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Research paper

Synthesis, binding assays, cytotoxic activity and docking studies of benzimidazole and benzothiophene derivatives with selective affinity for the CB2 cannabinoid receptor

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ABSTRACT

Herein we report the design, synthesis, bioinformatic and biological studies of benzimidazole and benzothiophene derivatives as new cannabinoid receptor ligands. To test the hypothesis that the lack of a hydrogen bond interaction between benzimidazole and benzothiophene derivatives with Lys192 reduces their affinity for CB1 receptors (as we previously reported) and leads to CB2 selectivity, most of the tested compounds do not exhibit hydrogen bond acceptors. All compounds displayed mostly CB2 selectivity, although this was more pronounced in the benzimidazoles derivatives. Furthermore, docking assays revealed a ∏-cation interaction with Lys109 which could play a key role for the CB2 selectivity index. The series displayed low toxicity on five different cell lines. Derivative **8f** presented the best binding profile (Ki = 0.08 μM), high selectivity index (KiCB1/KiCB2) and a low citoxicity. Interestingly, in cell viability experiments, using HL-60 cells (expressing exclusively CB2 receptors), all synthesised compounds were shown to be cytotoxic, suggesting that a CB2 agonist response may be involved.

1. Introduction

The Endocannabinoid System (ECS), a regulatory system consisting of lipid-derived signalling molecules, (i.e. the endocannabinoids), their G protein-coupled receptors (GPCR), and ligand-metabolizing enzymes (i.e., NAPE-PLD, DAGL, FAAH, MAGL), is emerging as a promising target in therapy. In the past decade, the ECS has been implicated in a wide range of physiological and pathological processes, both in the central and peripheral nervous systems [1]. Two different types of cannabinoid receptors (CBR), CB1 and CB2, have been discovered and cloned. CB1 receptors, previously known as central cannabinoid receptors, are mainly expressed in the brain [2] where they modulate the psychotropic effects of cannabinoids. CB2 receptors are primarily located at the surface of peripheral immune cells (monocytes, macrophages, neutrophils, B lymphocytes and T lymphocytes) [3], although a low amount of these receptors has been recently reported in the brain [4]. Due to the expression of CB2 receptors in the immune system, their activation elicits immunomodulatory responses, such as a downregulation of cytokines (IFN-γ and TNF-α) production during inflammatory processes [5].

As members of the class A (rhodopsin-like) G protein-coupled receptor (GPCR) superfamily, both CB1 and CB2 receptors are mostly coupled to heterotrimeric Gi/o proteins. As a consequence, the activation of cannabinoid receptors leads to the inhibition of adenylate cyclase and the following reduction in cyclic AMP accumulation in many tissues and models [6].

The development of novel cannabimimetic compounds

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represents a useful therapeutic strategy for the treatment of different pathological conditions, including obesity, cancer, inflammation, glaucoma and neuropathic pain [7–10]. It is noteworthy that most CB2 agonists interact with peripheral receptors, thus limiting the occurrence of psychoactive side effects typical of CB1 activation [11]. Therefore, in addition to pain modulation, CB2 ligands are also being actively investigated in a wide range of pathologies such as cancer [12], myocardial infarction [13], stroke [14], atherosclerosis [15], autoimmune [16] and neurodegenerative disorders [17], gastrointestinal inflammation [18], hepatic injury [19,20], fibrosis [21], and kidney [22] and bone [23] disorders.

Extensive knowledge of the structure and function of the ECS allowed the development of numerous ligands for the cannabinoid receptors. Based on the chemical structure, five groups of CBR agonists have been identified: (1) *classical cannabinoids* (CCs); (2) *non-classical cannabinoids* (NCCs); (3) *hybrid cannabinoids* (SAH); (4) *aminoalkylindoles* (AAIs); and (5) *diarylpyrazoles*. Among these, *aminoalkylindoles* (AAIs) represents the most relevant class of ligands. AAIs were developed by Sterling Winthrop as potential non-steroidal anti-inflammatory agents; however, these analogues exhibited anti-nociceptive properties that were eventually attributed to the interaction with cannabinoid receptors [24]. Two known AAI are shown in Fig. 1: a potent agonist which displays a similar selectivity for CB1 and CB2 receptors called WIN-55,212-2, and AM630 which is a CB2 selective inverse agonist.

Benzimidazole and benzothiophene represent very important frameworks and pharmacophores in drug discovery [25–37]. Benzimidazoles with affinity and pharmacological activity at both CB1 and CB2 receptors have been reported [38–44].

On the other hand, there are benzothiophene derivatives which display affinity for CB2 receptors [45]. Johnson et al. has developed a series of benzothiophenes with inhibitory activity over the fatty acid amide hydrolase enzyme (FAAH) which is involved in anandamide degradation [46].

Our research group has been interested in the synthesis and Structure-Activity Relationship (SAR) of benzimidazoles and other derivatives as ligands for CB receptors [47–49]. Our compounds increase the supply of available ligands and are synthetically easy to obtain in good yields. Furthermore, to extend these studies, the aim of this work was to explore the SAR of new benzimidazoles and benzothiophenes in CB1 and CB2 receptors, in order to identify structural requirements that can allow the synthesis of novel selective ligands for the peripheral CB2 receptors in view of their high therapeutic potential. All of the synthesised compounds were evaluated in their binding affinity to Human recombinant CB1/CB2 cannabinoid receptors. 22 of the 33 synthesised compounds were able to bind selectively to CB2 receptors with inhibition constants (K_i) in the low micromolar range and presented a good selectivity index ($K_iCB1/K_iCB2 > 6.0$). Finally, cell viability assays were performed for all compounds with the purpose to establish the toxicity parameters in different cell lines. In human Acute Promyelocytic Leukaemia cells (HL60), which express a high density of CB2 receptors [3,50], all compounds were found to be toxic with EC_{50} values lower than Ki's obtained in CB2 binding assays, suggesting that these compounds may act as agonists on CB2 receptors.

2. Results and discussion

2.1. Design criteria to develop of CB ligands

The design and development of new CB ligands was guided by the following rationale criteria: (i) A comparative reasoning led to an isosteric replacement of the indole ring in *aminoalkylindoles* (AAIs), by the aromatic frameworks benzimidazole and benzothiophene. (ii) Exploration of alkyl chains and rings at positions 1- and 3- of the indole core based on our previous 3D-QSAR (CoMFA) model [49], which demonstrated that bulky groups in those positions were favourable for CB2 affinity. (iii) Incorporation of fragments from known ligands at specific positions where *N*1 of benzimidazole and C-3 of benzothiophene were functionalised with 1-naphthoyl and 4-methoxybenzoyl moieties such as in WIN-55,212-2 and AM 630. (iv) Using a similar approach, position 2- of both heterocycles was functionalised with 4-methoxyphenyl moiety or aliphatic flexible hydrocarbon chains mimicking the lipophilic alkyl side chain of *Classical Cannabinoids* like THC. Furthermore, we have previously reported a crucial H-bond interaction between Lys 192 and related derivatives in the CB1 active site [47,48]. The presence or absence of H-bond acceptors at C2 (alkyl

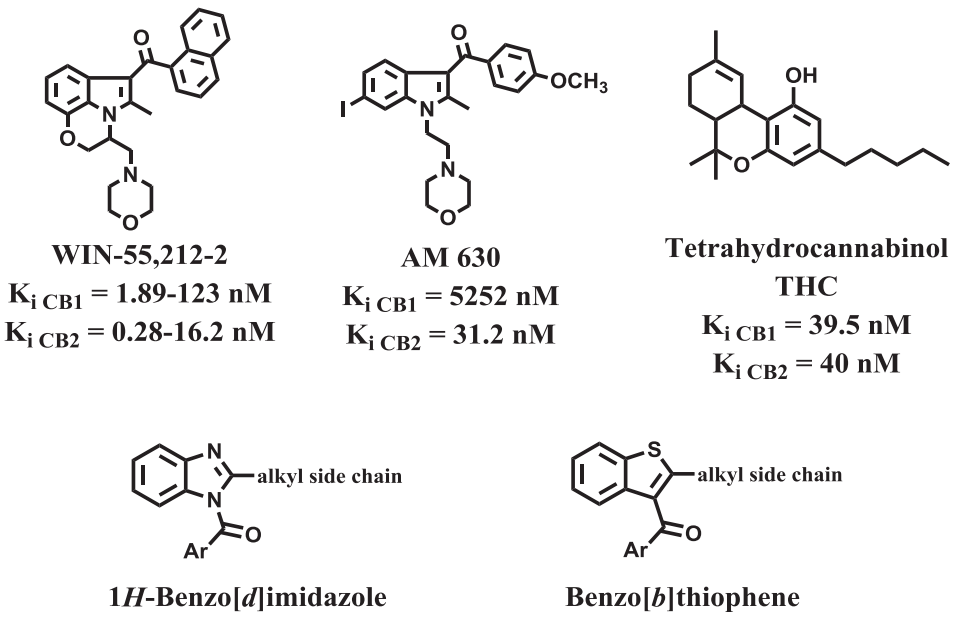


Fig. 1. Structures of WIN-55,212-2, AM630 (AAIs), (-)-*trans*-Δ^9-THC and general structures of benzo[*d*]imidazoles and benzo[*b*]thiophenes target compounds.

chains or 4-methoxyphenyl ring respectively) can provide us with valuable information into the precise role of this probable pivotal interaction.

Taking into account the four criteria mentioned above, Fig. 2 summarize the design strategy applied in the development of the new benzimidazole and benzothiophene derivatives.

2.2. Chemistry

(2-alkyl-1*H*-benzo[*d*]imidazol-1-yl)(naphthalene-1-yl)methanones (**8a-h,j**) and (4-methoxyphenyl)(2-alkyl-1*H*-benzo[*d*]imidazol-1-yl)methanone (**9a-h,j**) (Scheme 1) derivatives were obtained first according to the general condensation procedure of 1,2-phenylenediamine and different aliphatic aldehydes in acetonitrile [51] to obtain the corresponding 2-alkyl-1*H*-benzo[*d*]imidazoles (**7a-h**). Second, compounds **8a-j** and compounds **9a-j** were prepared by substitution of the chlorine atom of 1-naphthoyl chloride and 4-methoxybenzoyl chloride with the corresponding 1*H*-benzo[*d*]imidazoles (**7a-j**). These modifications were included with the aim of studying the importance of these two frameworks as both moieties are present in some known cannabinoid heterocyclic ligands [52]. Compounds **8f** and **9f** were synthesised under basic conditions (NaH) and reflux.

Derivative **7i** was obtained along with the by-product 1-(4-methoxybenzyl)-2-(4-methoxyphenyl)-1*H*-benzo[*d*]imidazole (**7i'**) produced by the double attack of 1,2-phenylenediamine on 4-methoxybenzaldehyde, forming both derivatives in various proportions (Scheme 2), as many authors have reported to occur with or without the use of catalysts [53–58].

Compound **7j** was synthesised using an alternative strategy (Scheme 3). 2-nitroaniline (**3**) was reacted with cyclobutanecarbonyl chloride (**4**) obtaining amide **5**. The nitro group of amide **5** was subsequently reduced by hydrazine hydrate catalysed with palladium charcoal to the corresponding *N*-(2-aminophenyl) cyclobutanecarboxamide (**6**) in a 99% yield [59]. Once compound **7j** was obtained by condensation and ring-closure of derivative **6** in glacial acetic acid, it was reacted with 1-naphthoyl chloride and 4-methoxybenzoyl chloride achieving compounds **8j** and **9j** respectively (Scheme 1).

On the other hand, (2-alkylbenzo[*b*]thiophen-3-yl)(aryl)methanones (**16k-n, 17k-n**) were synthesised from benzo[*b*]thiophene-2-carbaldehyde (**12**) (yields of 73% and 91% for steps **i** and **ii**, respectively) [60] (Scheme 4). In order to lengthen the aliphatic chains, different Grignard reagents were used to yield the alcohols **13k-n** [61]. The subsequent oxidation with PCC gave the intermediates **14k-n**. Then, by Huang- Minlon modified procedure of the Wolff-Kishner reaction, we obtained compounds **15k-n** [62,63]. Finally, the Friedel-Craft acylation of **15k-n** with 1-naphthoyl chloride and 4-methoxybenzoyl chloride allowed us to obtain compounds **16k-n** and **17k-n**, respectively [28,64].

Furthermore, in the Friedel-Craft reaction of benzo[*b*]thiophene (**18**) and 1-naphthoyl chloride, we obtained a mixture of two regioisomers acetylated in positions 2- and 3- (Scheme 5) [64].

Compounds **19** and **20** were separated by thin layer chromatography using hexane as a mobile phase. The structural characterisation and identification of both regioisomers were established on the basis of their spectral properties (^{1}H NMR, ^{13}C NMR, DEPT, COSY, HMQC, HMBC, and IR).

The synthesis of compounds **24** and **25** is described in Scheme 6. The first step consisted of a transition-metal-catalysed cross-coupling Negishi reaction under anaerobic conditions (yield 18%) [65]. In this step, the homocoupling by-product 4,4'-dimethoxy-1,1-biphenyl was produced and large amounts of the starting 2-bromobenzo[*b*]thiophene were recovered. Once the cross-coupling product **23** was isolated, a Friedel-Crafts reaction was performed with the same acid chlorides that had been used previously, to achieve the desired products **24** and **25**.

2.3. Binding assays and CB1 molecular docking

All compounds were evaluated for their ability to bind to human recombinant CB1 and CB2 receptors. IC_{50} and K_i values are shown in Table 1. Binding assays revealed that 22 of 33 synthesized compounds were able to bind selectively to CB2 receptor in a low micromolar range and with a relatively high selectivity index ($K_iCB1/K_iCB2 > 6.0$). Seven compounds exhibited variable affinity for both CB1 and CB2 receptors and only four compounds did not display significant affinity for CB receptors (**8a**, **8b, 9a** and **9d**).

Molecular docking experiments were performed in both CB1 and CB2 receptor models (Figs. 3 and 4). Benzimidazoles and benzothiophenes substituted with a 1-naphthoyl moiety and aliphatic chains (**8b-h, 8j** and **16k-n**) showed all their heterocyclic rings overlapping (Fig. 3A–B), and their aliphatic chains located nearby to the amino group of Lys 192 (except for compound **8a,**

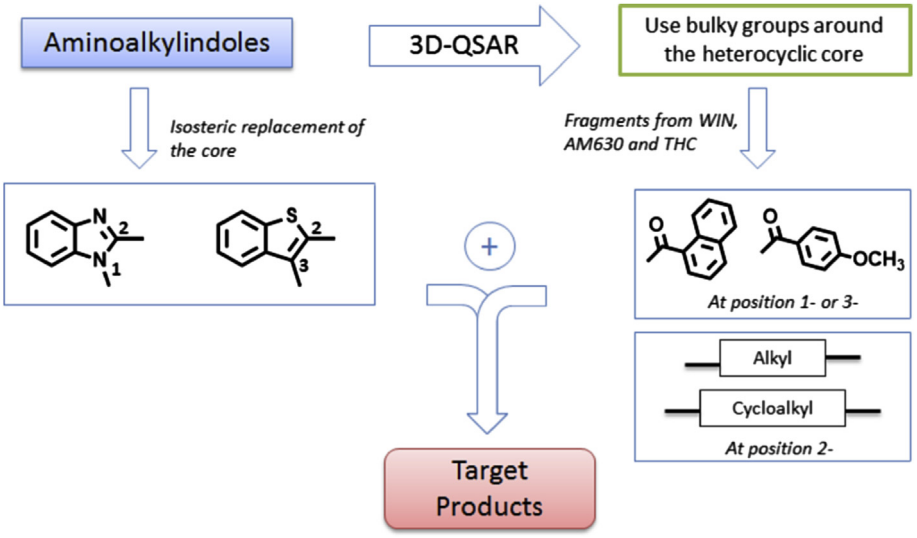
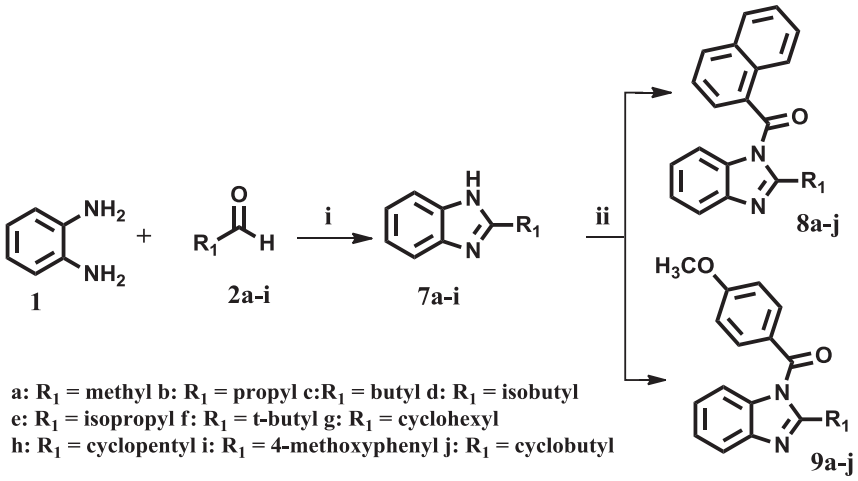
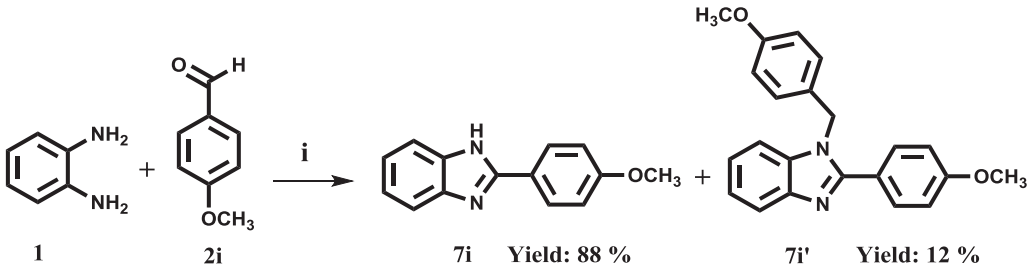


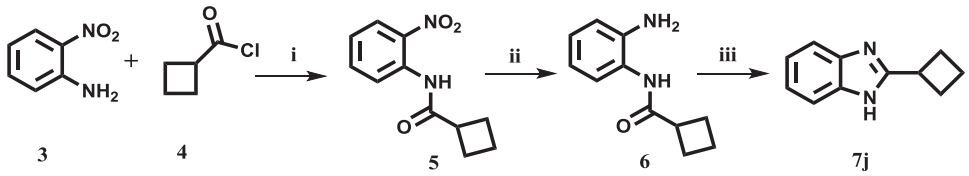
Fig. 2. Design strategy for the obtaining of new cannabinoid ligands of general structure 1*H*-Benzo[*d*]imidazole and Benzo[*b*]thiophene.



Scheme 1. Synthesis of benzo[*d*]imidazoles. Reagents and conditions: (i) CH_3CN, rt, 24h. (ii) 1-naphthoyl chloride or 4-methoxybenzoyl chloride, NEt_3, N_2, dry THF, rt, 8h. For compounds **8f** and **9f**, conditions used were 1-naphthoyl chloride or 4-methoxybenzoyl chloride N_2, dry THF, NaH reflux.



Scheme 2. Reagents and conditions: (i) CH_3CN, rt, 24h.



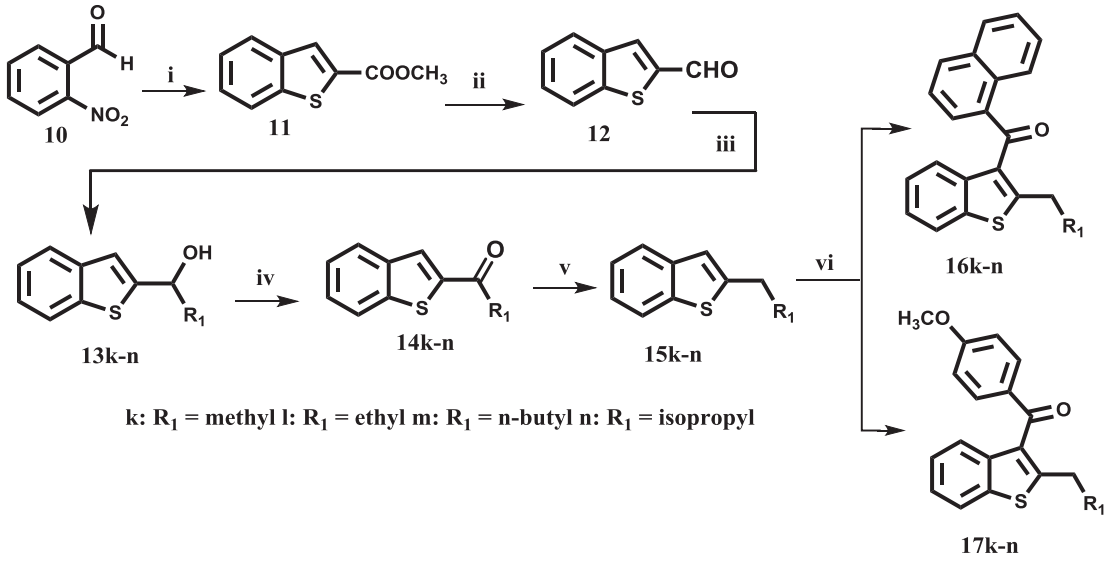
Scheme 3. Reagents and conditions: (i) NaH, N_2, dry THF, rt, 2h. (ii) H_2NNH_2, Pd-C, ethanol, 1h, reflux (iii) glacial CH_3COOH, 118 °C, 2h.

which has a short methyl group). As expected, these derivatives were unable to form a hydrogen bond interaction with Lys 192. Therefore, the essential interaction would involve a T-shaped spatial arrangement between the 1-naphthoyl moieties and Phe 174 (Fig. 3A–B). Binding assays showed that benzimidazoles **8c**, **8g**, **8i** and **8j** exhibited affinity for the CB1 receptor. Nevertheless, almost all benzothiophenes revealed affinity for both CB receptors except for compound **19**, suggesting that a 1-naphthoyl framework at position 3- of a benzothiophene ring substituted with aliphatic chains at position 2- represents a privileged substructure with the ability to bind to both receptors, losing selectivity [47].

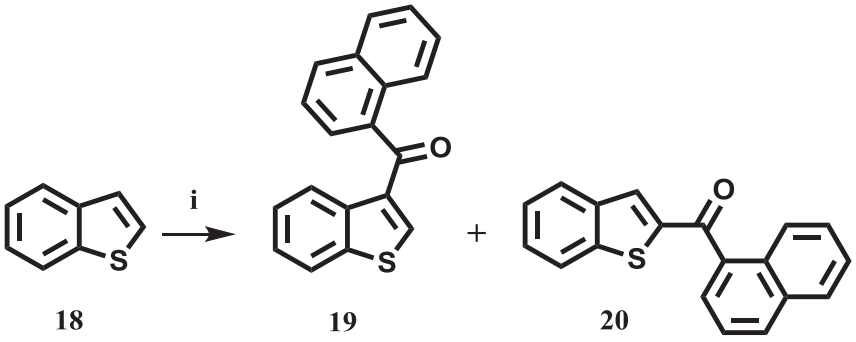
Derivatives substituted with 4-methoxybenzoyl moiety and aliphatic chains (**9a-e, 9g-h, 9j** and **17k-n**) adopted a common and superimposable binding alignment (Fig. 3C–D). Remarkably, none of these compounds displayed affinity for CB1 receptors, except the benzothiophene **17n**, which presented a weak affinity for CB1 and was selective for CB2 receptors. Methoxy groups of all derivatives were close to Lys 192 but not enough to establish a H-bond interaction with the amino group of this residue (>2.0 Å); thus, 4-methoxybenzoyl could be interacting through a ∏-cation interaction with Lys 192, and benzimidazole rings of derivatives **9a-e, 9g-h** and **9j** through a ∏-∏ interaction with Phe 170.

Benzimidazole compounds substituted exclusively with aromatic rings (**7i'**, **8i**, and **9i**) showed different patterns of behaviour in their affinities (Table 1). Derivative **8i** containing a 1-naphthoyl substituent and a 4-methoxyphenyl moiety was the only one of these three benzimidazoles that showed affinity for CB1 receptors. Similar to compounds **8c**, **8h** and **8j**, derivative **8i** appeared to interact with Phe 174 trough a T-shaped interaction. Therefore, it is possible that the combination of a hydrogen bond interaction with Lys 192 and a T-shaped interaction with Phe 174 in a bi-fused aromatic heterocyclic ring affords affinity for CB1 receptors, in agreement with the finding that all benzothiophene derivatives with a 1-naphthoyl in their structure (except for **19**) displayed affinity for CB1 receptors. Benzimidazoles **7i'** and **9i** were selective for CB2 receptors with high selectivity indexes. Both compounds exhibited a hydrogen bond and a ∏-cation interaction with Lys 192, but due their lack of the 1-naphthoyl ring, they were unable to interact with Phe 174.

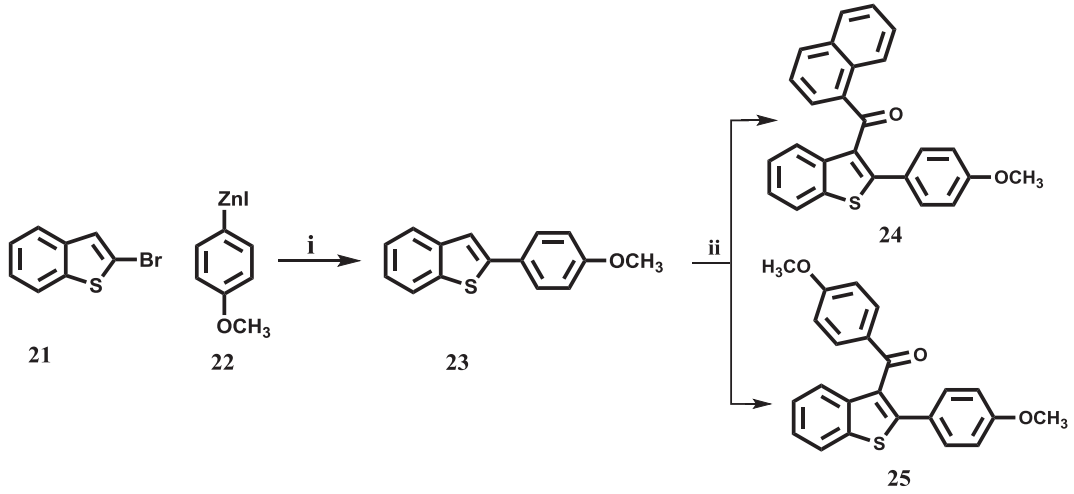
Results from experimental binding assays revealed that both



Scheme 4. Reagents and conditions: i) $SHCH_2COOCH_3$, K_2CO_3, DMF, 80 °C, 2h. ii) DIBAH, dry THF, -78 °C, N_2, 1h. iii) different Grignard reagents, dry THF, N_2, 1h. iv) PCC, CH_2Cl_2, rt, 1h. v) H_2NNH_2, KOH, ethylene glycol, 197 °C, 24h. vi) 1-naphthoyl chloride or 4-methoxybenzoyl chloride, $SnCl_4$, dry $Cl(CH_2)_2Cl$, rt, 6h.



Scheme 5. Reagents and conditions: (i) 1-Naphthoyl chloride, $SnCl_4$, dry $ClCH_2CH_2Cl$, rt, 6h.



Scheme 6. Reagents and conditions: (i) $Pd(PPh_3)_4$, N_2 dry THF, rt, 6h. ii) 1-naphthoyl chloride or 4-methoxybenzoyl chloride, $SnCl_4$, dry $ClCH_2CH_2Cl$, rt, 6h.

heterocyclic derivatives were mostly selective for CB2 receptors. Nonetheless, benzimidazoles tended to display greater CB2 selectivity compared with benzothiophenes, which is consistent with the results reported by other researchers [39–43].

Considering the high number of CB2 selective ligands obtained in this study, a CB2 homology model receptor was constructed, in order to study their spatial arrangement in docking assays.

Table 1
Radioligand displacement results for benzimidazole and benzothiophene derivatives.[a]

Comp.	CB1		CB2		Selectivity index
	IC_{50} (μM)	Ki (μM)	IC_{50} (μM)	Ki (μM)	(K_iCB1/K_iCB2)
7i'	>10	>10	2.7 ± 1.2	0.7 ± 0.3	>14
8a	>10	>10	>10	>10	–
8b	>10	>10	>10	>10	–
8c	9.7 ± 0.13	5.9 ± 0.8	1.7 ± 0.4	0.4 ± 0.1	14.7
8d	>10	>10	0.5 ± 0.2	0.1 ± 0.05	>100
8e	>10	>10	2.1 ± 0.7	0.5 ± 0.2	>20
8f	>10	>10	0.3 ± 0.1	0.08 ± 0.03	>125
8g	4.9 ± 0.9	2.9 ± 0.5	1.7 ± 0.1	0.4 ± 0.03	7.25
8h	>10	>10	2.9 ± 0.4	0.7 ± 0.1	>14
8i	8.1 ± 0.7	4.9 ± 0.4	7.7 ± 0.5	1.9 ± 0.1	2.57
8j	9.7 ± 1.6	5.8 ± 0.9	5.5 ± 3.0	1.4 ± 0.7	4.14
9a	>10	>10	>10	>10	-
9b	>10	>10	7.6 ± 1.7	1.9 ± 0.4	>5.26
9c	>10	>10	6.3 ± 2.6	1.6 ± 0.7	>6.25
9d	>10	>10	>10	>10	-
9e	>10	>10	3.2 ± 1.2	0.8 ± 0.3	>12.5
9f	>10	>10	2.4 ± 1.3	0.6 ± 0.3	>16.7
9g	>10	>10	2.8 ± 0.7	0.7 ± 0.2	>14
9h	>10	>10	2.2 ± 0.3	0.5 ± 0.07	>20
9i	>10	>10	5.9 ± 1.0	1.5 ± 0.2	>6.7
9j	>10	>10	3.6 ± 0.3	0.9 ± 0.2	>11
16k	8.4 ± 0.7	5.1 ± 0.4	2.9 ± 1.2	0.7 ± 0.3	7.28
16l	1.0 ± 0.2	0.6 ± 0.1	4.3 ± 2.3	1.0 ± 0.5	0.6
16m	0.8 ± 0.03	0.5 ± 0.01	1.3 ± 0.2	0.3 ± 0.06	1.7
16n	1.2 ± 0.00	0.7 ± 0.00	2.0 ± 1.1	0.5 ± 0.2	1.4
17k	>10	>10	6.6 ± 1.3	1.6 ± 0.3	>6.2
17l	>10	>10	1.2 ± 0.5	0.3 ± 0.2	>33
17m	>10	>10	1.2 ± 0.5	0.3 ± 0.1	>33
17n	7.6 ± 0.4	4.6 ± 0.2	0.7 ± 0.4	0.1 ± 0.1	46
19	>10	>10	1.1 ± 0.2	0.2 ± 0.06	>50
20	4.5 ± 0.2	2.7 ± 0.1	4.1 ± 2.3	1.0 ± 0.6	2.7
24	8.4 ± 0.2	5.1 ± 0.1	1.4 ± 0.4	0.3 ± 0.1	17
25	1.1 ± 0.3	0.7 ± 0.1	0.4 ± 0.1	0.1 ± 0.04	7.0

[a] Data are expressed as means ± SEM of n = 3 independent experiments. Affinity was calculated by using [^{3}H]CP-55,940 as radioligand on human CB1 and CB2 cannabinoid receptors. K_i and IC_{50} are expressed in μM.

2.4. Binding assays and CB2 molecular docking

Heterocyclic derivatives **7i'**, **9i**, **24** and **25** substituted with two aromatic rings presented high selectivity index values for CB2 receptor. Moreover, compounds **8i**, **24** and **25** also showed affinity for CB1 receptor according to a possible interaction with CB1 amino acids Lys 192 and Phe 174. These residues and the 1-naphthoyl moiety could play a significant role in the loss of CB2 receptor affinity. It is worth mentioning that in CB2 docking assays all these derivatives exhibited a ∏-cation interaction between Lys 109 and the 4-methoxyphenyl rings, except for compound **9i** that displayed a different orientation, interacting with Lys 109 through the benzimidazole ring.

Molecular docking results suggested that benzimidazoles **8a-e** and **8g** (Fig. 4A) interact primarily with hydrophobic amino acids. Furthermore, a ∏-cation interaction between Lys 109 and the 1-naphthoyl moieties was observed for each derivative. Compounds **8f**, **8h** and **8j** showed different spatial arrangements relative to compounds **8a-e** and **8g** (Fig. 4B), but all of them reveal similar orientations where a ∏-cation interaction with Lys 109 is still possible through their benzimidazole rings. Derivative **8j** orientates the carbonyl group towards the carboxylate group of Glu 181 (2.62 Å), which could be related to its higher Ki value (Ki CB2 = 1.4 μM, Table 1). Compounds **8a** and **8b** did not bind to the CB2 receptor, suggesting that short aliphatic chains do not efficiently interact with hydrophobic amino acids, hence lengthening the alkyl chain is an important requirement.

Complexes formed by derivatives **9a-e, 9g** and **9h** could stabilise by means of a ∏-cation interaction between Lys 109 and the benzimidazole rings. As also observed in CB1 docking assays, benzimidazole **9f** exhibited a different orientation directing the *t*-butyl group towards hydrophobic amino acids. Despite this different orientation of **9f** in the CB2 binding site, a ∏-cation interaction could still occur between Lys 109 and the 4-methoxybenzoyl framework.

Benzothiophenes **17k-n** (Fig. 4C), like benzimidazoles **9a-e, 9g** and **9h** (Fig. 4D), showed high selectivity indexes for CB2 receptor. The docking poses obtained for these derivatives favoured two main interactions: a ∏-cation between Lys 109 and the benzothiophene rings, and a hydrogen bond interaction between carbonyl groups and Thr 114. Therefore, these two interactions may be contributing to the CB2 binding selectivity.

Benzothiophenes **16k-n** revealed a similar alignment in docking assays; nonetheless, no ∏-cation interaction with Lys 109 was observed for these derivatives. These results suggest that Lys 109 is an important amino acid for CB2 selectivity; in fact, these derivatives showed the lowest selectivity indexes among the tested compounds, in agreement with the selectivity loss of benzothiophenes substituted at position 3- with a 1-naphthoyl group and aliphatic chains at position 2-, as we already discussed above. It is also possible to see this behaviour in compounds **19** and **20**, which do not have aliphatic chains in their structures. Compound **20** are arranged in the same manner that compounds **16k-n**, thus being unable to interact with Lys 109 loses selectivity for CB2 receptors (K_iCB1/K_iCB2 = 2.7). On the other hand, compound **19** adopted a different orientation interacting with Lys 109 and Thr 114 through a ∏-cation and a hydrogen bond interaction respectively (like compounds **17k-n**) and exhibited a high selectivity index value,

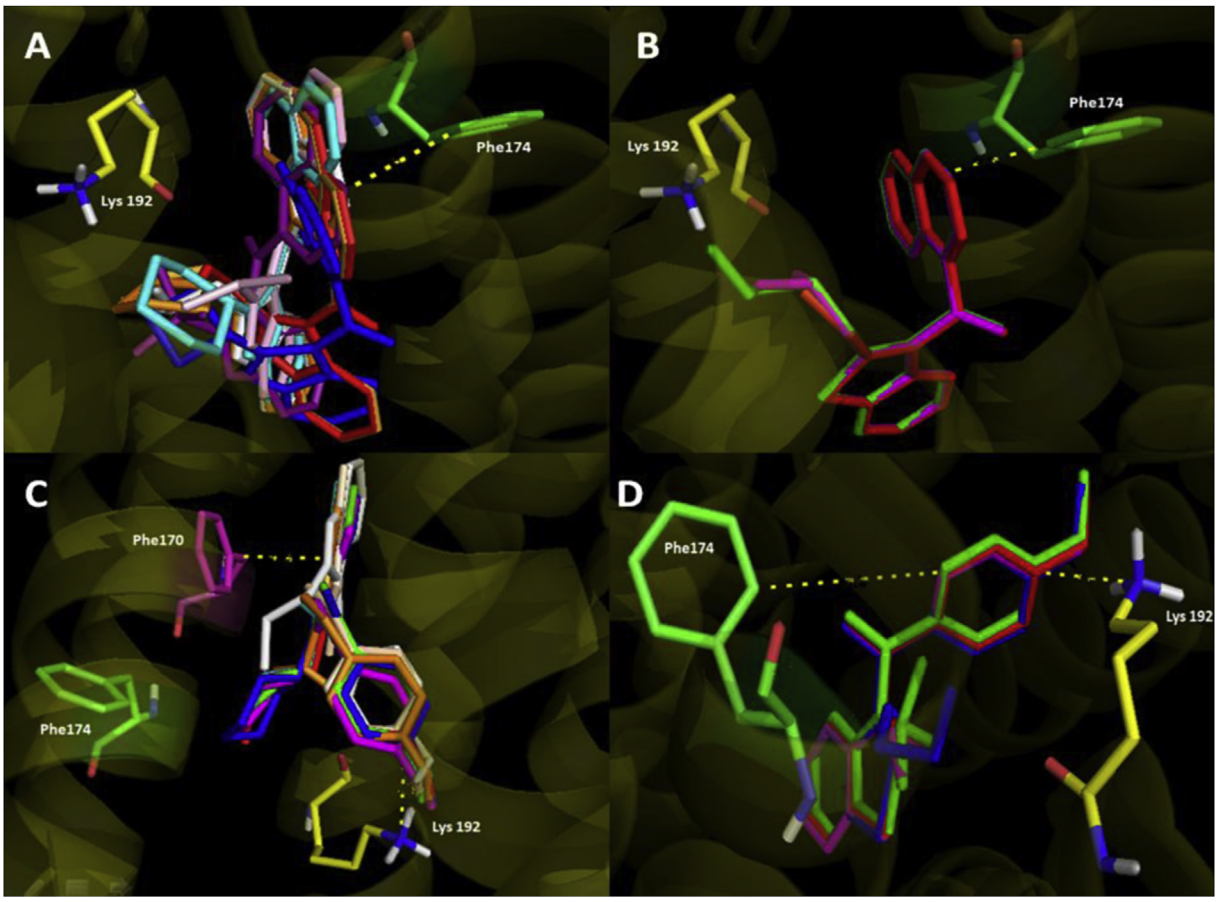


Fig. 3. Predicted binding mode and predicted intermolecular interactions with the CB1 receptor for **A. 8b-h, 8j** (T-shaped); **B. 16k-n** (T-shaped); **C. 9a-e, 9g-h, 9j** (π-cation with Lys192; π-π with Phe170) and **D. 17k-n** (π-cation with Lys192).

highlighting the role of these amino acid residues.

The alignment of the primary amino acid sequences of wild-type CB1 and CB2 cannabinoid receptors indicates a conserved lysine residue in the third transmembrane domain that corresponds to Lys 192 in the CB1 receptor and Lys 109 in the CB2 receptor. Therefore, it seems that both residues have a key role in the binding of some cannabinoid ligands and this was examined by testing the synthetic series of this work.

Lys 109 was shown to have little effect on known agonist binding or signalling of CB2 receptor [66], even though this particular lysine is crucial for the binding of HU-210 and CP-55,940 but not WIN-55,212-2 to the CB1 receptor [67]. A double mutation, Lys 109 to Ala, and Ser 112 to Gly (K3.28AS3.31G), resulted in the complete loss of affinity for other studied agonists, and for WIN-55,212-2 and JWH-015 as expected. However, downstream signalling by WIN-55,212-2 was drastically reduced, suggesting an improper coupling of this mutant [68].

2.5. Cell viability assays

Given the relationship between CB1 and CB2 agonists and cell death, the *in vitro* toxicity and antitumor efficacy of the synthesised compounds in several neoplastic cell lines were evaluated. All molecules were tested at different concentrations (0.001 μM–10 μM) and EC_{50} values were determined (Table 2). Etoposide and the well-known canabinoid agonist WIN 55,212-2 were used as positive control compounds. VERO cells were used as a non-neoplastic cells model.

Table 2 shows that all compounds were poorly cytotoxic on HeLa, H1975 and VERO cell lines. These results are in agreement with a low expression of CB2 receptors in lung, cervix and kidney tissues. However, in colon cancer cells (HCT116), where the CB2 receptor is highly expressed, some compounds showed a cytotoxic profile. Acute Promyelocytic Leukaemia cells (HL-60) exclusively express CB2 receptors, thus they were used to evaluate the cytotoxic effects of the synthesised compounds and the possibility of a CB2-mediated agonist-induced cell death mechanism [69,70]. Table 2 shows cytotoxicity values for synthesised compounds, focusing our analysis to pharmacologically attractive derivatives in terms of affinity.

WIN 55,212-2 showed a low EC_{50} value for HL-60 compared with other cell lines. Likewise, all compounds were cytotoxic on HL60 cells with EC_{50} values lower than the Ki obtained by CB2 binding assays, suggesting that these compounds may act as potent agonists at the CB2 receptor and could therefore be considered new, specific and promising derivatives for the treatment of cancer caused by Human Acute Promyelocytic Leukaemia cells. Moreover compared to WIN 55,212-2, our synthesised compounds displayed a greater selectivity index among HL-60 cells and the other cell lines.

Derivatives **8a**, **8b**, **9a**, and **9d** showed a Ki value > 10 μM on CB2 receptors. Moreover, cytotoxic potencies displayed by these four derivatives on HL-60 cells do not showed a linear correlation between CB2 affinity (K_i) and the EC_{50} on HL-60 cells, thus they were regarded as outliers. Cytotoxic activities of compounds **8a**, **8b**, **9a**, and **9d** could suggest a probable underlying SAR of the dataset. A

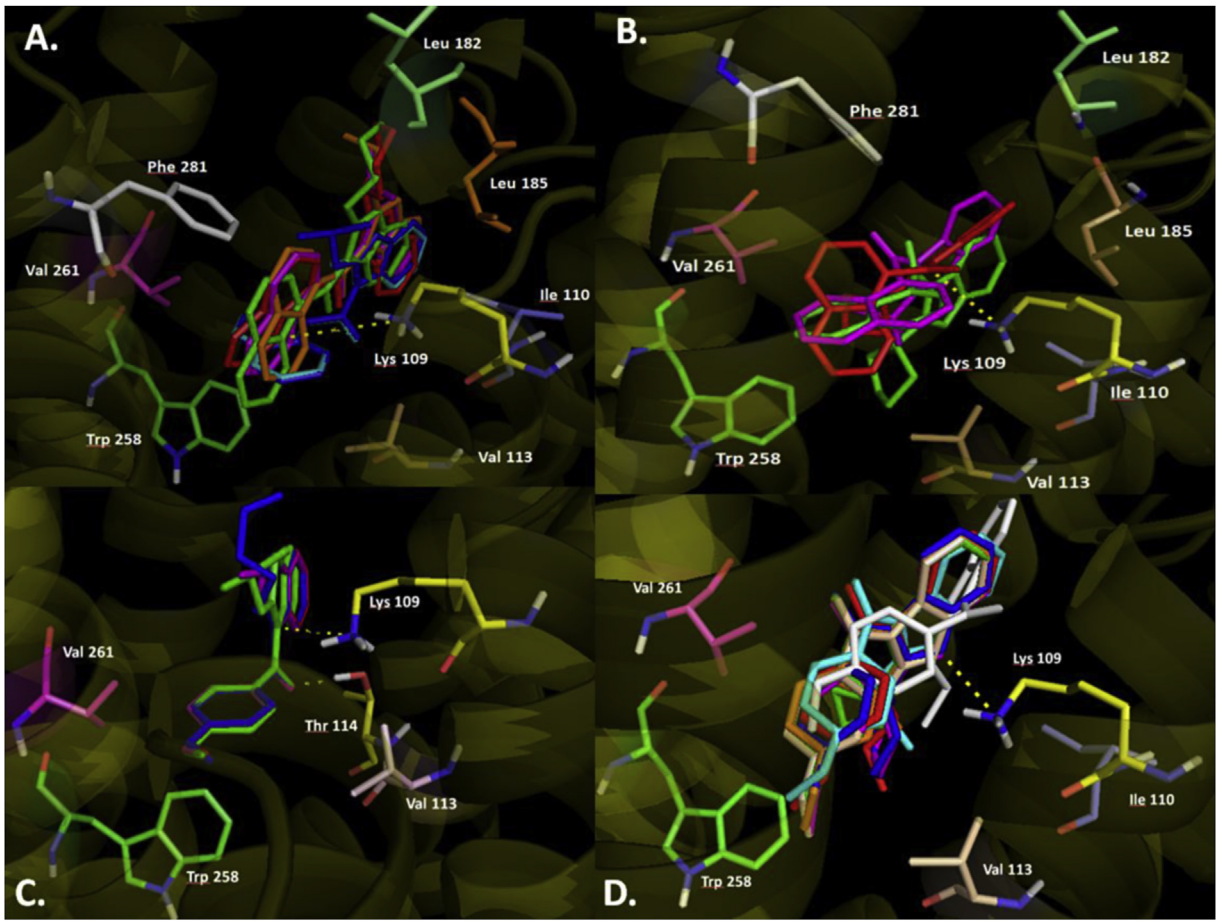


Fig. 4. Predicted binding mode and predicted intermolecular interactions with the CB2 receptor for **A. 8a-e** and **8g** (π-cation with Lys109); **B. 8f, 8h** and **8j** (π-cation with Lys109); **C. 17k-n** (π-cation with Lys109 and H-bonding with Thr114) and **D. 9a-e, 9g** and **9h** (π-cation with Lys109).

chemical interpretation of this SAR discontinuity is not provided on this work and could contribute to our ongoing efforts focused on cancer and cannabinoid agonists.

All compounds showed selective binding to CB2 receptor with K_i values in a low micromolar range and with a high selectivity index, except **8i**, **9b**, **16l**, **16m**, **16n** and **20**, which displayed high affinity for both CB receptors. As shown in Table 2, all derivatives were cytotoxic to HL-60 with similar EC_{50} values. Benzimidazoles and benzothiophenes, that contained the 4-methoxybenzoyl framework in their structures, presented slightly lower EC_{50} values in cell viability assays and showed low K_i values as well. It is worth noting that differences in K_i binding values and cytotoxic EC_{50} values were not significant for all tested compounds, with all of them being in the same concentration range. Interestingly, we found a linear correlation between the effect on cell viability and CB2 binding affinity of synthesized compounds, which were grouped into six different families according to their structural characteristics; whether they possess a benzimidazole or benzothiophene core, and considering their substituents at positions 2 and 3. Fig. 5 shows a clear tendency, where the families of compounds with higher affinity for CB2 receptor were also the most cytotoxic for HL-60 neoplastic cells. This might be attributed to the agonist activity of the compounds.

Compound **8f**, with the highest CB2 affinity and a high selectivity index, exhibited a low toxicity profile for all cell lines except HL-60. In view of this result, **8f** may represent a promising hit compound. Further experiments directed to assess the Absorption, Distribution, Metabolism and Excretion (ADME) and other drug-like properties are being performed based on the structure of this compound.

2.6. Structure activity relationship study

As can be seen from Table 1 compound **8f** had the best binding profile, displaying the lowest Ki value for CB2 receptor. Furthermore, **8f** showed to be toxic for HL-60 suggesting that it could behave as an agonist for this receptor. In order to assess the structural features related to affinity and selectivity for CB2 receptors, a Structure-Activity Relationship (SAR) analysis was conducted.

All derivatives were analysed in order to find common patterns and the specific requirements that could give good affinity and selectivity for CB2 receptors.

Our study indicates that both heterocyclic derivatives are mostly selective for CB2 receptors, being benzimidazoles more selective than benzothiophenes. Chemical substituents at position 2- of benzimidazoles and benzothiophenes can be interesting to explore. For example, compound **25** was one of the 6 compounds with the highest binding profile and carried an electronegative substitution at this position, which is in agreement with a ∏-cation interaction with Lys 109. On the other hand, the three compounds with the lower binding affinities (**9b**, **9c** and **17k**) did not have electron withdrawing or strong electron donating substituents at position 2- of their benzimidazole or benzothiophene rings, suggesting that

Table 2
Antiproliferative activity of compounds against different cell lines.[a]

Comp.	IC_{50} (μM)				
	HeLa	H1975-1	HTC116	VERO	HL-60
7i'	3.06	>10	1.75	>10	0.057
8a	>10	>10	>10	>10	0.093
8b	>10	>10	2.40	>10	0.100
8c	>10	>10	0.56	4.10	0.105
8d	>10	>10	2.17	>10	0.094
8e	>10	>10	4.41	7.23	0.070
8f	>10	>10	>10	>10	0.130
8g	>10	>10	3.02	>10	0.220
8h	>10	>10	>10	>10	0.106
8i	>10	>10	1.70	>10	0.146
8j	>10	>10	>10	>10	0.180
9a	>10	>10	0.01	1.23	0.057
9b	>10	>10	0.12	0.30	0.102
9c	>10	>10	>10	0.17	0.061
9d	>10	>10	3.56	>10	0.034
9e	>10	>10	0.19	>10	0.630
9f	>10	>10	0.02	>10	0.055
9g	>10	>10	>10	1.64	0.085
9h	>10	>10	>10	>10	0.076
9i	>10	>10	>10	>10	0.175
9j	>10	>10	>10	>10	0.047
16k	>10	>10	>10	>10	0.099
16l	>10	>10	>10	>10	0.130
16m	>10	>10	>10	>10	0.170
16n	>10	>10	>10	>10	0.072
17k	>10	>10	>10	>10	0.046
17l	>10	>10	>10	>10	0.080
17m	>10	>10	>10	>10	0.083
17n	>10	>10	>10	>10	0.066
19	>10	>10	>10	>10	0.058
20	>10	>10	>10	>10	0.051
24	>10	>10	>10	>10	0.071
25	>10	>10	>10	>10	0.076
WIN 55,212-2	0.98	3.8	0.92	0.31	0.007
Etoposide	8.2	9.5	2.8	1.5s	2.1

[a] HeLa, cervical cancer cells; H1975-1, non-small lung cancer cells; HCT116R, human colon carcinoma cells; VERO, kidney epithelial non-neoplastic cells; HL60 Human acute promyelocytic leukaemia cells. Data are expressed as means ± SEM of n = 3 independent experiments.

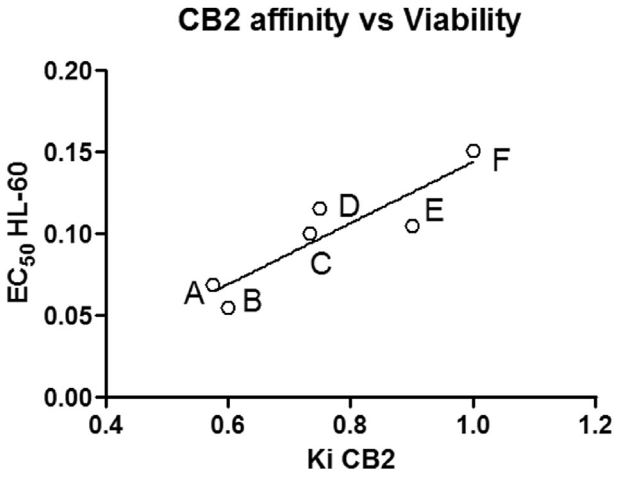


Fig. 5. Each point represents the mean values of Ki CB2 and EC_{50} in HL-60 for each family of synthesised compounds. **A**: **17k-n**; **B**: **19-20**; **C**:**16k,l,n**; **D**: **8c,e,h,j**; **E**: **7i',8i,9i,24-25**; **F**: **9b,c,e-h,j**.

electron rich groups at this position could play a key role in CB2 binding affinity.

It is unclear if the size, presence or absence of the chemical substituent at position 2- is important for affinity, since almost all compounds showed binding affinity for CB2 receptors except compounds **8a**, **8b** and **9a** which bear short aliphatic chains. Derivative **9d** with an isobutyl substituent did not show CB affinity either, while compound **19** lacking a substituent at position 2- displayed a low Ki value, suggesting that a chemical group at this position could be important but not essential for CB2 affinity. As was already mentioned above, the lack of CB2 binding affinity observed in compounds **8a**, **8b** and **9a** could be explained by the presence of short aliphatic chains that provide low lipophilicity, which is in agreement with docking experiments showing that short aliphatic chains were not able to establish efficient interactions with hydrophobic amino acids in the CB2 binding cavity. Therefore given the above, bulky aliphatic substituents would be better suited than large unbranched chains for CB2 affinity.

In general, compounds bearing the 4-methoxybenzoyl moieties such as compounds **9c**, **9i** and **17k** showed low binding affinity (unlike compound **8i**), whereas those with the 1-naphthoyl framework presented a better binding affinity profiles. It is possible that the length of the 1-naphthoyl moiety, rather than its volume, was crucial. That is, increasing the distance of a substituent from the heterocyclic framework could be detrimental for binding affinity. Moreover, in order to obtain new compounds with increased binding affinity, substitution of 4-methoxybenzoyl or 1-naphthoyl frameworks with chemical functions such as −OH, $-NH_2$ or −COOH would be advisable. Based on docking results, these functional groups may interact with the hydrophilic amino acid Ser 285 in compounds **7i'**, **8a-j, 9i 17k-n**, **24** and **25**, or with Tyr 190 in compounds **9a-h, 9j**, **16k-n**, **19** and **20**. Finally, Fig. 6 summarises the Structure-Activity Relationship derived from this work.

3. Conclusions

We developed a series of novel CB2 receptor ligands with high selectivity indexes based on two main heterocyclic rings; benzo[*d*] imidazole and benzo[*b*]thiophene emulating the known *amino-alkylindole* cannabinoid ligands through an isosteric replacement of the indole ring. The importance of a hydrogen bond interaction with Lys 192 in CB1 receptor was examined. Compounds bearing aliphatic chains at position 2- of the heterocyclic rings were synthesised to eliminate hydrogen bond acceptors, inhibiting the ability to interact with Lys 192 through a hydrogen bond interaction. On the other hand, compounds with a 4-methoxyphenyl at position 2- capable of interacting through a hydrogen bond with Lys 192, showed CB1 affinity, supporting the key role of this amino acid in the binding of ligands to CB1 receptors. Moreover, all compounds with high selectivity indexes for CB2 receptors showed a ∏-cation interaction with Lys 109, being this conserved residue crucial for CB2 binding affinity. Additionally, compound **8f** showed the best binding profile with the lowest Ki value for CB2, suggesting that substituents in compound **8f**, that is a bulky aliphatic chain at position 2- and a bulky aromatic ring with the correct spacer at *N*1, favour CB2 affinity.

Cell viability experiments determined low toxicity of our compounds in four out of five different cell lines. However, compounds with the 4-methoxybenzoyl moiety in their structure were shown to be the most toxic derivatives. Furthermore, in the HL-60 cell line, which expresses mostly CB2 receptors, all of the synthesised compounds were cytotoxic, suggesting that an agonist-mediated mechanism at CB2 receptors may be involved. We are planning to further direct our research towards the development of functional assays of these compounds, in order to conclusively establish their agonist profile. The results obtained in this study are of great relevance and could lead to the design, development and discovery of even more selective, potent and nontoxic CB2 ligands.

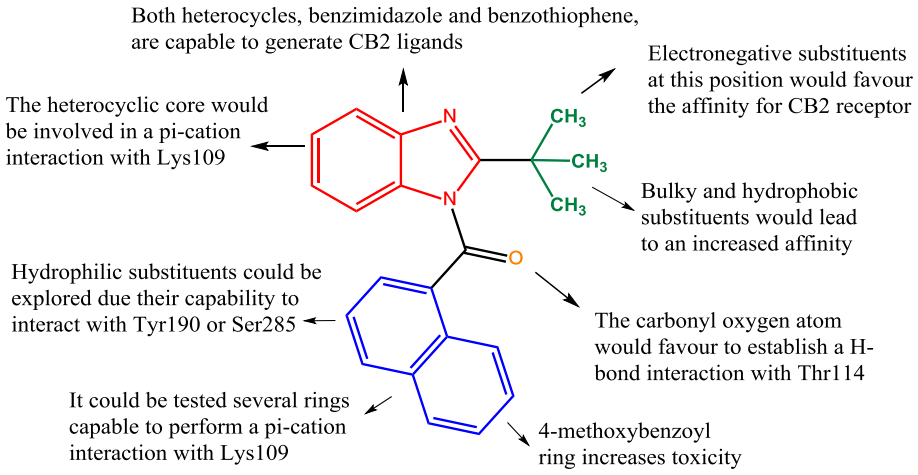


Fig. 6. SAR derived from this work, where hit compound **8f** is represented.

4. Experimental

4.1. Chemistry

4.1.1. Materials

All starting materials and solvents were purchased from commercial suppliers and used without further purification. Solvents were dried by reflux over sodium overnight and freshly distilled before use. Reactions over nitrogen atmosphere were carried out filling the reaction apparatuses through a gas flow of the corresponding, commercially available gas and afterwards closing the filled reaction system with a gas filled balloon. Thin layer chromatography (TLC) was performed on silica gel on aluminum foils with fluorescent indicator at 254 nm. For column chromatography silica gel 60 (particle size: 0.063–0.200 mm or 0.035–0.070 mm) was used. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. Infrared spectra were recorded on a Bruker Vector 22 spectrophotometer using KBr discs. Nuclear magnetic resonance spectra were recorded on a Bruker AM-400 instrument using $CDCl_3$ or DMSO-d_6 solutions containing tetramethylsilane as internal standard. Chemical shifts are expressed in parts per million (ppm) downfield from TMS, J values are given in Hertz for solutions in $CDCl_3$ or DMSO-d_6 unless otherwise indicated; multiplicity are abbreviated as: s: singlet; d: doublet; t: triplet; q: quartet; p: quintet; m: multiplet; dd: doublet of doublet; br: broad; and so on. The purity of compounds was determined by TLC and elemental analysis carried out on a FISONS EA 1108 CHNS-O analyzer.

4.1.2. General procedure for the synthesis of 2-alkyl(or -aryl)-1H-benzo[d]imidazole (**7a-h**)

To a solution of 1,2-phenylenediamine **1** (560 mg, 5.2 mmol) in CH_3CN (100 mL) in a reaction flask under aerobic conditions and magnetic stirring the corresponding aldehydes **2a-j** (0.3 mL, 5.2 mmol) were slowly added dropwise. After addition, the reaction was stirred at room temperature for 24 h after which the CH_3CN was removed in a rotary evaporator in vacuo. The solid residue was purified by column chromatography on silica gel with a mixture of CH_2Cl_2:ethyl acetate (2:1) as eluent to yield compounds **7a-h**.

*4.1.2.1. 2-Methyl-1H-benzo[d]imidazole (**7a**).* White solid. Yield = 46%. mp: 170.8–172.4 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 2.66 (s, 3H), 7.22 (dd, J_1 = 8,0 Hz, J_2 = 3,2 Hz, 2H), 7.53 (dd, J_1 = 8,0 Hz, J_2 = 3,2 Hz, 2H), 10.15 (br, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 15,03, 114,59 (2C), 122,25 (2C), 138,81 (2C), 151,64. IR (KBr) cm^{-1}: 3385.5, 1460.4, 744.9. Anal calcd for $C_8H_8N_2$ (m.wt. = 132.16): C = 72.70, H = 6.10, N = 21.20. Found: C = 72.62, H = 6.25, N = 21.08.

*4.1.2.2. 2-Propyl-1H-benzo[d]imidazole (**7b**).* White solid. Yield = 31%.mp: 152.3–154.9 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.12 (t, J_1 = 7.3 Hz, 3H), 2.05 (sextuplet, J_1 = 7.32 Hz, 2H), 3.11 (t, J_1 = 7.3 Hz, 2H), 7.36 (dd, J_1 = 8.0 Hz J_2 = 3.2 Hz, 2H), 7.71 (dd, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.94, 21.87, 31.36, 114.71 (2C), 122.17 (2C), 138.77 (2C), 155.71. IR (KBr) cm^{-1}: 3449.8, 1420.9, 749.0. Anal calcd for $C_{10}H_{12}N_2$ (m.wt. 160.22 g/mol): C = 74.97, H = 7.55, N = 17.48. Found: C = 75.19, H = 7.62, N = 17.14.

*4.1.2.3. 2-Butyl-1H-benzo[d]imidazole (**7c**).* White solid. Yield = 61%. mp: 150.9–15.4. °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.91 (t, J_1 = 7.7 Hz, 3H), 1.42 (sextuplet, J_1 = 7.7 Hz, 2H), 1.88 (p, J_1 = 7.7 Hz, 2H), 2.99 (t, J_1 = 7.7 Hz, 2H), 7.24 (dd, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H), 7.58 (q, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H), 10.35 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.85, 22.56, 29.20, 30.45, 114.74 (2C), 122.28 (2C), 138.62 (2C), 155.37. IR (KBr) cm^{-1}: 3438.6, 1419.8, 750.7. Anal calcd for $C_{11}H_{14}N_2$ (m.wt. = 174.24 g/mol): C = 75.82, H = 8.10, N = 16.08. Found: C = 75.30, H = 8.35, N = 15.82.

*4.1.2.4. 2-Isobutyl-1H-benzo[d]imidazole (**7d**).* White solid. Yield = 56%. mp: 190.3–191.2 °C ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.01 (d, J_1 = 6.6 Hz, 6H), 2.35–2.23 (m, 1H), 2.86 (d, J_1 = 7.3 Hz, 2H), 7.25 (dd, J_1 = 8.0 Hz, J_2 = 3.1 Hz, 2H), 7.59 (dd, J_1 = 8.0 Hz, J_2 = 3.1 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 22.64, 28.70, 38.60, 114.76 (2C), 122.18 (2C), 138.72 (2C), 154.84. IR (KBr) cm^{-1}: 3450.0, 1425.3, 747.4. Anal calcd for $C_{11}H_{14}N_2$ (m.wt. = 174.24 g/mol): C = 75.82, H = 8.10, N = 16.08. Found: C = 75.44, H = 8.42, N = 15.97.

*4.1.2.5. 2-Isopropyl-1H-benzo[d]imidazole (**7e**).* White solid. Yield = 77%. mp: 234.7–236.3 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.48 (d, J_1 = 7.0 Hz, 6H), 3.32 (heptuplet, J_1 = 7.0 Hz, 1H), 7.20 (dd, J = 8.0 Hz, J_2 = 3.2 Hz, 2H), 7.55 (dd, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H), 9.40 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 21.78 (2C), 29.23, 114.80 (2C), 122.25 (2C), 138.41 (2C), 160.41. IR (KBr) cm^{-1}: 3442.6, 1415.0, 743.4. Anal calcd for $C_{10}H_{12}N_2$ (m.wt. = 160.22 g/mol): C = 74.97, H = 7.55, N = 17.48. Found: C = 74.66, H = 7.78, N = 17.41.

*4.1.2.6. 2-(tert-butyl)-1H-benzo[d]imidazole (**7f**).* White solid.

Yield = 36%. mp: 289.9–291.5 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.49 (s, 9H), 7.15 (dd, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H), 7.56 (dd, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 29.40 (3C), 33.32, 114.96 (2C), 121.31 (2C), 130.85 (2C), 162.63.IR (KBr) cm^{-1}: 3423.8, 1408.8, 748.6. Anal calcd for $C_{11}H_{14}N_2$ (m.wt. = 174.24 g/mol): C = 75.82, H = 8.10, N = 16.08, Found: C = 74.84, H = 8.22, N = 15.93.

*4.1.2.7. 2-Cyclohexyl-1H-benzo[d]imidazole (**7g**).* White solid. Yield = 74%. mp: 285.3–286.5 °C. ^{1}H RMN (400 MHz, DMSO-d_6) δ ppm: 1.27 (qt, J_1 = 12.0 Hz, J_2 = 3.2 Hz, 1H), 1.38 (qt, J_1 = 12.4 Hz, J_2 = 3.0 Hz, 2H), 1.60 (qd, J_1 = 12.2 Hz, J_2 = 2.8 Hz, 2H), 1.70 (dt, J_1 = 12.2 Hz, J_2 = 3.2 Hz, 1H), 1.79 (dt, J_1 = 12.8 Hz, J_2 = 3.3 Hz, 2H), 2.01 (dd, J_1 = 13.1 Hz, J_2 = 2.2 Hz, 2H), 2.83 (tt, J_1 = 11.5 Hz, J_2 = 3.5 Hz, 1H), 7.09 (m, 2H), 7.39 (d, J_1 = 7.2 Hz, 1H), 7.51 (d, J_1 = 7.2 Hz, 1H), 12.10 (s, 1H). ^{13}C RMN (400 MHz, DMSO-d_6) δ ppm: 25.53, 25.59 (2C), 31.26 (2C), 37.71, 110.73, 118.20, 120.70, 121.32, 134.19, 143.09, 158.89. IR (KBr) cm^{-1}: 3448.5, 1421.4, 736.7. Anal calcd for $C_{13}H_{16}N_2$ (m.wt. = 200.28 g/mol): C = 77.96, H = 8.05, N = 13.99. Found: C = 78.17, H = 8.36, N = 14.21.

*4.1.2.8. 2-Cyclopentyl-1H-benzo[d]imidazole (**7h**).* White solid. Yield = 67%. mp: 259.6–261.9 °C. ^{1}H RMN (400 MHZ, $CDCl_3$) δ: 1.59–1.55 (m, 2H), 1.71–1.65 (m, 2H), 1.85–1.80 (m, 2H), 2.02–1.97 (m, 2H), 3.16 (p, J_1 = 8.4 Hz, 1H), 6.99 (dd, J_1 = 8.0 Hz, J_2 = 3.1 Hz, 2H), 7.36 (dd, J_1 = 8.0 Hz J_2 = 3.1 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 25.30 (4C), 31.98 (C), 121.12 (4C), 158.82 (3C). IR (KBr) cm^{-1}: 3450.1, 1421.8, 744.7. Anal calcd for $C_{12}H_{14}N_2$ (m.wt. = 186.25 g/mol): C = 77.38 H = 7.58, N = 15.04. Found: C = 76.77, H = 7.85, N = 15.41.

*4.1.2.9. 2-(4-methoxyphenyl)-1H-benzo[d]imidazole (**7i**).* Light red solid. Yield = 88%. mp: 194.6–196.0 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 3.78 (s, 3H), 6.90 (d, J_1 = 8.4 Hz, 2H), 7.12 (m, 2H), 7.54 (m, 2H), 8.06 (d, J_1 = 8.4 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 55.27, 114.13 (2C), 114.34 (2C), 122.01 (2C), 122.94, 127.16 (2C), 128.33 (2C), 152.08, 160.90. IR (KBr) cm^{-1}: 3448.5, 1437.2, 1252.0, 736.3. Anal calcd for $C_{14}H_{12}N_2O$ (m.wt. = 224.26 g/mol): C = 74.98, H = 5.39, N = 12.49. Found: C = 74.55, H = 5.89, N = 12.14.

*4.1.2.10. 1-(4-methoxybenzyl)-2-(4-methoxyphenyl)-1H-benzo[d] imidazol (**7i'**).* Light brown solid. Yield = 12%. mp: 125.4–127.0 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 3.69 (s, 3H), 3.75 (s, 3H), 5.29 (s, 2H), 6.76 (d, J_1 = 8,8 Hz, 2H), 6.88 (d, J_1 = 8,4 Hz, 2H), 6.88 (d, J_1 = 8,4 Hz, 2H), 6.94 (d, J_1 = 8,4 Hz, 2H), 7.14 (d, J_1 = 7,6 Hz, 2H) 7,23-7,18 (m, 1H), 7.55 (d, J_1 = 8,8 Hz, 2H), 7,76 (d, J_1 = 8,0 Hz, 1H, Ha). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 47.98, 55.38, 55.46, 110.54, 114.30 (2C), 114.53 (2C), 119.78, 122.51, 122.64, 122.85, 127.32 (2C), 128.57, 130.80 (2C), 136.18, 143.21, 154.21, 159.23, 161.02. IR (KBr) cm^{-1}: 1460.8, 1245.2. Anal calcd for $C_2H_{20}N_2O_2$ (m.wt. = 344.41 g/mol): C = 76.72, H = 5.85, N = 8.13. Found: C = 76.56, H = 6.07, N = 8.27.

*4.1.3. N-(2-nitrophenyl)cyclobutanecarboxamide (**5**)*

To a solution of 2-nitroaniline **3** (318 mg, 1.7 mmol) and NaH (318 mg, 1.7 mmol) in dry THF (100 mL) in a reaction flask under anaerobic conditions (N_2) and magnetic stirring was slowly added dropwise cyclobutanecarbonyl chloride (0.2 mL, 1.7 mmol). After addition, the reaction was stirred at room temperature for 2 h after which the THF was removed in a rotary evaporator in vacuo. The solid residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to yield compound **5**.

Yellow solid. Yield = 95%. mp: 62.3–64.4 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.86 (m, 1H), 1.97 (sextuplet, J_1 = 9.4 Hz, 1H), 2.30–2.19 (m, 2H), 2.35 (p, J_1 = 9.8 Hz, 2H), 3.22 (p, J_1 = 8.5 Hz, 1H), 7.09 (t, J_1 = 7.8 Hz, 1H), 7.58 (t, J_1 = 7.8 Hz, 1H), 8.14 (d, J_1 = 8.5 Hz, 1H), 8.76 (d, J_1 = 8.5 Hz, 1H), 10.25 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 18.07, 25.47 (2C), 41.78, 122.22, 123.10, 125.87, 135.31, 136.14, 136.32, 174.33. IR (KBr) cm^{-1}: 3353.0, 1670.0, 1545.4, 1335.8. Anal calcd for $C_{11}H_{12}N_2O_3$ (m.wt = 220.22 g/mol): C = 59.99, H = 5.49, N = 12.72. Found: C = 59.99, H = 5.77, N = 12.14.

*4.1.4. N-(2-aminophenyl)cyclobutanecarboxamide (**6**)*

In a three-necked round-bottomed flask with a mechanical stirrer were placed reagent *N*-(2-nitrophenyl)cyclobutanecarboxamide (**5**) (364 mg. 1,65 mmol) and ethanol (100 mL). After warming to 50 °C 0.1 g of palladium charcoal (previously moistened with ethanol) was added. H_2NNH_2 (0.17 mL 5.7 mmol) was incorporated dropwise during 5 min. At this point an additional 0.1 g palladized charcoal was added too, and the mixture was heated until the ethanol refluxes gently. After 1 h the supernatant liquor was almost colorless. Palladized charcoal was removed by filtration using suction and celite. The filtrate was concentrated under reduced pressure in a rotary evaporator. Then the solid was resuspended in CH_2Cl_2 and washed with 50 mL. of H_2O (3 x 50 mL). Finally, $CaCl_2$ anhydrous was filtered and CH_2Cl_2 was removed in a rotary evaporator in vacuo yielding compound **6**.

Brown solid. Yield = 99%. mp: 145.0–147.7 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.87–1.73 (m, 1H), 1.93 (heptuplet, J_1 = 9.2 Hz, 1H), 2.16–2.04 (m, 2H), 2.29 (pd, J_1 = 9.4 Hz, J_2 = 2.0 Hz, 2H), 3.21 (p, J_1 = 8.4 Hz, 1H), 4.04 (br, 2H), 6.61 (t, J_1 = 7.6 Hz, 1H), 6.68 (d, J_1 = 7.9 Hz, 1H), 6.90 (t, J_1 = 7.6 Hz, 1H), 7.12 (d, J_1 = 7.9 Hz, 1H), 8.60 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 17.59, 24.74 (2C), 39.21, 116.61, 117.54, 123.70, 124.89, 125.73, 140.63, 173.27. IR (KBr) cm^{-1}: 3436.9, 3412.4, 3263.6, 1641.4. Anal calcd for $C_{11}H_{14}N_2O$ (m.wt. = 190.24 g/mol): C = 69.45, H = 7.42, N = 14.73. Found: C = 68.94, H = 7.80, N = 14.81.

*4.1.5. 2-Cyclobutyl-1H-benzo[d]imidazole (**7j**)*

The Brown solid *N*-(2-aminophenyl)cyclobutanecarboxamide (**6**) (350 mg. 1,84 mmol) was dissolved in glacial acetic acid (80 mL). The solution was heated at 65 °C for 2 h. Then, reaction was washed with a $NaHCO_3$ water solution until pH 7–8. The mixture was extracted with ethyl acetate (3 x 100 mL) and the organic portions were dried with anhydrous $NaHCO_3$. Finally $NaHCO_3$ was filtered and the solvent was removed in rotary evaporator in vacuo, yielding compound **7j**.

White solid. Yield = 90%. mp: 228.9–229.6 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.94–1.86 (m, 1H), 2.03 (sextuplet, J_1 = 9.2 Hz, 1H), 2.38–2.28 (m, 2H), 2.45 (pd J_1 = 9.2 Hz, J_2 = 2.0 Hz, 2H), 3.69 (p, J_1 = 8,0 Hz, 1H), 7.08 (dd, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H), 7.45 (dd, J_1 = 8.0 Hz, J_2 = 3.2 Hz, 2H), 11.70 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 18.28, 27.63 (2C), 33.87, 121.13 (4C), 157.96 (3C). IR (KBr) cm^{-1}: 3448.6, 1419.8, 750.1. Anal calcd for $C_{11}H_{12}N_2$ (m.wt = 172.23 g/mol). C = 76.71, H = 7.02, N = 16.27. Found: C = 77.03, H = 7.14, N = 16.53.

4.1.6. General procedure for the synthesis of (2-alkyl-1H-benzo[d] imidazol-1-yl)(naphthalene-1-yl)methanone 8a-j and synthesis of (4-methoxyphenyl)(2-methyl-1H-benzo[d]imidazol-1-yl) methanone 9a-j

To a solution of the corresponding 2-alkyl(or-aryl)-1*H*-benzo[*d*] imidazole (**7a-j**) (164 mg. 1,2 mmol) and triethylamine (1.17 mL 1.25 mmol) in dry THF (50 mL) under anaerobic conditions (N_2) and magnetic stirring was added dropwise 1-naphthoyl chloride or 4-methoxybenzoyl chloride. After addition, reaction was stirred at room temperature for 8 h. until a precipitate appeared (triethylammonium chloride), after which it was removed by filtration. The filtrate was concentrated under reduced pressure in a rotary evaporator. The residue was purified by thin layer chromatography with CH_2Cl_2 as eluent to yield target compounds **8a-j** and **9a-j**.

For the synthesis of compounds **8j** and **9f** were used NaH (1,2 mmol) and reflux for 8 h.

*4.1.6.1. (2-Methyl-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl) methanone (**8a**).* Yellow solid. Yield = 89%. mp: 91.1–93.2 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 2.47 (s, 3H), 6.68 (d, $J_1 = 8.0$ Hz, 1H), 6.91 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.12 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.39 (t, $J_1 = 7.8$ Hz, 1H), 7.46–7.42 (m, 2H), 7.51 (dd, $J_1 = 7.1$ Hz, $J_2 = 1.1$ Hz, 1H), 7.58 (d, $J_1 = 8.0$ Hz, 1H), 7.83 (dd, $J_1 = 7.2$ Hz, $J_2 = 1.6$ Hz, 1H), 7.93 (dd, $J_1 = 7.2$ Hz, $J_2 = 1.6$ Hz, 1H), 7.98 (d, $J_1 = 8.2$ Hz, 1H).^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 17.76, 113.91, 119.41, 124.15 (2C), 124.39, 124.83, 127.17, 128.03, 128.41, 128.79, 130.17, 131.61, 133.05, 133.47, 133.76, 142.40, 153.15, 168.34. IR (KBr) cm^{-1}: 1713.9, 1453.4. Anal calcd for $C_{19}H_{14}N_2O$ (m.wt = 286.33 g/mol): C = 79.70, H = 4.93, N = 9.78. Found: C = 79.32, H = 5.46, N = 9.95.

*4.1.6.2. Naphthalen-1-yl(2-propyl-1H-benzo[d]imidazol-1-yl)methanone (**8b**).* Yellow oil. Yield = 79%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.87 (t, $J_1 = 7.6$ Hz, 3H), 1.79 (sextuplet $J_1 = 7.6$ Hz, 2H), 2.92 (t, $J_1 = 7.6$ Hz, 2H), 6.45 (d, $J_1 = 8.0$ Hz, 1H), 6.86 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.12 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.39 (t, $J_1 = 7.6$ Hz, 1H), 7.49–7.45 (m, 2H), 7.51 (dd, $J_1 = 7.2$ Hz, $J_2 = 0.8$ Hz, 1H), 7.64 (d, $J_1 = 8.0$ Hz, 1H), 7.86 (dd, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H), 8.0 (d, $J_1 = 8.8$ Hz, 1H), 8.03 (d, $J_1 = 7.2$ Hz, 1H).^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.97, 21.48, 32.46, 113.70, 119.6, 124.01, 124.23, 124.54, 124.83, 127.22, 128.53, 128.62, 128.85, 130.37, 131.40, 133.43, 133.53, 133.87, 142.32, 157.18, 168.40. IR (KBr) cm^{-1}: 1702.1, 1536.8. Anal calcd for $C_{21}H_{18}N_2O$ (m.wt = 314.38 g/mol): C = 80.23, H = 5.77, N = 8.91. Found: C = 80.27, H = 6.04, N = 8.77.

*4.1.6.3. (2-Butyl-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl)methanone (**8c**).* Yellow oil. Yield = 75%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.77 (t, $J_1 = 7.6$ Hz, 2H), 0.77 (t, $J_1 = 7.6$ Hz, 2H), 1.25 (sextuplet, $J_1 = 7.6$ Hz, 2H), 1.73 (p, $J_1 = 7.6$ Hz, 2H), 2.91 (t, $J_1 = 7.8$ Hz, 2H), 6.49 (d, $J_1 = 8.0$ Hz, 1H), 6.86 (td, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H), 7.12 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.39 (t, $J_1 = 7.2$ Hz, 1H), 7.50–7.43 (m, 2H), 7.52 (dd, $J_1 = 7.2$ Hz, $J_2 = 0.8$ Hz, 1H), 7.62 (d, $J_1 = 8.0$ Hz, 1H), 7.85 (dd, $J_1 = 8.8$ Hz, $J_2 = 1.2$ Hz, 1H), 8.03–7.99 (m, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.72, 22.57, 30.08, 30.40, 113.72, 119.63, 123.95, 124.18, 124.55, 124.82, 127.20, 128.49, 128.53, 128.83, 130.37, 131.52, 133.34, 133.60, 133.87, 142.53, 157.30, 168.42. IR (KBr) cm^{-1}: 1707.4, 1535.8. Anal calcd for $C_{22}H_{20}N_2O$ (m.wt = 328.41 g/mol): C = 80.46, H = 6.14, N = 8.53. Found: C = 80.21, H = 6.67, N = 8.18.

*4.1.6.4. (2-Isobutyl-1H-benzo[d]imidazol-1-yl)(naphthalene-1-yl) methanone (**8d**).* Yellow oil. Yield = 84%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.84 (d, $J_1 = 6.4$ Hz, 6H), 2.16 (m, $J_1 = 6.4$ Hz, 1H), 2.79 (d, $J_1 = 6.4$ Hz, 2H), 6.45 (d, $J_1 = 8.0$ Hz, 1H), 6.83 (td, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.09 (td, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.36 (t, $J_1 = 7.6$ Hz, 1H), 7.47–7.41 (m, 2H), 7.48 (dd, $J_1 = 7.2$ Hz $J_2 = 1.1$ Hz, 1H), 7.61 (d, $J_1 = 8.0$ Hz, 1H), 7.83 (dd, $J_1 = 7.6$ Hz, $J_2 = 2.0$ Hz, 1H), 7.98 (d, $J_1 = 8.2$ Hz, 1H), 8.02 (dd, $J_1 = 7.6$ Hz, $J_2 = 1.2$ Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 22.50 (2C), 28.12, 39.17, 113.58, 119.65, 123.85, 124.07, 124.50, 124.75, 127.15, 128.47, 128.69, 128.78, 130.38, 131.34, 133.40, 133.61, 133.82, 142.46, 156.31, 168.41. IR (KBr) cm^{-1}: 1697.74, 1460.8. Anal calcd for $C_{22}H_{20}N_2O$ (m.wt = 328.41 g/mol): C = 80.46, H = 6.14, N = 8.53. Found: C = 80.80, H = 6.64, N = 8.39.

*4.1.6.5. (2-Isopropyl-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl) methanone (**8e**).* Yellow solid. Yield = 85%. mp: 131.4–133.3 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.37 (d, $J_1 = 6.8$ Hz, 6H), 3.54 (heptuplet, $J_1 = 6.8$ Hz, 1H), 6.31 (d, $J_1 = 8.0$ Hz, 1H), 6.80 (td, $J_1 = 8.0$ Hz, $J_2 = 0.4$ Hz, 1H), 7.08 (t, $J_1 = 7.6$ Hz, 1H), 7.37 (t, $J_1 = 8.0$ Hz, 1H), 7.48–7.45 (m, 2H), 7.50 (dd, $J_1 = 7.2$ Hz, $J_2 = 0.8$ Hz, 1H), 7.64 (d, $J_1 = 8.0$ Hz, 1H), 7.85 (dd, $J_1 = 7.6$ Hz, $J_2 = 2.0$ Hz, 1H), 7.99 (d, $J_1 = 8.2$ Hz, 1H), 8.10–8.07 (m, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 21.75 (2C), 28.77, 113.49, 119.79, 123.80, 123.99, 124.64, 124.81, 127.20, 128.54, 128.84, 128.93, 130.48, 131.38, 133.59, 133.71, 133.93, 142.46, 162.29, 168.47. IR (KBr) cm^{-1}: 1707.8, 1455.4. Anal calcd for $C_{21}H_{18}N_2O$ (m.wt = 314.38 g/mol): C = 80.23, H = 5.77, N = 8.91. Found: C = 80.37, H = 5.57, N = 8.92.

*4.1.6.6. (2-(tert-butyl)-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl) methanone (**8f**).* White solid. Yield = 25%. mp: 168.1–171.0 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.59 (s, 9H), 6.19 (d, $J_1 = 8.0$ Hz, 1H), 6.79 (t, $J_1 = 7.7$ Hz, 1H), 7.09 (t, $J_1 = 7.7$ Hz, 1H), 7.36 (t, $J_1 = 7.4$ Hz, 1H), 7.56 (t, $J_1 = 7.0$ Hz, 2H), 7.62 (t, $J_1 = 7.4$ Hz, 1H), 7.67 (d $J_1 = 8.0$ Hz, 1H), 7,92 (d, $J_1 = 8.2$ Hz, 1H), 8.06 (d, $J_1 = 8.2$ Hz, 1H), 8.56 (d, $J_1 = 8.2$ Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 29.83 (3C), 35.94, 112.66, 119.87, 123.37, 123.56, 124.92, 125.17, 127.35, 129.06, 129.10, 130.43, 131.21, 131.35, 134.28, 135.04, 135.27, 141.55, 163.83, 170.03. IR (KBr) cm^{-1}: 1711.54, 1455.6. Anal calcd for $C_{22}H_{20}N_2O$ (m.wt = 328.41 g/mol): C = 80.46, H = 6.14, N = 8.53. Found: C = 80.26, H = 6.14, N = 8.74.

*4.1.6.7. (2-Cyclohexyl-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl) methanone (**8g**).* Yellow solid. Yield = 66%. mp: 132.8–135.8 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.26–1.04 (m, 3H), 1.64–1.57 (m, 1H), 1.69 (td, $J_1 = 11.7$ Hz, $J_2 = 3.1$ Hz, 4H), 2.01 (d, $J_1 = 11.7$ Hz, 2H), 3.09 (tt, $J_1 = 11.4$ Hz, $J_2 = 3.1$ Hz, 1H), 6.46 (d, $J_1 = 8.4$ Hz, 1H), 6.86 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.12 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.51–7.48 (m, 2H), 7.42 (t, $J_1 = 8.0$ Hz, 1H), 7.54 (dd, $J_1 = 7.2$ Hz, $J_2 = 1.2$ Hz, 1H), 7.65 (d, $J_1 = 8.0$ Hz, 1H), 7.89 (dd. $J_1 = 7.0$ Hz, $J_2 = 2.8$ Hz, 1H), 8.04 (d, $J_1 = 8.0$ Hz, 1H), 8.09–8.07 (m, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 25.95, 26.29 (2C), 32.20 (2C), 38.42, 113.68, 119.81, 123.86, 124.07, 124.65, 124.87, 127.25, 128.57, 128.85, 128.88, 130.52, 131.56, 133.54, 133.60, 133.95, 142.62, 161.33, 168.66. IR (KBr) cm^{-1}: 1698.8, 1454.0. Anal calcd for $C_{24}H_{22}N_2O$ (m.wt = 354.44 g/mol): C = 81.33, H = 6.26, N = 7.90. Found: C = 81.48, H = 6.38, N = 8.31.

*4.1.6.8. (2-Cyclopentyl-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl) methanone (**8h**).* Yellow solid. Yield = 81%. mp: 114.2–115.8 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.59–1.49 (m, 2H), 1.82–1.73 (m, 2H), 2.03 (qd, $J_1 = 8.2$ Hz, $J_2 = 2.0$ Hz, 4H), 3.58 (p, $J_1 = 8.2$ Hz, 1H), 6.37 (d, $J_1 = 8.0$ Hz, 1H), 6.85 (td, $J_1 = 8.0$ Hz, $J_2 = 1.2$ Hz, 1H), 7.12 (td, $J_1 = 8.0$ Hz, $J_2 = 0.8$ Hz, 1H), 7.43 (t, $J_1 = 8.0$ Hz, 1H), 7.54–7.49 (m, 2H), 7.55 (dd, $J_1 = 7.2$ Hz, $J_2 = 0.8$ Hz, 1H), 7.64 (d, $J_1 = 8.0$ Hz, 1H), 7.89 (dd, $J_1 = 8.2$ Hz, $J_2 = 4.6$ Hz, 1H), 8.04 (d, $J_1 = 8.2$ Hz, 1H), 8.13–8.10 (m 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 25.84 (2C), 32.55 (2C), 39.71, 113.48, 119.75, 123.78, 124.04, 124.75, 124.88, 127.26, 128.60, 128.90, 129.08, 130.59, 131.51, 133.63, 133.94, 133.99, 142.53, 161.15, 168.62. IR (KBr) cm^{-1}: 1698.2, 1454.3. Anal calcd for $C_{23}H_{20}N_2O$ (m.wt = 340.42 g/mol): C = 81.15, H = 5.92, N = 8.23. Found: C = 80.68, H = 5.47, N = 8.36.

*4.1.6.9. (2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl)methanone (**8i**).* White solid. Yield = 23%. mp: 149.3–152.0 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 3.57 (s, 3H), 6.45 (d, $J_1 = 8.8$ Hz, 2H), 7.20 (t, $J_1 = 6.8$ Hz, 1H), 7.24 (t, $J_1 = 7.6$ Hz, 1H), 7.30 (d, $J_1 = 8.8$ Hz, 2H), 7.34 (t, $J_1 = 7.6$ Hz, 1H), 7.43 (d, $J_1 = 6.8$ Hz, 1H), 7.52–7.46 (m, 2H), 7.54 (d, $J_1 = 7.6$ Hz, 1H) 7.76 (d, $J_1 = 8.0$ Hz, 1H), 7.81–7.79 (m, 2H), 8.15 (d $J_1 = 8.4$ Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 55.36, 113.51 (2C), 113.88, 120.13, 123.04, 124.41, 124.72, 124.93, 126.97, 128.60, 128.75, 130.24, 130.49 (2C), 131.15, 131.18, 133.70. 133.85, 134.84, 142.87, 154.41, 160.64, 169.09. IR (KBr) cm^{-1}: 1707.9, 1465.0, 1256.2. Anal calcd for $C_{25}H_{18}N_2O_2$

(m.wt = 378.42 g/mol): C = 79.35, H = 4.79, N = 7.40. Found: C = 79.02, H = 4.61, N = 7.73.

*4.1.6.10. (2-Cyclobutyl-1H-benzo[d]imidazol-1-yl)(naphthalen-1-yl) methanone (**8j**).* Yellow oil. Yield = 87%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.93–1.78 (m, 2H), 2.26–2.14 (m, 2H), 2.50 (pd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 3.81 (p, J_1 = 8.5 Hz, 1H), 6.48 (d, J_1 = 8.0 Hz, 1H), 6.87 (td, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 7.13 (td, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 7.50–7.44 (m, 2H), 7.41 (t, J_1 = 8.0 Hz, 1H), 7.51 (dd, J_1 = 7.2 Hz, J_2 = 1.0 Hz, 1H), 7.66 (d, J_1 = 8.0 Hz, 1H), 7.88 (dd, J_1 = 7.2 Hz, J_2 = 2.8 Hz, 1H), 8.01 (d, J_1 = 7.6 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 18.32, 27.74 (2C), 35.26, 113.69, 119.77, 123.99, 124.19, 124.65, 124.84, 127.22, 128.49, 128.67, 128.85, 130.40, 131.52, 133.36, 133.76, 133.89, 142.69, 159.86, 168.18. IR (KBr) cm^{-1}: 1698.7, 1454.18. Anal calcd for $C_{22}H_{18}N_2O$ (m.wt = 326.39 g/mol): C = 80.96, H = 5.56, N = 8.58. Found: C = 80.80, H = 6.05, N = 8.75.

*4.1.6.11. (4-methoxyphenyl)(2-methyl-1H-benzo[d]imidazol-1-yl) methanone (**9a**).* Yellow oil. Yield = 74%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 2.62 (s, 3H), 3.82 (s, 3H), 6.82 (d, J_1 = 8.0 Hz, 1H), 6.90 (d, J_1 = 8.8 Hz, 2H), 7.02 (t, J_1 = 7.8 Hz, 1H), 7.16 (t, J_1 = 7.8 Hz, 1H), 7.61 (d, J_1 = 8.0 Hz, 1H), 7.67 (d, J_1 = 8.8 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 16.71, 55.70, 113.17, 114.36 (2C), 119.38, 123.47, 123.73, 125.19, 132.82 (2C), 134.11, 142.38, 153.15, 164.58, 167.85. IR (KBr) cm^{-1}: 1685.4, 1426.6, 1261.4. Anal calcd for $C_{16}H_{14}N_2O_2$ (m.wt = 266.29 g/mol): C = 72.16, H = 5.30, N = 10.52. Found: C = 72.33, H = 5.58, N = 10.91.

*4.1.6.12. (4-methoxyphenyl)(2-propyl-1H-benzo[d]imidazol-1-yl) methanone (**9b**).* White solid. Yield = 53%. mp: 60.7–62.9 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.91 (t, J_1 = 7.4 Hz, 3H), 1.78 (sextuplet, J_1 = 7.4 Hz, 2H), 2.96 (t, J_1 = 7.4 Hz, 2H), 3.81 (s, 3H), 6.74 (d, J_1 = 8.0 Hz, 1H), 6.88 (d, J_1 = 8.8 Hz, 2H), 6.99 (t, J_1 = 7.7 Hz, 1H), 7.15 (t, J_1 = 7.7 Hz, 1H), 7.63 (d, J_1 = 8.0 Hz, 1H), 7.66 (d, J_1 = 8.8 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.95, 21.46, 31.49, 55.70, 113.01, 114.39 (2C), 119.51, 123.36, 123.56, 125.20, 132.89 (2C), 134.16, 142.33, 156.88, 164.65, 167.95. IR (KBr) cm^{-1}: 1685.7, 1426.7, 1262.5. Anal calcd for $C_{18}H_{18}N_2O_2$ (m.wt = 294.35 g/mol): C = 73.45, H = 6.16, N = 9.52. Found: C = 73.76, H = 6.30, N = 9.30.

*4.1.6.13. (2-Butyl-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl) methanone (**9c**).* White solid. Yield = 45%. mp: 66.2–68.3 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.82 (t, J_1 = 7.6 Hz, 3H), 1.32 (sextuplet, J_1 = 7.6 Hz, 2H), 1.75 (p, J_1 = 7.6 Hz, 2H), 2.98 (t, J_1 = 7.6 Hz, 2H), 3.82 (s, 3H), 6.75 (d, J_1 = 8.0 Hz, 1H), 6.90 (d, J_1 = 8.8 Hz, 2H), 7.0 (td, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 7.16 (td, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 7.64 (d, J_1 = 8.0 Hz, 1H), 7.68 (d, J_1 = 8.8 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.81, 22.57, 29.39, 30.14, 55.74, 113.04, 114.52 (2C), 119.56, 123.38, 123.59, 125.28, 132.91 (2C), 134.20, 142.40, 157.15, 164.67, 168.01. IR (KBr) cm^{-1}: 1685.5, 1417.7, 1261.75. Anal calcd for $C_{19}H_{20}N_2O_2$ (m.wt = 308.37 g/mol): C = 74.00, H = 6.54, N = 9.08. Found: C = 74.36, H = 6.34, N = 9.01.

*4.1.6.14. (2-Isobutyl-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl) methanone (**9d**).* Yellow oil. Yield = 50%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.84 (d, J_1 = 7.2 Hz, 6H), 2.11 (m, J_1 = 7.2 Hz, 1H), 3.82 (s, 3H), 2.91 (d, J_1 = 7.2 Hz, 2H), 6.75 (d, J_1 = 8.0 Hz, 1H), 6.89 (d, J_1 = 8.8 Hz, 2H), 7.01 (td, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 7.17 (td, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H), 7.67 (d, J_1 = 8.8 Hz, 3H).^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 22.54 (2C), 28.38, 38.07, 55.75, 113.00, 114.47 (2C), 119.52, 123.47, 123.66, 125.15, 133.00 (2C), 134.15, 142.07, 156.27, 164.77, 168.02. IR (KBr) cm^{-1}: 1685.6, 1427.1, 1263.0. Anal calcd for $C_{19}H_{20}N_2O_2$ (m.wt = 308.37 g/mol): C = 74.00, H = 6.54, N = 9.08. Found: C = 73.82, H = 6.80, N = 8.98.

*4.1.6.15. (2-Isopropyl-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl) methanone (**9e**).* White solid. Yield = 43%. mp: 78.9–80.7 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.36 (d, J_1 = 6.8 Hz, 6H), 3.49 (heptuplet J_1 = 6.8 Hz, 1H), 6.71 (d, J_1 = 8.4 Hz, 1H), 3.83 (s, 3H), 6.91 (d, J_1 = 8.8 Hz, 2H), 7.00 (t, J_1 = 7.8 Hz, 1H), 7.16 (t, J_1 = 7.8 Hz, 1H), 7.69 (d, J_1 = 8.8 Hz, 3H).^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 21.70 (2C), 27.99, 55.78, 112.94, 114.49 (2C), 119.74, 123.37, 123.50, 125.31, 133.06 (2C), 134.36, 142.32, 161.96, 164.80, 168.17. IR (KBr) cm^{-1}: 1687.0, 1455.4, 1262.5. Anal calcd for $C_{18}H_{18}N_2O_2$ (m.wt = 294.35 g/mol): C = 73.45, H = 6.16, N = 9.52. Found: C = 74.01, H = 6.77, N = 10.04.

*4.1.6.16. (2-(tert-butyl)-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl)methanone (**9f**).* White solid. Yield = 21%. mp: 146.3–148.1 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 7.72 (d, J_1 = 8.4 Hz, 2H), 7.70 (d, J_1 = 7.8 Hz, 1H), 7.15 (t, J_1 = 7.8 Hz, 1H), 6.99 (t, J_1 = 7.8 Hz, 1H), 6.89 (d, J_1 = 8.4 Hz, 2H), 6.62 (d, J_1 = 7.8 Hz, 1H), 3.82 (s, 3H), 1.48 (s, 9H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 29.89 (3C), 35.85, 55.85, 112.04, 114.74 (2C), 119.73, 123.01, 123.26, 125.20, 133.73 (2C), 135.77, 141.43, 163.08, 165.30, 169.96. IR (KBr) cm^{-1}: 1703.99, 1454.1, 1261.5. Anal calcd for $C_{19}H_{20}N_2O_2$ (m.wt = 308.37 g/mol): C = 74.00, H = 6.54, N = 9.08. Found: C = 74.36, H = 6.43, N = 8.76.

*4.1.6.17. (2-Cyclohexyl-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl)methanone (**9g**).* White solid. Yield = 77%. mp: 101.8–103.4 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.30–1.20 (m, 3H), 1.70–1.63 (m, 3H), 1.73–1.75 (m, 2H), 2.01 (d, J_1 = 12.8 Hz, 2H), 3.14 (tt, J_1 = 11.6 Hz, J_2 = 3.2 Hz, 1H), 3.81 (s, 3H), 6.68 (d, J_1 = 8.0 Hz, 1H), 6.89 (d, J_1 = 8.8 Hz, 2H), 6.97 (t, J_1 = 7.6 Hz, 1H), 7.14 (t, J_1 = 7.6 Hz, 1H), 7.66 (d, J_1 = 8.0 Hz, 1H), 7.66 (d, J_1 = 8.8 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 25.95, 26.22 (2C), 32.03 (2C), 37.47, 55.74, 112.89, 114,41 (2C), 119.65, 123.23, 123.40, 125.28, 132.99 (2C), 134.13, 142.39, 161.03, 164.72, 168.13. IR (KBr) cm^{-1}: 1700.5, 1453.8, 1254.6. Anal calcd for $C_{21}H_{22}N_2O_2$ (m.wt = 334.41 g/mol): C = 75.42, H = 6.63, N = 8.38. Found: C = 76.09, H = 7.19, N = 8.89.

*4.1.6.18. (2-Cyclopentyl-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl)methanone (**9h**).* White solid. Yield = 29%. mp: 71.1–73.2 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.62–1.51 (m, 2H), 1.82–1.72 (m, 2H), 2.09–1.93 (m, 4H), 3.52 (p, J_1 = 8.4 Hz, 1H), 3.83 (s, 3H), 6.72 (d, J_1 = 8.0 Hz, 1H), 6.89 (d, J_1 = 8.8 Hz, 2H), 6.99 (t, J_1 = 7.6 Hz, 1H), 7.15 (t, J_1 = 7.6 Hz, 1H), 7.66 (d, J_1 = 8.0 Hz, 1H), 7.69 (d, J_1 = 8.8 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 25.84 (2C), 32.51 (2C), 38.90, 55.77, 112.84, 114.46 (2C), 119.61, 123.27, 123.48, 125.40, 133.06 (2C), 134.50, 142.34, 160.79, 164.75, 168.23. IR (KBr) cm^{-1}: 1697.8, 1454.9, 1260.5. Anal calcd for $C_{20}H_{20}N_2O_2$ (m.wt = 320.38 g/mol): C = 74.98, H = 6.29, N = 8.74. Found: C = 74.71, H = 6.61, N = 9.01.

*4.1.6.19. (4-methoxyphenyl)(2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)methanone (**9i**).* White solid. Yield = 42%. mp: 150.3–152.1 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 3.71 (s, 3H), 3.77 (s, 3H), 6.77 (t, J_1 = 8.8 Hz, 4H), 7.16 (d, J_1 = 8.0 Hz, 1H), 7.26 (t, J_1 = 7.4 Hz, 2H), 7.53 (d, J_1 = 8.8 Hz, 2H), 7.66 (d, J_1 = 8.8 Hz, 2H), 7.77 (d, J_1 = 8.0 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 55.43, 55.75, 112.70, 114.10 (2C), 114.37 (2C), 119.99, 122.84, 124.14, 125.23, 130.72 (2C), 133.42 (2C), 135.28, 143.06, 153.96, 161.01, 164.74, 168.55. IR (KBr) cm^{-1}: 1708.24, 1459.6, 1255.3. Anal calcd for $C_{22}H_{18}N_2O_3$ (m.wt = 358.39 g/mol): C = 73.73, H = 5.06, N = 7.82. Found: C = 73.57, H = 5.46, N = 7.40.

*4.1.6.20. (2-Cyclobutyl-1H-benzo[d]imidazol-1-yl)(4-methoxyphenyl)methanone (**9j**).* White solid. Yield = 58%. mp: 96.9–98.2 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 2.00–1.82 (m, 2H),

2.32–2.24 (m, 2H), 2.49 (pd, J_1 = 9.2 Hz, J_2 = 2.4 Hz, 2H), 3.81 (p, J_1 = 8.6 Hz, 1H), 3.81 (s, 3H), 6.78 (d, J_1 = 8.0 Hz, 1H), 6.88 (d, J_1 = 8.8 Hz, 2H), 7.00 (t, J_1 = 7.8 Hz, 1H), 7.14 (t, J_1 = 7.8 Hz, 1H), 7.63 (d, J_1 = 8.8 Hz, 2H), 7.67 (d, J_1 = 8.0 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 18.45, 27.83 (2C), 34.37, 55.69, 113.04, 114.34 (2C), 119.60, 123.42, 123.59, 125.27, 132.81 (2C), 134.24, 142.49, 159.68, 164.57, 167.73. IR (KBr) cm^{-1}: 1696.2, 1453.8, 1262.0. Anal calcd for $C_{19}H_{18}N_2O_2$ (m.wt = 306.36 g/mol): C = 74.49, H = 5.92, N = 9.14. Found: C = 74.17, H = 6.23, N = 9.52.

*4.1.7. Methyl benzo[b]thiophene-2-carboxylate (**11**)*

To a solution of 2-nitrobenzaldehyde (14.4 g. 0.095 mol) and K_2CO_3 (13.17 g. 0.095 mol) under magnetic stirring in DMF (40 mL) was added dropwise methyl thioglycolate (8.6 mL 0.095 mol). After addition, the reaction was stirred for 2 h at reflux, then was carried out at room temperature and finally poured into water (250 mL) yielding a precipitate which it was removed by filtration and taken to dryness. The solid correspond to pure product **11**.

Yellow solid. Yield = 73%. mp: 68.3–70.5 °C. ^{1}H RMN (400 MHz, DMSO-d_6) δ ppm: 8.22 (s, 1H), 8.07 (d, J_1 = 8.0 Hz, 1H), 8.04 (d, J_1 = 8.0 Hz, 1H), 7.54 (t, J_1 = 7.4 Hz, 1H), 7.48 (t, J_1 = 7.4 Hz, 1H), 3.90 (s, 3H)· ^{13}C RMN (400 MHz, DMOS-d6) δ ppm: 52.63, 123.01, 125.23, 125.92, 127.37, 130.94, 132.60, 138.47, 141.27, 162.45. IR (KBr) cm^{-1}: Anal calcd for $C_{10}H_8O_2S$ (m.wt = 192.23 g/mol): C = 62.48, H = 4.19, S = 16.68. Found: C = 62.44, H = 4.12, S = 16.30.

*4.1.8. Benzo[b]thiophene-2-carbaldehyde (**12**)*

To a solution of methyl benzo[*b*]thiophene-2-carboxylate **11** (13.3 g. 0.07 mol) in dry THF at −78 °C. was added very slowly dropwise DIBAH (70 mL. 0.07 mol) for 10 min. The reaction was stirred for 1 h at low temperature upon complete consumption of **11**. The crude reaction mixture is poured directly with vigorous stirring into 360 mL of ice cold HCl (1 M). The aqueous phase is extracted with diethyl ether (3x 100 mL) and the combined organic extracts were washed with saturated aqueous NaCl solution. After drying over anhydrous Na_2SO_4, filtration and removal of the solvent under reduced pressure were performed. The residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to obtain compound **12**.

Orange crystals. Yield = 91%. mp: 35.5–37.2 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 7.44 (td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 7.51 (td, J_1 = 7.6 Hz, J_2 = 1.2 Hz, 1H), 7.90 (d, J_1 = 8.0 Hz, 1H), 7.95 (d, J_1 = 8.0 Hz, 1H), 10.11 (s, 1H), 8.03 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 123.44, 125.39, 126.41, 128.31, 134.65, 138.66, 142.81, 143.46, 188.87. IR (KBr) cm^{-1}: 1669.7. Anal calcd for C_9H_6OS (m.wt = 162.21 g/mol): C = 66.64, H = 3.73, S = 19.77. Found: C = 67.01, H = 3.78, S = 19.25.

4.1.9. General procedure for the synthesis of 1-(benzo[b]thiophen-2-yl)alkyl-1-ol 13k-n

To a solution of benzo[*b*]thiophene-2-carbaldehyde **12** (1.1 g. 6.7 mmol) under anaerobic conditions (N_2), magnetic stirring and dry THF (60 mL) was added dropwise the corresponding Grignard reagents (5 mL. 0.015 mol). The reaction was stirred at room temperature for 1 h then mixture was poured into a cold aqueous solution of HCl (0.1 M) and transferred to a 500 mL separatory funnel, the mixture was extracted with ethyl acetate (3x 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and filtered. Solvent was removed by rotary evaporation under reduce pressure and the residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to yield target compounds **13k-n**.

*4.1.9.1. 1-(benzo[b]thiophen-2-yl)ethanol (**13k**).* White solid. Yield = 98%. mp: 62.0–64.1 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.62 (d, J_1 = 6.5 Hz, 3H), 2.39 (d, J_1 = 2.8 Hz, 1H), 5.15 (dd, J_1 = 6.1 Hz, J_2 = 2.8 Hz, 1H), 7.14 (s, 1H) 7.27 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.32 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.68 (dd, J_1 = 7.1 Hz, J_2 = 1.4 Hz, 1H), 7.79 (dd, J_1 = 8.0 Hz, J_2 = 0.8 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 25.16, 66.92, 119.58, 122.58, 123.57, 124.25, 124.38, 139.40, 139.69, 150.63. IR (KBr) cm^{-1}: 3298.9, 11067.4. Anal calcd for $C_{10}H_{10}OS$ (m.wt = 178.25 g/mol): C = 67.38, H = 5.65, S = 17.99. Found: C = 67.46%; H = 5.35, S = 17.71.

4.1.9.2. 1-(benzo[b]thiophen-2-yl)propan-1-ol (13l). Yellow oil. Yield = 75%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.98 (t, J_1 = 7.4 Hz, 3H), 1.99–1.83 (m, 2H), 2.28 (d, J_1 = 3.5 Hz, 1H), 4.89 (td, J_1 = 6.5 Hz, J_2 = 3.5 Hz, 1H), 7.15 (s, 1H), 7.28 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.32 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.7 (d, J_1 = 7.2 Hz, 1H), 7.79 (d, J_1 = 7.8 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 10.08, 32.02, 72.41, 120.34, 122.60, 123.54, 124.23, 124.36, 139.47, 139.65, 149.38. IR (KBr) cm^{-1}: 3373.1, 1071.8. Anal calcd for $C_{11}H_{12}OS$ (m.wt = 192.28 g/mol): C = 68.71, H = 6.29, S = 16.68. Found: C = 69.14, H = 6.64, S = 16.69.

*4.1.9.3. 1-(benzo[b]thiophen-2-yl)pentan-1-ol (**13m**).* White crystals. Yield = 80%. mp: 58.7–59.9 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.88 (t, J_1 = 7,0 Hz, 3H), 1.46–1.25 (m, 4H), 1.94–1.78 (m, 2H), 2.40 (s, 1H), 4.93 (t, J_1 = 6.0 Hz, 1H), 7.13 (s, 1H), 7.29 (tt, J_1 = 7.6 Hz, J_2 = 1.6 Hz, 2H), 7.68 (d, J_1 = 7.3 Hz, 1H), 7.78 (d, J_1 = 7.7 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 14.08, 22.59, 27.87, 38.78, 71.03, 120.21, 122.59, 123.52, 124.20, 124.33, 139.44, 139.63, 149.73. IR (KBr) cm^{-1}: 3264.10, 1014.6. Anal calcd for $C_{13}H_{16}OS$ (m.wt = 220.33 g/mol): C = 70.87%; H = 7.32%; S = 14.55%. Found: C = 71.22, H = 7.64, S = 14.66.

*4.1.9.4. 1-(benzo[b]thiophen-2-yl)-2-methylpropan-1-ol (**13n**).* Yellow oil. Yield = 51%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.91 (d, J_1 = 6,7 Hz, 3H), 1.06 (d, J_1 = 6,7 Hz, 3H), 2.06 (dq, J_1 = 13.5 Hz, J_2 = 6.7 Hz, 1H), 2.21 (s, 1H), 4.67 (d, J_1 = 6.7 Hz, 1H), 7.15 (s, 1H), 7.28 (td, J_1 = 7.3 Hz, J_2 = 1.2 Hz, 1H), 7.33 (td, J_1 = 7.3 Hz, J_2 = 1.2 Hz, 1H), 7.70 (dd, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.80 (dd, J_1 = 7.7 Hz, J_2 = 0.6 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 18.18 (2C), 19.13, 35.73, 120.90, 122.55, 123.48, 124.18, 124.33, 139.50, 139.57, 148.54. IR (KBr) cm^{-1}: 3342.8, 1019.0. Anal calcd for $C_{12}H_{14}OS$ (m.wt = 206.3 g/mol): C = 69.86, H = 6.84, S = 15.54. Found: C = 70.09, H = 7.14, S = 16.11.

4.1.10. General procedure for the synthesis of 1-(benzo[b]thiophen-2-yl)alkyl-1-one 14k-n

To a solutions of the corresponding 1-(benzo[b]thiophen-2-yl) alkyl-1-ol **13k-n** (1.08 g. 6.0 mmol) in CH_2Cl_2 under magnetic stirring was added pyridinium chlorochromate (2,6 g. 0,012 mol). After addition, reaction was stirred for 1 h at room temperature. The mixture was filtered and concentrated under reduced pressure in a rotary evaporator. The residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to yield target compounds **14k-n**.

*4.1.10.1. 1-(benzo[b]thiophen-2-yl)ethanone (**14k**).* White solid. Yield = 96%. mp: 89.0–91.3 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 2.64 (s, 3H), 7.39 (td, J_1 = 7.6 Hz, J_2 = 0.8 Hz, 1H), 7.44 (td, J_1 = 7.6 Hz, J_2 = 0.8 Hz, 1H), 7.85 (t, J_1 = 9.2 Hz, 2H), 7.91 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 26.84, 123.04, 125.07, 125.99, 127.50, 129.76, 139.19, 142.67, 144.01, 192.30.IR (KBr) cm^{-1}: 1660.4. Anal calcd for $C_{10}H_8OS$ (m.wt = 176.23 g/mol): C = 68.15, H = 4.58, S = 18.19. Found: C = 68.76, H = 4.69, S = 18.58.

4.1.10.2. 1-(benzo[b]thiophen-2-yl)propan-1-one (14l). White crystals. Yield = 99%. mp: 82.5–84.3 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.27 (t, J_1 = 7.3 Hz, 3H), 3.03 (q, J_1 = 7.3 Hz, 2H), 7.39 (t,

J_1 = 7.4 Hz, 1H), 7.45 (t, J_1 = 7.4 Hz, 1H), 7.86 (t, J_1 = 7.5 Hz, 2H), 7.93 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 8.60, 32.59, 123.06, 125.04, 125.93, 127.36, 128.75, 139.25, 142.43, 143.65, 195.4. IR (KBr) cm^{-1}: 1664.9. Anal calcd for $C_{11}H_{10}OS$ (m.wt = 190.26 g/mol): C = 69.44, H = 5.30, S = 16.85. Found: C = 70.02, H = 5.60, S = 17.07.

4.1.10.3. 1-(benzo[b]thiophen-2-yl)pentan-1-one (14 m). White crystals. Yield = 95%. mp: 98.1–100.6 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.96 (t, J_1 = 7.6 Hz, 3H), 1.44 (sextuplet, J_1 = 7.6 Hz, 2H), 1.77 (p, J_1 = 7.6 Hz, 2H), 3.00 (t, J_1 = 7.6 Hz, 2H), 7.40 (t, J_1 = 7.4 Hz, 1H), 7.45 (t, J_1 = 7.4 Hz, 1H) 7.87 (t, J_1 = 8.0 Hz, 2H), 7.95 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 14.02, 22.59, 26.98, 39.14, 123.10, 125.06, 125.97, 127.40, 128.88, 139.29, 142.54, 144.04, 195.16. IR (KBr) cm^{-1}: 1659.4. Anal calcd for $C_{13}H_{14}OS$ (m.wt = 218.31 g/mol): C = 71.52, H = 6.46, S = 14.69. Found: C = 71.93. H = 6.80, S = 15.01.

*4.1.10.4. 1-(benzo[b]thiophen-2-yl)-2-methylpropan-1-one (**14n**).* Oil, Orange Oil. Yield = 93%. ^{1}H RMN (400 MHz, CDCl3) δ ppm: 1.29 (d, *J*1 = 6.9 Hz, 6H), 3.51 (heptuplet, J_1 = 6.9 Hz, 1H), 7.39 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.44 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.89–7.83 (m, 2H), 7.96 (s, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 19.53 (2C), 37.16, 123.02, 125.01, 125.95, 127.34, 128.70, 139.31, 142.56, 143.17, 199.00. IR (KBr) cm^{-1}: 1664.8. Anal calcd for $C_{12}H_{12}OS$ (m.wt = 204.29 g/mol): C = 70.55, H = 5.92, S = 15.70. Found: C = 70.23, H = 6.09, S = 16.09.

*4.1.11. General procedure for the synthesis of 2-alkylbenzo[b] thiophene **15k-n***

To the corresponding 1-(benzo[*b*]thiophen-2-yl)alkyl-1-one **14k-n** (0.90 g. 5.2 mL) were dissolved en 10 mL. of ethylene glycol. Then 8 eq. of KOH was added (2.3 g. 0.04 mol) previously dissolved in 5 mL. of ethylene glycol by heating. To this mixture $H_2NNH_2xH_2O$ (0.8 mL 0,02 mol) was added. Reaction was stirred for 24 h at 197 °C. Reaction was poured into water (150 mL) and transferred to a 250 mL separatory funnel; the mixture was extracted with dichloromethane (3x100 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and filtered. Solvent was removed by rotary evaporation under reduce pressure and the residue was purified by column chromatography on silica gel with CH_2Cl_2 as eluent to obtain compounds **15k-n**.

*4.1.11.1. 2-ethylbenzo[b]thiophene (**15k**).* Yellow oil. Yield = 70%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.35 (t, J_1 = 7.5 Hz, 3H), 2.90 (qd, J_1 = 7.5 Hz, J_2 = 1.1 Hz, 2H), 6.96 (s, 1H), 7.21 (td, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 7.27 (td, J_1 = 7.5 Hz, J_2 = 1.2 Hz, 1H), 7.63 (d, J_1 = 7.6 Hz, 1H), 7.73 (d, J_1 = 8.2 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 15.54, 24.23, 119.78, 122.23, 122.80, 123.47, 124.14, 139.35, 140.39, 148.39. IR (KBr) cm^{-1}: 2967.5–2928.2. Anal calcd for $C_{10}H_{10}S$ (m.wt = 162.25 g/mol): C = 74.03, H = 6.21, S = 19.76. Found: C = 74.20, H = 6.48, S = 19.24.

4.1.11.2. 2-propylbenzo[b]thiophene (15l). Yellow oil. Yield = 70%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.99 (t, J_1 = 7.5 Hz, 3H), 1.75 (sextuplet, J_1 = 7.5 Hz, 1H), 2.85 (t, J_1 = 7.5 Hz, 2H), 6.96 (s, 1H), 7.22 (t, J_1 = 7.4 Hz, 1H), 7.28 (t, J_1 = 7.4 Hz, 1H), 7.63 (d, J_1 = 7.8 Hz, 1H), 7.73 (d, J_1 = 7.9 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.81, 24.49, 32.94, 120.63, 122.22, 122.77, 123.44, 124.12, 139.47, 140.37, 146.69. IR (KBr) cm^{-1}: 2959.8-2930.0-2870.9. Anal calcd for $C_{11}H_{12}S$ (m.wt = 176.28 g/mol): C = 74.95, H = 6.86, S = 18.19. Found: C = 74.69, H = 6.89, S = 18.18.

*4.1.11.3. 2-pentylbenzo[b]thiophene (**15m**).* Yellow oil. Yield = 44%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.89 (t, J_1 = 6.9 Hz, 3H), 1.39–1.28 (m, 4H), 1.71 (dq, J_1 = 14.7 Hz, J_2 = 7.6 Hz, 2H), 2.83 (t, J_1 = 7.6 Hz, 2H), 7.19 (t, J_1 = 7.5 Hz, 1H), 6.93, (s, 1H), 7.25 (t, J_1 = 7.5 Hz, 1H), 7.61 (d, J_1 = 7.9 Hz, 1H), 7.71 (d, J_1 = 7.9 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 14.11, 22.55, 30.86, 30.92, 31.41, 120.49, 122.19, 122.74, 123.41, 124.09, 139.44, 140.37, 146.92. IR (KBr) cm^{-1}: 2956.1–2856.0. Anal calcd for $C_{13}H_{16}S$ (m.wt = 204.33 g/mol): C = 76.41, H = 7.89, S = 15.69. Found: C = 76.17, H = 8.34, S = 15.83.

*4.1.11.4. 2-isobutylbenzo[b]thiophene (**15n**).* Yellow oil. Yield = 63%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.01 (d, J_1 = 7.0 Hz, 6H), 2.02 (tq, J_1 = 13.5 Hz, J_2 = 7.0 Hz, 1H), 2.76 (d, J_1 = 7.0 Hz, 2H), 6.97 (s, 1H), 7.25 (t, J_1 = 7.4 Hz, 1H), 7.31 (t, J_1 = 7.4 Hz, 1H), 7.67 (d, J_1 = 8.0 Hz, 1H), 7.77 (d, J_1 = 8.0 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ: 22.39 (2C), 30.37, 40.13, 121.43, 122.13, 122.74, 123.40, 124.05, 139.62, 140.29, 145.51. IR (KBr) cm^{-1}: 2956.7-2925.5-2868.3. Anal calcd for $C_{12}H_{14}S$ (m.wt = 190.30 g/mol): C = 75.74, H = 7.42, S = 16.85. Found: C = 75.43, H = 7.64, S = 16.87.

4.1.12. General procedure for the synthesis of (2-alkylbenzo[b] thiophen-3-yl)(aryl)methanone 16k-n, 17k-n, benzo[b]thiophen-3-yl(naphthalen-1-yl)methanone 19, benzo[b]thiophen-2-yl(naphthalen-1-yl)methanone 20, (2-(4-methoxyphenyl)benzo[b] thiophen-3-yl)(naphthalen-1-yl)methanone 24 and (4-methoxyphenyl)(2-(4-methoxyphenyl)benzo[b]thiophen-3-yl) methanone 24

To a magnetic stirred solution under anaerobic conditions (N_2) of 1-naphthoyl chloride (0.1 mL 0.7 mmol) or 4-methoxybenzoyl chloride (0,1 mL. 0.8 mmol) and tin (IV) chloride (0.1 mL 1.0 mmol) in 1,2-dichloroethane (50 mL) was added dropwise the corresponding 2-alkylbenzo[*b*]thiophene **15k-n** (111,6 mg. 0.8 mmol), benzo[*b*]thiophene **18** (111.6 mg 0.8 mmol) or 2-(4-methoxyphenyl)benzo[*b*]thiophene **22** (94.2 mg 0.30 mmol) dissolved in 1,2-dichloroethane (10 mL). The mixture was stirred at room temperature under anaerobic conditions (N_2) for 6 h. Later, reaction was poured into a cold aqueous solution of $NaHCO_3$ (1 M) and transferred to a 250 mL. separatory funnel, then the mixture was extracted with ethyl acetate (3x 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and filtered. Solvent was removed by rotary evaporation under reduce pressure and the residue was purified by thin layer chromatography with CH_2Cl_2 (or hexane for compounds **18** and **19**) as eluent to yield target compounds **16k-n**, **17k-n**, **19**, **20**, **24** and **25**.

*4.1.12.1. (2-ethylbenzo[b]thiophene-3-yl)(naphthalene-1-yl)methanone (**16k**).* Yellow oil. Yield = 79%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.19 (t, J_1 = 7.5 Hz, 3H), 2.78 (q, J_1 = 7.5 Hz, 2H), 7.20 (t, J_1 = 7.8 Hz, 1H), 7.25 (t, J_1 = 7.8 Hz, 1H), 7.37 (t, J_1 = 8.2 Hz, 1H), 7.56–7.50 (m, 2H), 7.60–7.56 (m, 1H), 7.62 (d, J_1 = 8.2 Hz, 1H), 7.76 (d, J_1 = 7.8 Hz, 1H), 7.89 (d, J_1 = 8.3 Hz, 1H), 7.97 (d, J_1 = 8.2 Hz, 1H), 8.60 (d, J_1 = 8.3 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 16.16, 23.68, 121.95, 127.73, 124.41, 124.69, 124.98, 125.67, 126.71, 128.09, 128.59, 129.83, 130.63, 132.83, 132.91, 134.09, 137.26, 137.64, 139.09, 155.96, 194.28. IR (KBr) cm^{-1}: 1644.9. Anal calcd for $C_{21}H_{16}OS$ (m.wt = 316.42 g/mol): C = 79.71, H = 5.10, S = 10.13. Found: C = 79.39, H = 5.25, S = 10.53.

*4.1.12.2. Naphthalen-1-yl(2-propylbenzo[b]thiophen-3-yl)methanone (**16l**).* Yellow oil. Yield = 87%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.76 (t, J_1 = 7.4 Hz, 3H), 1.62 (sextuplet, J_1 = 7.4 Hz, 2H), 2.72 (t, J_1 = 7.4 Hz, 2H), 7.17 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.23 (td, J_1 = 7.2 Hz, J_2 = 1.2 Hz, 1H), 7.33 (t, J_1 = 8.2 Hz, 1H), 7.53–7.47 (m, 2H), 7.59–7.53 (m, 1H), 7.61 (dd, J_1 = 8.2 Hz, J_2 = 1.2 Hz, 1H), 7.73 (d, J_1 = 7.7 Hz, 1H), 7.86 (dd, J_1 = 8.8 Hz, J_2 = 1.2 Hz, 1H), 7.94 (d, J_1 = 8.2 Hz, 1H), 8.61 (d, J_1 = 8.5 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.75, 26.16, 31.91, 121.86, 123.67, 124.37, 124.62, 124.87, 125.62, 126.64, 128.02, 128.53, 129.77, 130.61, 132.75, 133.43, 134.02, 137.17, 137.70, 139.03, 154.10, 194.26. IR (KBr) cm^{-1}: 1647.1. Anal

calcd for $C_{22}H_{18}OS$ (m.wt = 330.44 g/mol): C = 79.96, H = 5.49, S = 9.70. Found: C = 79.44, H = 5.66, S = 10.06.

*4.1.12.3. Naphthalen-1-yl(2-pentylbenzo[b]thiophen-3-yl)methanone (**16m**).* Yellow oil. Yield = 94%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.75 (t, J_1 = 7.0 Hz, 3H), 1.16–1.05 (m, 4H), 1.59 (p, J_1 = 7.6 Hz, 2H), 7.20 (td, J_1 = 7.3 Hz, J_2 = 1.2 Hz, 1H), 7.25 (td, J_1 = 7.3 Hz, J_2 = 1.2 Hz, 1H), 7.37 (t, J_1 = 8.2 Hz, 1H), 7.57–7.50 (m, 2H), 7.61–7.57 (m, 1H), 7.62 (dd, J_1 = 8.2 Hz, J_2 = 1.2 Hz, 1H), 7.75 (dd, J_1 = 7.3 Hz, J_2 = 0.8 Hz, 1H), 7.89 (d, J_1 = 7.6 Hz, 1H), 7.97 (d, J_1 = 8.2 Hz, 1H), 8.58 (dd, J_1 = 8.3 Hz, J_2 = 0.8 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.19, 22.21, 30.04, 31.38, 31.63, 121.89, 123.72, 124.41, 124.67, 124.94, 125.66, 126.68, 128.04, 128.56, 129.73, 130.64, 132.75, 133.31, 134.07, 137.29, 137.72, 139.09, 154.55, 194.33. IR (KBr) cm^{-1}: 1646.7. Anal calcd for $C_{24}H_{22}OS$ (m.wt = 358.50 g/mol): C = 80.41, H = 6.19, S = 8.94. Found: C = 80.13, H = 6.03, S = 9.20.

*4.1.12.4. (2-isobutylbenzo[b]thiophen-3-yl)(naphthalene-1-yl)methanone (**16n**).* Yellow oil. Yield = 88%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.81 (d, J_1 = 6.8 Hz, 6H), 1.93 (ninth, J_1 = 6.8 Hz, 1H), 2.67 (d, J_1 = 6.8 Hz, 2H), 7.19 (t, J_1 = 8.0 Hz, 1H), 7.27 (t, J_1 = 8.0 Hz, 1H), 7.38 (t, J_1 = 8.2 Hz, 1H), 7.56–7.47 (m, 2H), 7.61–7.56 (m, 1H), 7.63 (d, J_1 = 8.2 Hz, 1H), 7.77 (d, J_1 = 8.0 Hz, 1H), 7.91 (d, J_1 = 8.2 Hz, 1H), 8.00 (d, J_1 = 8.2 Hz, 1H), 8.62 (d, J_1 = 8.3 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 22.46 (2C), 31.41, 38.80, 121.90, 123.69, 124.44, 124.70, 124.90, 125.70, 126.73, 128.17, 128.61, 130.14, 130.74 (2C), 132.96, 134.10, 137.03, 137.91, 139.11, 152.89, 194.56. IR (KBr) cm^{-1}: 1649.2. Anal calcd for $C_{23}H_{20}OS$ (m.wt = 344.47 g/mol): C = 80.19, H = 5.85, S = 9.31. Found: C = 79.64, H = 6.13, S = 9.70.

*4.1.12.5. (2-ethylbenzo[b]thiophen-3-yl)(4-methoxyphenyl)methanone (**17k**).* Yellow oil. Yield = 81%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 1.28 (t, J_1 = 7.5 Hz, 3H), 2.87 (q, J_1 = 7.5 Hz, 2H), 3.82 (s, 3H), 6.90 (d, J_1 = 8.7 Hz, 2H), 7.28–7.19 (m, 2H), 7.45 (d, J_1 = 7.4 Hz, 1H), 7.76 (d, J_1 = 7.4 Hz, 1H), 7.82 (d, J_1 = 8.7 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 16.18, 23.30, 55.49, 113.90 (2C), 121.99, 123.16, 124.16, 124.58, 131.32, 131.95, 132.18 (2C), 137.94, 139.08, 151.30, 163.95, 192.37. IR (KBr) cm^{-1}: 1648.8, 1260.5. Anal calcd for $C_{18}H_{16}O_2S$ (m.wt = 296.38 g/mol): C = 72.94, H = 5.44, S = 10.82. Found: C = 72.92, H = 5.31 S = 11.20.

*4.1.12.6. (4-methoxyphenyl)(2-propylbenzo[b]thiophen-3-yl)methanone (**17l**).* Yellow oil. Yield = 78%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.90 (t, J_1 = 7.6 Hz, 3H), 1.71 (sextuplet, J_1 = 7.6 Hz, 2H), 2.84 (t, J_1 = 7.6 Hz, 2H), 3.83 (s, 3H), 6.90 (d, J_1 = 8.9 Hz, 2H), 7.23 (pd, J_1 = 7.2 Hz, J_2 = 1.4 Hz, 2H), 7.42 (d, J_1 = 7.2 Hz, 1H), 7.76 (d, J_1 = 7.2 Hz, 1H), 7.82 (d, J_1 = 8.9 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.80, 25.02, 31.68, 55.52, 113.94 (2C), 121.97, 123.21, 124.18, 124.56, 131.42, 132.23 (2C), 132.63, 138.11, 139.12, 149.59, 164.00, 192.44. IR (KBr) cm^{-1}: 1649.3, 1260.3. Anal calcd for $C_{19}H_{19}O_2S$ (m.wt = 310.41 g/mol): C = 73.52, H = 5.84, S = 10.33. Found: C = 73.50, H = 5.93, S = 10.54.

*4.1.12.7. (4-methoxyphenyl)(2-pentylbenzo[b]thiophen-3-yl)methanone (**17m**).* Yellow oil. Yield = 84%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.82 (t, J_1 = 7.6 Hz, 3H), 1.30–1.18 (m, 4H), 1.68 (p, J_1 = 7.6 Hz, 2H), 2.85 (t, J_1 = 7.6 Hz, 2H), 3.83 (s, 3H), 6.90 (d, J_1 = 8.9 Hz, 2H), 7.24 (pd, J_1 = 7.2 Hz, J_2 = 1.4 Hz, 2H), 7.43 (d, J_1 = 7.2 Hz, 1H), 7.77 (d, J_1 = 7.2 Hz, 1H), 7.82 (d, J_1 = 8.9 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 13.92, 22.31, 29.69, 31.34, 31.42, 55.53, 113.92 (2C), 121.97, 123.20, 124.16, 124.56, 131.42, 132.23 (2C), 132.48, 138.08, 139.12, 149.97, 163.97, 192.46. IR (KBr) cm^{-1}: 1649.6, 1260.3. Anal calcd for $C_{21}H_{22}O_2S$ (m.wt = 338.46 g/mol): C = 74.52, H = 6.55, S = 9.47. Found: C = 74.60, H = 7.15, S = 9.75.

*4.1.12.8. (2-isobutylbenzo[b]thiophen-3-yl)(4-methoxyphenyl)methanone (**17n**).* Yellow oil. Yield = 88%. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 0.89 (d, J_1 = 7.3 Hz, 6H), 1.96 (hp, J_1 = 7.3 Hz, 1H), 2.76 (d, J_1 = 7.3 Hz, 2H), 3.84 (s, 3H), 6.91 (d, J_1 = 8.9 Hz, 2H), 7.24 (pd, J_1 = 7.4 Hz, J_2 = 1.4 Hz, 2H), 7.40 (d, J_1 = 7.4 Hz, 1H), 7.78 (d, J_1 = 7.4 Hz, 1H), 7.82 (d, J_1 = 8.9 Hz, 2H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 22.47, 31.14 (2C), 38.62, 55.57, 113.98 (2C), 121.97, 123.28, 124.23, 124.56, 131.42, 132.33 (2C), 133.30, 138.28, 139.14, 148.63, 164.01, 192.58. IR (KBr) cm^{-1}: 1649.9, 1260.2. Anal calcd for $C_{20}H_{20}O_2S$ (m.wt = 324.44 g/mol): C = 74.04, H = 6.21, S = 9.88. Found: C = 74.50, H = 6.28, S = 10.08.

*4.1.12.9. (2-methylbenzo[b]thiophen-3-yl)(nahpthalen-1-yl)methanone (**19**).* White solid. Yield = 27.5%. mp: 106.0–108.0 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 7.52–7.44 (m 4H), 7.56 (t, J_1 = 7.6 Hz, 1H), 7.64 (d, J_1 = 7.0 Hz, 1H), 7.86 (s, 1H), 7.90 (t, J_1 = 8.6 Hz, 2H), 7.98 (d, J_1 = 8.0 Hz, 1H), 8.16 (d, J_1 = 7.6 Hz, 1H), 8.86 (d, J_1 = 8.0 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 122.50, 124.49, 125.70, 125.74, 125.82, 126.09, 126.63, 127.26, 127.38, 128.49, 130.87, 131.22, 133.88, 136.53, 137.41, 137.81, 140.31, 141.18, 192.35. IR (KBr) cm^{-1}: 1637.5. Anal calcd for $C_{19}H_{12}OS$ (m.wt = 288.36 g/mol): C = 79.14, H = 4.19, S = 11.12. Found: C = 79.61, H = 4.45, S = 11.55.

*4.1.12.10. (2-methylbenzo[b]thiophen-2-yl)(nahpthalen-1-yl)methanone (**20**).* White solid. Yield = 13.3%. mp: 138.0–140.5 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 7.29 (td, J_1 = 7.2 Hz, J_2 = 0.8 Hz, 1H), 7.39 (td, J_1 = 7.2 Hz, J_2 = 0.8 Hz, 1H), 7.49–7.42 (m, 3H), 7.59 (s, 1H), 7.71–7.68 (m, 2H), 7.86–7.82 (m, 2H), 7.95 (d, J_1 = 8.4 Hz, 1H), 8.11 (d, J_1 = 8.4 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 123.16, 124.46, 125.20, 125.62, 126.36, 126.77, 127.41, 127.55, 127.82, 128.53, 130.75, 131.65, 133.34, 133.92, 135.98, 139.14, 143.35, 144.95, 191.37. IR (KBr) cm^{-1}: 1634.6. Anal calcd for $C_{19}H_{12}OS$ (m.wt = 288.36 g/mol): C = 79.14, H = 4.19, S = 11.12. Found: C = 79.64, H = 4.67, S = 11.02.

*4.1.12.11. (2-(4-methoxyphenyl)benzo[b]thiophen-3-yl)(naphthalen-1-yl)methanone (**24**).* White solid. Yield = 25%. mp: 46.7–49.2 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 3.56 (s, 3H), 6.45 (d, J_1 = 8.8 Hz, 2H), 7.14 (t, J_1 = 7.6 Hz, 1H), 7.20 (d, J_1 = 8.8 Hz, 2H), 7.41–7.36 (m, 2H), 7.48 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H), 7.53 (dd, J_1 = 7.2 Hz, J_2 = 0.8 Hz, 1H), 7.60 (td, J_1 = 7.7 Hz, J_2 = 1.4 Hz, 1H), 7.77–7.75 (m, 2H), 7.86 (d, J_1 = 7.6 Hz, 1H), 8.00 (d, J_1 = 7.6 Hz, 1H), 8.74 (d, J_1 = 8.6 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 55.26, 113.66 (2C), 121.95, 124.07, 124.29, 125.11, 125.49, 125.69, 125.84, 126.38, 128.08, 128.42, 130.59 (2C), 130.81, 131.26, 132.75, 132.97, 133.79, 136.17, 138.59, 139.87, 149.76, 159.93, 195.17. IR (KBr) cm^{-1}: 1641.93, 1253.4. Anal calcd for $C_{26}H_{18}O_2S$ (m.wt = 394.48 g/mol): C = 79.16, H = 4.60, S = 8.13. Found: C = 79.28, H = 5.02, S = 8.34.

*4.1.12.12. (4-methoxyphenyl)(2-(4-methoxyphenyl)benzo[b]thiophen-3-yl)methanone (**25**).* White solid. Yield = 33%. mp: 47.5–49.8 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 3.66 (s, 3H), 3.70 (s, 3H), 6.69 (d, J_1 = 8.4 Hz, 4H), 7.26–7.24 (m, 2H), 7.30 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 2H), 7.56 (d, J_1 = 7.6 Hz, 1H), 7.70 (d, J_1 = 8.4 Hz, J_2 = 1.2 Hz 2H), 7.76 (d, J_1 = 7.6 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 55.37, 55.52, 113.81 (2C), 114.25 (2C), 122.00, 123.43, 124.84, 125.06, 125.97, 130.52 (2C), 131.13, 132.47 (2C), 138.76, 140.03, 145.00, 160.11, 163.90, 193.35. IR (KBr) cm^{-1}: 1648.0, 1257.1. Anal calcd for $C_{23}H_{18}O_3S$ (m.wt = 374.45 g/mol): C = 73.77, H = 4.85, S = 8.56. Found: C = 73.63, H = 5.15, S = 9.10.

*4.1.13. 2-(4-methoxyphenyl)benzo[b]thiophene (**23**)*

To a solution of tetrakis triphenylphosphine palladium (0) (82.0 mg 4mol %. 71.2 mmol) and 2-bromobenzo[*b*]thiophene (380.0 mg 1.7 mmol) in dry THF (100 mL) was added dropwise (4-

methoxyphenyl)zinc(II) iodide (3.5 mL 1,7 mmol) dissolved in dry THF. Reaction was stirred at room temperature under anaerobic conditions (N_2) for 6 h. Later, reaction was poured into a cold aqueous solution of HCl (3 M)) and transferred to a 250 mL. separatory funnel, then the mixture was extracted with diethyl ether (3x 100 mL). The combined organic phases were washed with saturated aqueous $NaHCO_3$ solution, then were dried over anhydrous Na_2SO_4 and filtered. Solvent was removed by rotary evaporation under reduce pressure and the residue was purified by thin layer chromatography with hexane as eluent to yield compound **23**.

White crystals. Yield = 18%. mp: 192.7–194.6 °C. ^{1}H RMN (400 MHz, $CDCl_3$) δ ppm: 3.84 (s, 3H), 6.94 (d, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 2H), 7.27 (td, J_1 = 7.7 Hz, J_2 = 1.2 Hz, 1H), 7.33 (td, J_1 = 7.7 Hz, J_2 = 1.2 Hz, 1H), 7.41 (s, 1H), 7.63 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 2H), 7.73 (d, J_1 = 7.5 Hz, 1H), 7.80 (d, J_1 = 7.9 Hz, 1H). ^{13}C RMN (400 MHz, $CDCl_3$) δ ppm: 55.53, 114.53 (2C), 118.36, 122.32, 123.40, 124.09, 124.59, 127.24, 127.91 (2C), 139.36, 141.06, 144.32, 159.98. IR (KBr) cm^{-1}: 1254.9. Anal calcd for $C_{15}H_{12}OS$ (m.wt = 240.32 g/mol): C = 79.97, H = 5.03, S = 13.34. Found: C = 79.67, H = 5.22, S = 13.69.

4.2. Biology

4.2.1. Binding assays CB1/CB2

For CB1 and CB2 receptor binding assays, the compounds were subjected to a preliminary screening using three different concentrations (1, 0.1 and 0.01 μM) of the synthesized compounds; HEK-293 cell membranes overexpressing either the human cannabinoid CB1 receptor (Perkin Elmer Inc., Waltham, Ma, USA, Product No.: 6110129400UA, Lot No.: 509–845-A), or the human recombinant cannabinoid CB2 receptor (Perkin Elmer Inc., Waltham, Ma, USA, Product No.: 6110129400UA, Lot No.: 509–845-A); and [^{3}H]-(-)*cis*-3-[2hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP-55,940; Kd = 500 pM) as the high affinity ligand. Compounds that displaced [^{3}H]CP-55,940 by more than 50% at 1 μM were further analyzed. Receptors were incubated with compounds at 30 °C for 1.5 h at different increasing concentrations using 0.5 nM of [^{3}H]CP-55,940. In all cases, K_i values were calculated applying Cheng- Prusoff equation [71]. Displacement curves were generated using GraphPad, GraphPad Software, Inc., La Jolla, CA, USA.

4.2.2. Cell viability assay

4.2.2.1. Cell cultures. Human cancer cell lines H1975 (lung), HTC116 (Colon), Hela (Cervix), and HL60 (leukemia) cells; and non-neoplastic cell line (VERO) were maintained at 5% CO_2 and 37 °C in RPMI 1640 medium, containing 5% Fetal Bovine Serum and 100 U/mL penicillin and 100 μg/mL streptomycin.

4.2.2.2. Viability assay. Cytotoxicity assays were performed by using the MTT reduction method. Briefly, cancer cell lines were plated in a flat-bottom 96-wells plate at 10,000 cells per well density. Then, the cells were incubated with synthetized compounds at different concentrations (0.001 μM–10 μM) in 200 μL of 5% fetal bovine serum-RPMI culture medium at 37 °C for 72 h. Etoposide was used as positive control. 10 μL of 5 mg/mL MTT was added and cells were incubated at 37 °C for 4 h, and then solubilized with 10% sodium dodecyl sulfate (SDS) in 0.1 mM HCl and incubated overnight at 37 °C. Formazan formation was measured at 570 nm in a multiwell microplate reader (StatFax 4200, Awareness Technology, Inc. Palm City, FL, USA).

4.3. Computational

4.3.1. CB1 receptor model

Human CB1 receptor model was used for all simulations with the Autodock 4.2 [72]. The model receptor was built by homology and presents more than 97% of the residues in allowed regions according to Ramachandran plot analysis. All other conditions were as previously published [47].

4.3.2. CB2 receptor model

The sequence of the human CB2 receptor was retrieved from UniProtKB database with accession number P34972. The crystal structures of human adenosine A2a receptor (PDB ID: 3EML) was retrieved from the Protein Data Bank (PDB) and selected as the template for homology modeling according to the results of BLAST search [73]. Subsequently multiple sequence alignment and CB2 model construction was performed using Modeller as implemented in Discovery Studio (DS) 2.1 with default parameters. A set of 100 models was generated, and the best model according to the internal scoring function of the program (PDF score) was subjected to energy minimization protocol in order to relax the structure and optimize bonds geometry. Model validation was performed using the tools available at the NIH SAVES server (http://nihserver.mbi.ucla.edu/SAVES/). The active site was defined using the amino acids which interact with the aminoalkylindoles WIN-55,212-2 and AM-630. Trp194 was designated as the centre of the grid since it has an important role in CB2 receptor ligand binding and inhibition of adenylyl cyclase (AC) activity [66,74–77].

4.3.3. Molecular docking

The geometries of all ligands involved in this study were fully optimized using the Hartree-Fock method with the standard basis set 6-31G*. All calculations were performed using Spartan' 08 [78]. All simulations were performed with Autodock 4.2 [72]. Grid maps were calculated using the autogrid option and were centered on the putative ligand-binding site considering the residues involved in the interaction of CB1 with WIN 55,212-2, as our group has previously reported [79] and in the case of CB2 receptor Trp164 was designated as the center of the grid. The volumes chosen for the grid maps were made up of 60 × 60 × 60 points, with a grid-point spacing of 0.375 Å. The docked compound complexes were built using the lowest docked-energy binding positions. The different complexes were visualised in Visual Molecular Dynamics program (VMD).

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