 8.08 (bs, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 14.4, 22.2, 28.7, 29.6, 55.4, 124.3, 127.9, 128.1, 130.0, 130.4, 130.5, 133.7, 135.6, 136.5, 136.9, 140.8, 149.9, 151.7, 159.9. Anal. Calcd for $C_{20}H_{18}Cl_2N_4OS$: C, 55.43; H, 4.19; N, 12.93; Found: C, 55.43; H, 4.27; N, 13.01.

*6.1.1.1.9. 7-Methyl-1-(2,4-dichlorophenyl)-N-cyclohexyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6i**).* General procedure **I** was used to convert **16** and cyclohexylamine into the title product. Purification by flash chromatography afforded **6i** (0.220 g, 82%) as a yellow solid. Rf = 0.60 ($CHCl_3$/MeOH 99:1); mp 102–103 °C; IR (ν/cm^{-1}): 3403, 3315, 3102, 1660; ^1H NMR ($CDCl_3$, ppm/δ): 1.10–1.65 (m, 6H), 1.73 (s, 3H), 1.90–2.10 (m, 4H), 3.85 (s, 2H), 3.90–4.00 (m, 1H), 6.63 (bs, 1H), 6.80 (s, 1H), 7.43 (dd, *J* = 8.4 Hz, 2.2 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.60 (s, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 14.5, 24.9, 25.6, 28.6, 33.1, 48.1, 124.3, 127.9, 128.2, 130.0, 130.2, 130.5, 133.7, 135.8, 136.2, 137.0, 141.8, 150.1, 151.7, 160.9. Anal. Calcd for $C_{22}H_{21}Cl_2N_3OS$: C, 59.20; H, 4.74; N, 9.41. Found: C, 59.22; H, 4.76; N, 9.49.

*6.1.1.1.10. 7-Methyl-1-(2,4-dichlorophenyl)-N-morpholinyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6j**).* General procedure **I** was used to convert **16** and *N*-aminomorpholine into the title product. Purification by flash chromatography afforded **6j** (0.110 g, 41%) as a yellow solid. Rf = 0.17 ($CHCl_3$/MeOH 99:1); mp 165–168 °C; IR (ν/cm^{-1}): 3401, 3246, 3091, 1670; ^1H NMR ($CDCl_3$, ppm/δ): 1.72 (s, 3H), 2.88–3.10 (m, 4H), 3.78–4.02 (m, 6H), 6.81 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 2.0 Hz, 1H), 7.66 (s, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 14.4, 28.5, 29.6, 56.0, 66.4, 124.4, 128.0, 128.1, 130.0, 130.2, 130.4, 130.6, 133.7, 135.7, 136.7, 136.8, 150.0, 151.8, 159.2. Anal. Calcd for $C_{20}H_{18}Cl_2N_4O_2S$: C, 53.46; H, 4.04; N, 12.47. Found: C, 53.50; H, 4.08; N, 12.40.

*6.1.1.1.11. 7-Methyl-1-(2,4-dichlorophenyl)-N-menthyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6k**).* General procedure **I** was used to convert **16** and L-menthylamine into the title product. Purification by flash chromatography afforded **6k** (0.187 g, 62%) as a yellow solid. Rf = 0.74 ($CHCl_3$/MeOH 99:1); mp 104–105 °C; IR (ν/cm^{-1}): 3390, 3278, 3066, 1662; ^1H NMR ($CDCl_3$, ppm/δ): 0.83 (s, 3H), 0.87 (s, 3H), 0.91 (s, 3H), 0.70–1.02 (m, 2H), 1.05–1.25 (m, 2H), 1.60–1.80 (m, 3H), 1.72 (s, 3H), 1.98–2.19 (m, 2H), 3.86 (s, 2H), 3.85–4.00 (m, 1H), 6.59 (d, *J* = 8.2 Hz, 1H), 6.80 (s, 1H), 7.41–7.61 (m, 3H); ^{13}C NMR ($CDCl_3$, ppm/δ): 14.5, 16.1, 21.1, 22.1, 23.8, 26.7, 28.6, 31.9, 34.5, 43.0, 47.8, 49.7, 124.2, 127.9, 128.1, 130.0, 130.1, 130.5, 133.7, 135.7, 136.5, 137.0, 141.7, 150.0, 151.6, 161.0; $[\alpha]_D^{20}$: −26.35° ($CHCl_3$, *c* = 0.16). Anal. Calcd for $C_{26}H_{29}Cl_2N_4OS$: C, 62.15; H, 5.82; N, 8.36. Found: C, 62.21; H, 5.79; N, 8.32.

*6.1.1.1.12. 7-methyl-1-(2,4-dichlorophenyl)-N-fenchyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6l**).* General procedure **I** was used to convert **16** and 1*R*,2*S*,4*R*-fenchylamine into the title product. Purification by flash chromatography afforded **6l** (0.180 g, 60%) as a yellow solid. Rf = 0.72 ($CHCl_3$/MeOH 99:1); mp 85–87 °C; IR (ν/cm^{-1}): 3415, 3091, 1668; ^1H NMR ($CDCl_3$, ppm/δ): 0.87 (s, 3H), 1.11 (s, 3H), 1.18 (s, 3H), 1.20–1.26 (m, 2H), 1.31–1.48 (m, 2H), 1.60–1.80 (m, 3H), 1.73 (s, 3H), 3.76–3.90 (m, 1H), 3.85 (s, 2H), 6.80 (s, 1H), 6.95 (d, *J* = 10.0 Hz, 1H), 7.44 (dd, *J* = 9.0 Hz, 1.8 Hz, 1H), 7.57 (d, *J* = 9.0 Hz, 1H), 7.60 (s, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 14.6, 19.8, 21.3, 26.0, 27.3, 28.6, 30.9, 39.5, 42.7, 48.1, 48.6, 63.1, 124.2, 127.8, 128.1, 129.9, 130.6, 133.7, 135.9, 136.3, 137.1, 141.6, 149.9, 151.5, 162.5; $[\alpha]_D^{20}$: −6.08° ($CHCl_3$, *c* = 0.23). Anal. Calcd for $C_{26}H_{27}Cl_2N_3OS$: C, 62.40; H, 5.44; N, 8.40. Found: C, 62.48; H, 5.46; N, 8.38.

*6.1.1.1.13. 6-Methyl-1-(4-methylbenzyl)-N-menthyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6m**).* General procedure **I** was used to convert **17** and L-menthylamine into the title product. Purification by flash chromatography afforded **6m** (0.138 g, 50%) as a yellow solid. Rf = 0.63 ($CHCl_3$/MeOH

99:1); mp 67–70 °C; IR (ν/cm^{-1}): 3399, 3303, 1658; ^{1}H NMR ($CDCl_3$, ppm/δ): 0.83–0.93 (m, 9H), 1.10–1.25 (m, 2H), 1.64–1.78 (m, 5H), 2.03–2.15 (m, 2H), 2.34 (s, 3H), 2.45 (s, 3H), 3.74 (s, 2H), 3.85–4.00 (m, 1H), 5.38 (s, 2H), 6.33 (s, 1H), 6.60 (d, J = 9.9 Hz, 1H), 7.10–7.21 (m, 4H); ^{13}C NMR ($CDCl_3$, ppm/δ): 16.0, 16.2, 21.1, 22.1, 23.8, 26.7, 28.8, 31.9, 34.5, 43.0, 47.8, 49.6, 55.0, 115.0, 127.3, 129.5, 129.6, 132.8, 134.5, 137.9, 139.8, 143.4, 147.8, 161.3; $[\alpha]_D^{20}$: −26.47° ($CHCl_3$, c = 0.13). Anal. Calcd for $C_{28}H_{35}N_3OS$: C, 72.85; H, 7.64; N, 9.10. Found: C, 72.87; H, 7.60; N, 9.14.

*6.1.1.1.14. 7-Methyl-1-(4-methylbenzyl)-N-(piperidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6n**).* General procedure **I** was used to convert **18** and *N*-aminopiperidine into the title product. Purification by flash chromatography afforded **6n** (0.193 g, 88%) as a yellow solid. Rf = 0.26 ($CHCl_3$/MeOH 99:1); mp 213–218 °C; IR (ν/cm^{-1}): 3234, 1648; ^{1}H NMR ($CDCl_3$, ppm/δ): 1.38–1.50 (m, 2H), 1.60–1.81 (m, 4H), 2.28 (s, 3H), 2.31 (s, 3H), 2.87 (t, J = 5.2 Hz, 4H), 3.79 (s, 2H), 5.55 (s, 2H), 6.83 (s, 1H), 6.97 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 7.59 (s, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.7, 21.0, 23.3, 25.3, 28.6, 55.3, 57.0, 124.2, 125.8, 127.6, 129.4, 130.9, 133.7, 135.6, 137.4, 139.3, 148.0, 151.2, 159.4. Anal. Calcd for $C_{23}H_{26}N_4OS$: C, 67.95; H, 6.45; N, 13.78. Found: C, 67.99; H, 6.43; N, 13.81.

*6.1.1.1.15. 7-Methyl-1-(4-methylbenzyl)-N-(homopiperidin-yl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6o**).* General procedure **I** was used to convert **18** and *N*-aminohomopiperidine into the title product. Purification by flash chromatography afforded **6o** (0.227 g, 90%) as a yellow solid. Rf = 0.29 ($CHCl_3$/MeOH 99:1); mp 188–191 °C; IR (ν/cm^{-1}): 3365, 3270, 1654; ^{1}H NMR ($CDCl_3$, ppm/δ): 1.55–1.82 (m, 8H), 2.28 (s, 3H), 2.31 (s, 3H), 3.12–3.19 (m, 4H), 3.79 (s, 2H), 5.54 (s, 2H), 6.83 (s, 1H), 6.97 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 8.03 (s, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.8, 21.0, 26.7, 27.0, 28.4, 55.4, 58.2, 124.2, 125.9, 127.7, 129.5, 130.8, 133.8, 135.7, 137.4, 139.4, 148.0, 151.3, 159.9. Anal Calcd for $C_{24}H_{28}N_4OS$: C, 68.54; H, 6.71; N, 13.32. Found: C, 68.60; H, 6.75; N, 13.33.

*6.1.1.1.16. 7-Methyl-1-(4-methylbenzyl)-N-(pyrrolidinyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6p**).* General procedure **I** was used to convert **18** and *N*-aminopyrrolidine hydrochloride into the title product adding 1.5 equiv of triethylamine to the mixture. Purification by flash chromatography afforded **6p** (0.174 g, 74%) as a yellow solid. Rf = 0.21 ($CHCl_3$/MeOH 99:1); mp 177–185 °C; IR (ν/cm^{-1}): 3234, 3052, 1662; ^{1}H NMR ($CDCl_3$, ppm/δ): 1.85–1.99 (m, 4H), 2.28 (s, 3H), 2.31 (s, 3H), 2.90–3.15 (m, 4H), 3.80 (s, 2H), 5.55 (s, 2H), 6.83 (s, 1H), 6.96 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 7.56 (br. s, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.8, 21.0, 22.2, 28.4, 55.4, 124.2, 125.8, 127.7, 129.5, 130.8, 133.7, 135.6, 137.4, 139.2, 148.1, 151.3, 160.3. Anal. Calcd for $C_{22}H_{24}N_4OS$: C, 67.32; H, 6.16; N, 14.27; Found: C, 67.42; H, 6.19; N, 14.20.

*6.1.1.1.17. 7-Methyl-1-(4-methylbenzyl)-N-cyclohexyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6q**).* General procedure **I** was used to convert **18** and cyclohexylamine into the title product. Purification by flash chromatography afforded **6q** (0.124 g, 51%) as a yellow solid. Rf = 0.61 ($CHCl_3$/MeOH 99:1); mp 188–194 °C; IR (ν/cm^{-1}): 3332, 3064, 1639; ^{1}H NMR ($CDCl_3$, ppm/δ): 1.20–1.45 (m, 4H), 1.60–1.73 (m, 4H), 1.95–2.05 (m, 2H), 2.28 (s, 3H), 2.31 (s, 3H), 3.78 (s, 2H), 3.83–3.98 (m, 1H), 5.55 (s, 2H), 6.75 (d, J = 8.2 Hz, 1H), 6.82 (s, 1H), 6.96 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.8, 21.0, 25.0, 25.6, 28.4, 29.7, 33.2, 48.0, 55.4, 124.2, 125.8, 127.7, 129.5, 130.5, 133.9, 135.8, 137.4, 140.2, 148.1, 151.2, 161.3. Anal. Calcd for $C_{24}H_{27}Cl_2N_3OS$: C, 71.08; H, 6.71; N, 10.36. Found: C, 71.16; H, 6.79; N, 10.31.

*6.1.1.1.18. 7-Methyl-1-(4-methylbenzyl)-N-morpholinyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6r**).* General procedure **I** was used to convert **18** and *N*-aminomorpholine into the title product. Purification by flash chromatography afforded **6r** (0.181 g, 74%) as a yellow solid. Rf = 0.14 ($CHCl_3$/MeOH 99:1); mp 218–222 °C; IR (ν/cm^{-1}): 3222, 1646; ^{1}H NMR ($CDCl_3$, ppm/δ): 2.29 (s, 3H), 2.32 (s, 3H), 2.95–3.05 (m, 4H), 3.79 (s, 2H), 3.80–3.98 (m, 4H), 5.56 (s, 2H), 6.85 (s, 1H), 6.98 (d, J = 8.0 Hz, 1H), 7.13 (d, J = 8.0 Hz, 2H), 7.64 8 (s, 1H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.7, 21.0, 28.3, 55.3, 55.9, 66.4, 124.3, 125.8, 127.6, 129.4, 130.9, 133.6, 135.5, 137.4, 139.0, 148.1, 151.2, 159.6. Anal. Calcd for $C_{22}H_{24}N_4O_2S$: C, 64.68; H, 5.92; N, 13.71. Found: C, 64.74; H, 5.99; N, 13.70.

*6.1.1.1.19. 7-Methyl-1-(4-methylbenzyl)-N-menthyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6s**).* General procedure **I** was used to convert **18** and L-menthylamine into the title product. Purification by flash chromatography afforded **6s** (0.197 g, 71%) as a yellow solid. Rf = 0.73 ($CHCl_3$/MeOH 99:1); mp 158–165 °C; IR (ν/cm^{-1}): 3397, 3297, 3100, 1658; ^{1}H NMR ($CDCl_3$, ppm/δ): ^{1}H NMR ($CDCl_3$, ppm/δ): 0.83–0.93 (m, 9H), 1.10–1.26 (m, 2H), 1.64–1.78 (m, 5H), 1.95–2.15 (m, 2H), 2.27 (s, 3H), 2.32 (s, 3H), 3.79 (s, 2H), 3.80–4.00 (m, 1H), 5.56 (s, 2H), 6.60 (d, J = 10.0 Hz, 1H), 6.83 (s, 1H), 6.97 (d, J = 7.8 Hz, 2H), 7.13 (d, J = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.7, 16.2, 21.0, 21.1, 22.1, 23.9, 26.7, 28.4, 31.9, 34.5, 43.1, 47.8, 49.5, 55.3, 124.1, 125.7, 127.7, 129.4, 130.4, 133.9, 135.7, 137.3, 140.2, 148.1, 151.2, 161.4; $[\alpha]_D^{20}$: −38.89° ($CHCl_3$, c = 0.30). Anal. Calcd for $C_{28}H_{35}N_3OS$: C, 72.85; H, 7.64; N, 9.10. Found: C, 72.90; H, 7.68; N, 9.18.

*6.1.1.1.20. 7-Methyl-1-(4-methylbenzyl)-N-fenchyl-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxamide (**6t**).* General procedure **I** was used to convert **18** and 1*R*,2*S*,4*R*-fenchylamine into the title product. Purification by flash chromatography afforded **6t** (0.098 g, 59%) as a yellow solid. Rf = 0.73 ($CHCl_3$/MeOH 99:1); mp 58–62 °C; IR (ν/cm^{-1}): 3417, 1668; ^{1}H NMR ($CDCl_3$, ppm/δ): 0.86 (s, 3H), 1.11 (s, 3H), 1.17 (s, 3H), 1.20–1.25 (m, 2H), 1.35–1.45 (m, 2H), 1.60–1.80 (m, 3H), 2.26 (s, 3H), 2.31 (s, 3H), 3.75–3.86 (m, 1H), 3.79 (s, 2H), 5.58 (s, 2H), 6.82 (s, 1H), 6.96–6.70 (m, 1H), 6.98 (d, J = 7.8 Hz, 2H), 7.12 (d, J = 7.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, ppm/δ): 15.7, 19.8, 21.0, 21.3, 26.0, 27.3, 28.4, 30.9, 39.4, 42.7, 48.1, 48.6, 55.4, 63.0, 124.1, 125.8, 127.7, 129.4, 130.3, 134.0, 135.8, 137.3, 139.9, 148.1, 151.2, 162.8; $[\alpha]_D^{20}$: −7.76° ($CHCl_3$, c = 0.32). Anal. Calcd for $C_{28}H_{33}N_3OS$: C, 73.17; H, 7.24; N, 9.14. Found: C, 73.22; H, 7.28; N, 9.10.

6.1.2. General procedure II: synthesis of acids. To a mixture of an ester **11**–**14** (7.00 mmol) in ethanol (36 mL) was added a solution of sodium hydroxide (10 equiv) in H_2O (36 mL). The resulting mixture was heated under refluxed 5 h. The mixture was allowed to cool to room temperature and then poured into water and acidified with 1 N hydrochloric acid. The precipitate was filtered, washed with water and air-dried to yield the analytically pure acid.

6.1.2.1. 6-Methyl-1-(2,4-dichlorophenyl)-1,4-dihydrothieno [2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylic acid. General procedure **II** was used to convert **11** into the title product **15** (2.311 g, 98%) as a white solid. Rf = 0.38 ($CHCl_3$/MeOH 8:2); mp: 255–258 °C; IR (ν/cm^{-1}): 3400, 1705; ^{1}H NMR (DMSO, ppm/δ): 2.43 (s, 3H), 3.79 (s, 2H), 6.55 (s, 1H), 7.50–7.95 (m, 3H), 12.72 (brs, 1H); ^{13}C NMR (DMSO, ppm/δ): 15.6, 28.8, 115.4, 128.7, 130.1, 130.2, 130.7, 134.3, 134.8, 135.6, 139.3, 143.8, 147.6, 148.3, 162.9. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S$: C, 52.62; H, 2.76; N, 7.67. Found: C, 52.68; H, 2.80; N, 7.69.

6.1.2.2. 7-Methyl-1-(2,4-dichlorophenyl)-1,4-dihydrothieno [2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylic acid. General procedure **II** was used to convert **12** into the title product **16** (2.147 g, 91%) as a white solid. Rf = 0.41 ($CHCl_3$/MeOH 8:2); mp: 200–202 °C; IR (ν/cm^{-1}): 3400, 3089, 1714; ^{1}H NMR (DMSO, ppm/δ): 1.62 (s, 3H), 3.79 (s, 2H), 7.10 (s, 1H), 7.71 (d, J = 7.8 Hz, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.99 (s, 1H), 13.1 (brs, 1H); ^{13}C NMR (DMSO, ppm/

δ): 13.9, 28.4, 125.4, 127.4, 128.6, 129.6, 131.2, 132.6, 135.4, 135.7, 136.7, 139.0, 148.9, 150.7, 162.9. Anal. Calcd for $C_{16}H_{10}Cl_2N_2O_2S$: C, 52.62; H, 2.76; N, 7.67. Found: C, 52.70; H, 2.81; N, 7.70.

6.1.1.2.3. 6-methyl-1-(4-methylbenzyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylic acid. General procedure **II** was used to convert **13** into the title product **17** (1.599 g, 90%) as a white solid. Rf = 0.45 ($CHCl_3$/MeOH 8:2); mp: 240–243 °C; IR (ν/cm^{-1}): 3600, 3440, 1685; ^{1}H NMR (DMSO, ppm/δ): 2.26 (s, 3H), 2.47 (s, 3H), 3.65 (s, 2H), 5.49 (s, 2H), 6.93 (s, 1H), 7.00-7.40 (m, 4H), 12.72 (brs, 1H); ^{13}C NMR (DMSO, ppm/δ): 15.8, 20.7, 28.7, 52.2, 115.8, 127.6, 129.3, 130.7, 133.7, 134.4, 137.2, 143.2, 143.3, 146.4, 163.1. Anal. Calcd for $C_{18}H_{16}N_2O_2S$: C, 66.65; H, 4.97; N, 8.64. Found: C, 66.70; H, 5.02; N, 8.61.

6.1.1.2.4. 7-methyl-1-(4-methylbenzyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylic acid. General procedure **II** was used to convert **13** into the title product **17** (1.724 g, 97%) as a white solid. Rf = 0.46 ($CHCl_3$/MeOH 8:2); mp: 200–202 °C; IR (ν/cm^{-1}): 3400, 3100, 1714; ^{1}H NMR (DMSO, ppm/δ): 2.23 (s, 6H), 3.67 (s, 2H), 5.60 (s, 2H), 6.93 (s, 1H), 7.00–7.40 (m, 4H); ^{13}C NMR (DMSO, ppm/δ): 15.2, 20.6, 28.3, 55.9, 117.6, 124.9, 125.8, 127.6, 129.3, 131.7, 134.5, 135.6, 136.7, 146.8, 150.0, 163.4. Anal. Calcd for $C_{18}H_{16}N_2O_2S$: C, 66.65; H, 4.97; N, 8.64. Found: C, 66.69; H, 5.05; N, 8.63.

6.1.1.3. General procedure III: synthesis of esters. To a stirred mixture of diketoester **9**,**10** (3.17 mmol) and the requisite phenyl hydrazine hydrochloride (1.2 equiv) in AcOH (6 mL) was heated under reflux for 8 h. The reaction was allowed to cool to room temperature and poured onto ice-water. The precipitate was filtered, washed with water and air-dried to yield the analytically pure esters.

6.1.1.3.1. Ethyl 6-methyl-1-(2,4-dichlorophenyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylate. General procedure **III** was used to convert **9** into the title product **11** (1.123 g, 97%) as a bright yellow solid. Rf = 0.42 ($CHCl_3$); mp: 162–166 °C; IR (ν/cm^{-1}): 1716; ^{1}H NMR ($CHCl_3$, ppm/δ): 1.43 (t, J = 7.4 Hz, 3H); 2.49 (s, 3H), 3.81 (s, 2H), 4.45 (q, J = 7.0 Hz, 2H), 6.46 (s, 1H), 7.40 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.61 (d, J = 2.2 Hz, 1H); ^{13}C NMR ($CHCl_3$, ppm/δ): 14.4, 16.0, 29.0, 61.2, 115.6, 128.0, 129.7, 130.1, 130.9, 131.2, 135.0, 135.6, 136.0, 139.1, 143.9, 148.0, 149.2, 162.3. Anal. Calcd for $C_{18}H_{14}Cl_2N_2O_2S$: C, 54.97; H, 3.59; N, 7.12. Found: C, 55.02; H, 3.55; N, 7.10.

6.1.1.3.2. Ethyl 7-Methyl-1-(2,4-dichlorophenyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylate. General procedure **III** was used to convert **9** into the title product **12** (1.123 g, 92%) as a white solid. Rf = 0.32 ($CHCl_3$); mp: 170–173 °C; IR (ν/cm^{-1}): 3095, 1731; ^{1}H NMR ($CHCl_3$, ppm/δ): 1.43 (t, J = 7.2 Hz, 3H); 1.73 (s, 3H), 3.82 (s, 2H), 4.45 (q, J = 7.2 Hz, 2H), 6.82 (s, 1H), 7.41 (dd, J = 8.4 Hz, 2.2 Hz, 1H), 7.50–7.70 (m, 2H); ^{13}C NMR ($CHCl_3$, ppm/δ): 14.4, 16.0, 28.6, 61.2, 124.4, 127.8, 128.3, 129.8, 130.6, 131.6, 133.7, 135.9, 136.6, 136.9, 138.9, 149.8, 151.0, 162.1. Anal. Calcd for $C_{18}H_{14}Cl_2N_2O_2S$: C, 54.97; H, 3.59; N, 7.12. Found: C, 55.03; H, 3.58; N, 7.17.

6.1.1.3.3. Ethyl 6-methyl-1-(4-methylbenzyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylate. General procedure **III** was used to convert **10** into the title product **13** (0.884 g, 86%) as a white solid. Rf = 0.29 ($CHCl_3$); mp: 128–132 °C; IR (ν/cm^{-1}): 1719; ^{1}H NMR ($CHCl_3$, ppm/δ): 1.42 (t, J = 7.2 Hz, 3H); 2.33 (s, 3H), 2.44 (s, 3H), 3.68 (s, 2H), 4.43 (q, J = 7.2 Hz, 2H), 5.47 (s, 2H), 6.26 (s, 1H), 7.17 (dd, J = 7.8 Hz, 4H); ^{13}C NMR ($CHCl_3$, ppm/δ): 14.4, 16.0, 21.1, 28.8, 55.6, 60.8, 115.4, 127.6, 129.5, 131.6, 132.6, 134.8, 136.8, 138.0, 143.5, 147.0, 147.4, 162.5. Anal. Calcd for $C_{20}H_{20}N_2O_2S$: C, 68.16; H, 5.72; N, 7.95. Found: C, 68.17; H, 5.77; N, 7.90.

6.1.1.3.4. Ethyl 7-methyl-1-(4-methylbenzyl)-1,4-dihydrothieno[2′,3′-4,5]cyclopenta[1,2c]pyrazole-3-carboxylate. General procedure **III** was used to convert **10** into the title product **14** (1.021 g, 95%) as a gray solid. Rf = 0.45 ($CHCl_3$); mp 61–63 °C; IR (ν/cm^{-1}): 3100, 1716; ^{1}H NMR ($CHCl_3$, ppm/δ): 1.42 (t, J = 7.2 Hz, 3H); 2.33 (s, 3H), 2.44 (s, 3H), 3.68 (s, 2H), 4.43 (q, J = 7.2 Hz, 2H), 5.47 (s, 2H), 6.26 (s, 1H), 7.17 (dd, J = 7.8 Hz, 4H); ^{13}C NMR ($CHCl_3$, ppm/δ): 14.4, 15.7, 21.0, 28.4, 55.7, 60.8, 124.3, 125.9, 127.6, 129.0, 132.2, 133.6, 135.1, 137.2, 137.3, 147.7, 150.6, 162.5. Anal. Calcd for $C_{20}H_{20}N_2O_2S$: C, 68.16; H, 5.72; N, 7.95. Found: C, 68.21; H, 5.72; N, 7.89.

6.1.1.4. General procedure IV: synthesis of diketoesters. Sodium (2.0 equiv) was added in small portion to dry ethanol (5 mL) and stirred until all the sodium had reacted. Ethyl oxalate (1.0 equiv) was added, followed by dropwise addition of a solution of appropriate thienoindanone (**7**,**8**) starting material (6 mmol) in dry ethanol (30 mL). The solution was stirred at room temperature for 5 h. The mixture was slowly poured over 2 N hydrochloride acid and the resulting precipitate was collected by filtration and washed with a small volume of ice-cold ethanol and water. The air-dried residue afforded the analytically pure product.

6.1.1.4.1. Ethyl 2-(2-methyl-4-oxo-5,6-dihydro-4H-cyclopenta[b]thiophen-5-yl)-2-oxoacetate. General procedure **IV** was used to convert **7** into the title product **9** (1.027 g, 62%) as a yellow solid. Rf = 0.68 ($CHCl_3$/MeOH 9:1); mp: 136–138 °C; IR (ν/cm^{-1}): 3100, 1716, 1666; ^{1}H NMR ($CHCl_3$, ppm/δ): 1.41 (t, J = 7.2 Hz, 3H); 2.53 (s, 3H), 3.97 (s, 2H), 4.39 (q, J = 7.2 Hz, 2H), 6.90 (s, 1H), 12.80 (brs, 1H); ^{13}C NMR ($CHCl_3$, ppm/δ): 14.2, 16.1, 30.2, 62.1, 117.0, 119.8, 145.4, 146.4, 150.5, 162.7, 165.1, 191.9. Anal. Calcd for $C_{12}H_{12}O_4S$: C, 57.13; H, 4.79. Found: C, 57.19; H, 4.83.

6.1.1.4.2. Ethyl 2-(3-methyl-4-oxo-5,6-dihydro-4H-cyclopenta[b]thiophen-5-yl)-2-oxoacetate. General procedure **IV** was used to convert **8** into the title product **10** (1.093 g, 66%) as a white solid. Rf = 0.64 ($CHCl_3$/MeOH 9:1); mp: 84–87 °C; IR (ν/cm^{-1}): 3417, 3097, 1718, 1660; ^{1}H NMR ($CHCl_3$, ppm/δ): 1.42 (t, J = 7.2 Hz, 3H); 2.28 (s, 3H), 3.77 (s, 2H), 4.41 (q, J = 7.2 Hz, 2H), 7.55 (s, 1H), 12.90 (brs, 1H); ^{13}C NMR ($CHCl_3$, ppm/δ): 13.5, 14.1, 19.7, 62.1, 115.6, 120.3, 132.5, 144.2, 150.5, 162.6, 166.5, 192.9. Anal. Calcd for $C_{12}H_{12}O_4S$: C, 57.13; H, 4.79. Found: C, 57.20; H, 4.85.

*6.1.1.4.3. 3-Methyl-5,6-dihydro-4H-cyclopenta[b]thiophen-4-one (**8**).* 3-(4-methylthiophen-2-yl)propanoic acid (**21**) (1 g, 5.88 mmol) was added in small portions to warm (75 °C) polyphosphoric acid (8.5 g). After the addition was complete, heating was continued at 75 °C for 1 h. Cooling to room temperature was followed by dilution with water and extraction with CH_2Cl_2. The combined organics were dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (PEt/EtOAc 1:1) gave **8** as a bright yellow solid (0.28 g, 32%). Rf = 0.89 ($CHCl_3$/MeOH 9:1); mp: °C; 55–58 °C; IR (ν/cm^{-1}): 1672; ^{1}H NMR ($CHCl_3$, ppm/δ): 2.49 (s, 3H), 2.89 (t, J = 5.1 Hz, 2H), 3.11 (t, J = 5.1 Hz, 2H), 6.79 (s, 1H); ^{13}C NMR ($CHCl_3$, ppm/δ): 13.5, 24.0, 41.8, 125.3, 132.4, 144.28, 171.0, 198.8. Anal. Calcd for C_8H_8OS: C, C, 63.13; H, 5.30. Found: C, 63.15; H, 5.32.

6.2. Pharmacology

6.2.1. Animals

All animal experiments were performed according to the UE guidelines for the care and use of experimental animals (CEE N° 86/609). Animals (Charles River, Calco, LC, Italy) were housed in the animal care quarters; temperatures were maintained at 22 ± 2 °C (humidity 55 ± 5%) on a 12 h light/dark cycle. Food and water were available ad libitum before to start with the experimental procedures.

6.2.2. Receptor binding studies

Male CD1 mice weighing 30–35 g were killed by cervical dislocation and the brain (minus cerebellum) and spleen were

rapidly removed and placed on an ice-cold plate. Brain and spleen tissues were used for CB1 and CB2 binding assays, respectively. After thawing, tissues were homogenated in 20 vol. (wt/v) of ice-cold TME buffer (50 mM Tris–HCl, 1 mM EDTA and 3.0 mM $MgCl_2$, pH 7.4). The homogenates were centrifuged at 1086 × *g* for 10 min at 4 °C, and the resulting supernatants were centrifuged at 45,000 × *g* for 30 min.

For radioreceptor binding experiments, the novel compounds were dissolved in dimethyl-sulfoxide (DMSO). DMSO concentration in the different assays never exceeded 0.1% (v/v) and was without effects.

[^{3}H]-CP-55,940 binding was performed by the previously described method [30]. [^{3}H]-CP-55,940 (specific activity 180 Ci/mmol) was purchased from Perkin Elmer Italia. Briefly, the membranes (30–80 μg of protein) were incubated with 0.5–1 nM of [^{3}H]-CP-55,940 for 1 h at 30 °C in a final volume of 0.5 mL of TME buffer containing 5 mg/mL of fatty acid-free bovine serum albumin. Non-specific binding was estimated in the presence of 1 μM of CP-55,940. All binding studies were performed in glass tubes pre-treated with Sigma-Cote (Sigma Chemical Co. Ltd., Poole, UK), in order to reduce non-specific binding. The reaction was blocked by rapid filtration through Whatman (GF/C filters presoaked in 0.5% polyethyleneimine (PEI) using a Brandell 36-sample harvester (Gaithersburg, MD, USA). Filters were washed five times with 4 mL aliquots of ice cold Tris HCl buffer (pH 7.4) containing 1 mg/mL BSA. The filter bound radioactivity was measured in a liquid scintillation counter (Trisarb 2900, Packard, Meridien, USA) with 4 mL of scintillation fluid (Ultima Gold MV, Packard).

Protein determination was performed by means of Bradford protein assay using BSA as a standard according to the protocol of the supplier (Bio-Rad, Milan, Italy).

All experiments were performed in triplicate and results were confirmed in at least five independent experiments. Data from radioligand inhibition experiments were analyzed by nonlinear regression analysis of a Sigmoid Curve using Graph Pad Prism program. IC_{50} values were derived from the calculated curves and converted to Ki values according to previously described procedures [39].

6.2.3. Intrinsic activity by in vitro assays

HL-60 cells were grown at 37 °C in humidified 5% CO_2 in RPMI 1640 Medium (Gibco-BRL, Gaithensburg, MA) supplemented with 10% Fetal Bovine Serum (FBS), 1 μg/mL Penicillin-Streptomycin, 2 mM L-Glutamine, 2.5 μg/mL Amphotericin B, and 50 μg/mL Gentamicin. Cells lines from the European Collection of Cell Cultures (ECACC), FBS, Penicillin-Streptomycin, L-Glutamine, Amphotericin B, Gentamicin, D-PBS and DMSO were purchased from Sigma–Aldrich (Milan, Italy).

HL-60 cells were treated with the compounds to be assayed or the reference compounds WIN55,212-2 or AM630 (Sigma–Aldrich, Milan, Italy). Tested and reference compounds were dissolved in culture medium with 1% DMSO and treatments were performed in a volume of 10 μL/mL of cell suspension.

P-ERK 1/2 expression was determined after HL-60 cell treatment. Western blot analysis was carried out according to the previously reported general procedure [30]. After appropriate time of exposure, cells were collected by centrifugation at 1000 × *g* and the resulting pellets were washed in ice-cold PBS buffer by centrifugation at 1000 × *g*. The pellet was lysed at 4 °C in HEPES based buffer containing other components [30]. The extracts were centrifuged at 10,000 × *g* for 15 min at 4 °C. The resulting supernatant was collected as total cell extracts. Extracted total proteins were quantified using a Quant-iT™ Protein Assay Kit (Invitrogen™) by a Qubit Quantitation platform system (Invitrogen™). Each western blot assay was carried out at fixed total protein amount of 40 μg. Separation was performed by 10% Bis–Tris Gel NuPAGE® Novex (Invitrogen) and nitrocellulose membranes (Bio-Rad). Blots were blocked through ChemiBLOCKER™ (Millipore) in TBST (0.1% Tween 20 in Tris borate saline, Bio-Rad) and probed with Anti-phospho-ERK 1/2 recombinant clone AW39R (Millipore) with a 1:1000 dilution in 1% BSA (Bovine Serum Albumin) in TBST (10 min). After three cycles of washing in TBST, membranes were probed with the secondary antibody Goat anti-Rabbit IgC – HRP (Invitrogen) with a 1:2000 dilution in ChemiBLOCKER™-TBST 1:4. After three cycles of washing in TBST, blots were treated for 5 min with Immobilon Western Chemiluminescent HRP substrate (Millipore) and immunoreactive bands were visualized by a Fujifilm Las-1000 analyzer (Raytest Isotopenmessgeräte GmbH). Immunoreactive band optical density was measured using AIDA 2.11 software (Raytest Isotopenmessgeräte GmbH).

Data were expressed as mean percentage of vehicle ±SEM (results of five separate experiments). Statistical analysis was performed by One-way ANOVA followed by Dunnet's multiple comparison using Graph Pad Prism 5 program (San Diego).

In a first step P-ERK 1/2 was determined after 15 min treatment of the HL-60 cells with the compounds to be assayed. Successively, to confirm the capability of the novel derivatives to act as CB2 agonists, a 5 min pre-treatment with the selective CB2 receptor antagonist AM630 (75 nM) was carried out before the exposure to the cells to the compounds to be tested or to the reference cannabinoid agonist WIN55,212-2 (75 nM).

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