This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Naphtahlene and 2,3-dihydrobenzo[b][1,4]dioxin Derivatives With Extended Side Chains as New Scaffolds of CB$_2$ Selective Ligands

Noha A. Osman$^a$, Amr H. Mahmoud$^b$, Christian D. Klein$^c$, Marco Allarà$^d$, Vincenzo Di Marzo$^d$, Khaled M. Abouzid$^b$, Ashraf H. Abadi$^a$*

$^a$Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt.

$^b$Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt.

$^c$Medicinal Chemistry, Institute of Pharmacy and Molecular Biotechnology IPMB, Heidelberg University, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany.

$^d$Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy.

*To whom correspondences should be addressed: email: ashraf.abadi@guc.edu.eg; Tel: +22-2759716; Fax: +202-27581041
Abstract

Herein, we report the synthesis of naphthalene, dihydrobenzodioxine and fluorene derivatives with extended side chains and their biological evaluation as ligands of CB₁ and CB₂ receptors. Compounds 6a and 18b showed Kᵢ values in the sub-micromolar range and relatively high selectivity indices towards CB₂. Active compounds represent new scaffolds with such type of activity. Moreover, a multiple XED-based field templates model has been constructed for activity interpretation of some of the synthesised compounds. The reported ligands can serve as a template to develop new CB₂ ligands with enhanced potency and selectivity and the computational model can be applied for SAR interpretation &/or structure optimization of any other CB₂-selective ligand(s).

Keywords: CB₂ selective ligands; naphthalene derivatives; dihydrobenzodioxine derivatives; fluorine derivatives
Introduction

The therapeutic utility as well as the recreational use of the hemp, Cannabis sativa, has been known for as long as history has been recorded. However, only during the past decade has interest in the Cannabinoid field suddenly aroused. This interest is the result of the diverse pathological conditions that can be treated by cannabinoids including fertility, learning, anxiety, pain, inflammation, cancer, emesis, appetite enhancement or suppression, and neurological disorders. Such effects have been found to be obtained by the modulation of two GPCR subtypes; CB₁ and CB₂. The CB₁ receptor is highly expressed in the brain and mediates many of the neurobehavioural and psychotropic effects of ∆⁹-tetrahydrocannabinol (THC), the primary bioactive cannabinoid of Cannabis sativa. However, the CB₂ receptors are mainly expressed by cells of the immune system, spleen and bone and can be used as a target for the treatment of inflammation, pain, immunological disorders and osteoporosis. It is thus obvious that compounds with mixed CB₁/CB₂ receptor activity elicit several therapeutic effects but produce unwanted psychotropic side effects resulting from central activation of the CB₁ receptor. For this reason research activities are currently directed towards the development of CB₂-selective ligands for their diverse therapeutic utility without eliciting neurobehavioural side effects and potential for abuse.

The primary aim of the present work was to synthesize and study the SAR of novel chemical classes of compounds that have the ability to selectively bind to CB₂ receptors. Choice of the scaffolds was based on structure of ligands previously synthesized by our group, as well as, other previously reported ligands in the literature. Ligands reported earlier were anandamide analogues obtained by modifications of the fatty acyl chain and/or the ethanolamide “tail”.

1, 2, 3, 4, 5, 6, 7, 8, 9
Since anandamide (AEA) is a very flexible molecule while tetrahydrocannabinol (THC) and cannabinoid analogues, including tricyclic and bicyclic cannabinoids, are rigid compounds\textsuperscript{7}. Our new series of compounds contain an aromatic moiety that mimics the rigid portion of THC while having a flexible chain as in AEA (Figure 1).

**Insert Figure 1**

In this study, we also constructed a multiple XED-based field templates model to interpret the activity of some of the already-synthesised ligands in the shadow of the field similarity with known selective ligands. It is worth noting that it can be used for SAR interpretation &/or structure optimization of any other CB\textsubscript{2}-selective ligand(s). The details of this model are given in the supplementary data.

**Results & Discussion**

**Chemistry**

Four different synthetic Schemes were carried out to give different new classes of compounds to be reported for the first time in literature. The newly synthesized compounds belong to one of the following classes: 5-(2-Aryl)-penta-2,4-dienoic acid amide derivatives; 6-(Aryloxy)-hexanoic acid amide derivatives; Ethanesulfonic acid [5-(aryloxy/thio)-pentyl]-amide derivatives; and 1-Ethyl-3-[5-(aryloxy/thio)-pentyl]-urea or [5-(Aryloxy/thio)-pentyl]-urea derivatives.

Reaction of the aromatic aldehyde with acetaldehyde in presence of acetic anhydride yielded the corresponding acrylaldehyde derivative. Reaction of the latter with trimethylphosphonoacetate in the presence of KHMDS as a base, THF as a solvent and at low
temperature yielded the corresponding 5-arylpenta-2,4-dienoate methyl ester (Scheme 1). As reported in literature, the Horner–Wadsworth–Emmons reaction favors the formation of E-alkenes, this is confirmed from the $^1$H-NMR spectrum of compounds 3 a-c which showed a peak corresponding to the alpha carbon proton at around 6.00 PPM with a coupling constant of 15. Hydrolysis of the ester to the acid by a base followed by coupling to the respective amine in the presence of HATU along with Hunig's base yielded the target amides 5 a, b; 6 a-c; and 7 a-c.

Reaction of the alpha or beta naphthol with ethyl bromohexanoate yielded the corresponding ether 9 a, b which upon reaction with the primary amine particularly ethanol amine yielded the corresponding amide 10 a, b (Scheme 2). To improve the yield of the side chain introduction reactions, compounds 9 a, b were hydrolyzed to the corresponding acid followed by coupling with the respective amine, yielding compounds 12 a, b and 13 a, b (Scheme 3).

Insert Schemes 1-4

Aromatic derivatives with phenolic hydroxyl or thiol are reacted with 5-bromovaleronitrile followed by reduction with LiAlH$_4$ to give the corresponding amine. The latter reacted with sodium cyanate to give the unsubstituted urea derivative 19 c, d; ethylisocyanate to give the ethyl substituted urea derivative 18 a, c or ethanesulfonyl chloride to yield the ethyl substituted sulfonamide derivative 17 a-d (Scheme 4).

1.1. Biology and molecular modeling

Membranes from HEK-293 cells transfected with the human recombinant CB$_1$ receptor ($B_{\text{max}}$=2.5 pmol/mg protein) and human recombinant CB$_2$ receptor ($B_{\text{max}}$=4.7 pmol/mg protein) were incubated with $[^3]$H-CP-55,940 (0.14 nM / $K_d$ = 0.18 nM and 0.084 nM / $K_d$ = 0.31 nM,
respectively for CB₁ and CB₂ receptor) as the high affinity ligand and displaced with 10 µM of the newly synthesized compounds. IC₅₀ values were determined for compounds showing >50% displacement at 10 µM. All compounds were tested following the procedure described by the manufacturer (Perkin Elmer, Italy). Displacement curves were generated by incubating drugs with [³H]-CP-55,940 for 90 minutes at 30 °C. Kᵢ values were calculated by applying the Cheng-Prusoff equation to the IC₅₀ values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compound. Data are means ±SEM of at least n=3 experiments⁷, Table 1.

**Insert Table 1**

Regarding the SAR of compounds having the general structure 5-(2-Aryl)-penta-2,4-dienoic acid amide 5 a,b; 6 a-c; and 7 a-c, the following could be concluded: (a) The presence of a bicyclic “head” (either naphthyl or 2,3-dihydrobenzo[b][1,4]dioxin) seems to represent optimum size for binding to the CB₂ receptor since substitution with a bulkier fluorene ring (5b, 6b, 7b) dramatically diminished the ability to bind to CB₂ receptors. (b) As per the amidic “tail”, it was found that the presence of an ethanolamide (5a) or a cyclopropylamide (6a) tail gave good CB₂ binding affinities only in compounds containing a naphthyl head and not otherwise. It is also obvious that cyclopropylamide derivatives (6a) are relatively more potent than their ethanolamide counterparts (5a), which agrees with literature data.¹⁰ On the other hand, incorporation of an ethyl glycinate ester tail showed CB₂ activity only in the presence of a 2,3-dihydrobenzo[b][1,4]dioxin head (7c) but not otherwise.
In an attempt to study the effect of the “linker length, unsaturation and heteroatom incorporation” on activity; compounds with the general structure 6-(Aryloxy)-hexanoic acid amide (Scheme 2 & 3) were synthesized. None of these proved to show any CB₂ activity despite of attaining the bicyclic head that proved to be active in Scheme 1. This might be due to the incorporation of an O atom in the “linker” between the aryl head and the amidic “tail” and increasing the linker length from a 5-atom linker (Scheme 1 compounds) to a 7-atom one. This therefore indicates that both linker length and type crucially influence CB₂ activity.

For Scheme 4 compounds, the following points can be summarized: (a) Presence of an O atom in the linker (either in the alpha position or as its regioisomer; the beta position) yielded compounds that were unable to effectively bind to CB₂ receptors, regardless of the aryl head type. (b) In contrast to this, substitution with a heteroatom S in the beta-position of the aryl head (alpha position has not been investigated) yielded compounds with relatively much higher CB₂ binding affinities (17b, 18b), independent of the tail type. (c) When the sulphonamide tail (17b) was replaced by an ethyl urea moiety (18b), the CB₂ binding affinity was greatly enhanced producing a Kᵢ that is practically less than half that of the original compound (17b).

As mentioned earlier, the design of our new chemical scaffolds was partly based on the structure of previously discovered CB₂ ligands that were reported by our group.⁷ Of these, compound XI (Figure 2) ((E)-6-Naphthalen-2-yl-4-oxohex-5-enoic acid ethanolamide) showed a log (SI) of 1 where SI is calculated as Kᵢ (CB₁)/Kᵢ (CB₂) and Kᵢ (CB₁) was >10,000 and Kᵢ (CB₂) was 1000 nM.
Field templaner was used to create, align and profiling the electronic field templates for five
reference CB2-selective ligands (supplementary information S1) and fifty four different ligands
belonging to different CB2 ligands categories (supplementary information S2-S10) to create
consensus field templates. In addition, the field templates of the compounds synthesized in this
work were also created. Extended electronic distribution (XED) field templates encode more
information than traditional pharmacophore features as it does not belong to a particular class
of ligands, and the field patterns are calculated directly from the structure of a conformation
and it considers the steric restrictions around this group and the nature of the substituents that
are attached to the molecule (donating or withdrawing groups). The developed compounds
showed maximum similarity against F29 (supplementary information, Figure S6) where, the
total similarity was 0.7. F29 is a selective CB2 ligand with the code name is MDA75 and has a log
(SI) of 1.37, with Ki (CB1) is >10,000 nM and Ki (CB2) is 422 nM. Compounds 6a and 18b have
been shown to have the highest field similarity against MDA7. This is correlated with their
biological evaluation. Thus, compound 18b showed a log (SI) of 1.3, where Ki (CB1) is >10,000
nM and Ki (CB2) is approximately 500 nM (almost half that of the previously discovered
compound, XI).

**Conclusion**

A new class of selective ligands to CB2 receptors is reported. The ligands are made up of three
parts aryl head, alkyl/alkene spacer and small alkyl/yclicic substituted amide/urea tail. The
active compounds are of similarity to the CB$_2$ selective ligand MDA7 which possesses a 3,6-disubstituted dihydrobenzofuran scaffold. This might be a way to decide about the activity of future derivatives.

**Supplementary data**

Supplementary data associated with this article includes details of the chemical synthesis, molecular modeling and biological testing.

**References**


Table 1: Results of the radioligand binding assays of the synthesized compounds

<table>
<thead>
<tr>
<th>Cpd #</th>
<th>Structure</th>
<th>Max Tested on CB1 (µM) (% displacement)</th>
<th>IC₅₀ on CB1 (µM)</th>
<th>Kᵢ on CB1 (µM)</th>
<th>Max Tested on CB2 (µM) (% displacement)</th>
<th>IC₅₀ on CB2 (µM)</th>
<th>Kᵢ on CB2 (µM)</th>
<th>Selectivity Index (Kᵢ CB₁/Kᵢ CB₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td></td>
<td>10 (+4.91%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (54.60%)</td>
<td>4.81</td>
<td>1.27</td>
<td>7.87</td>
</tr>
<tr>
<td>6a</td>
<td></td>
<td>10 (18.54%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (58.49%)</td>
<td>3.02</td>
<td>0.81</td>
<td>12.19</td>
</tr>
<tr>
<td>7a</td>
<td></td>
<td>10 (+2.24%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (48.49%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>5b</td>
<td></td>
<td>10 (0.00%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (24.17%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>6b</td>
<td></td>
<td>10 (13.62%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (36.11%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>7b</td>
<td></td>
<td>10 (+5.47%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (39.06%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>6c</td>
<td></td>
<td>10 (+6.18%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (26.47%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>7c</td>
<td></td>
<td>10 (4.77%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (55.11%)</td>
<td>9.69</td>
<td>2.56</td>
<td>3.90</td>
</tr>
<tr>
<td>10a</td>
<td></td>
<td>10 (3.65%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (3.60%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>12a</td>
<td></td>
<td>10 (44.80%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (31.37%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>13a</td>
<td></td>
<td>10 (+6.46%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (25.47%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>10b</td>
<td></td>
<td>10 (+26.97%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (14.78%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>12b</td>
<td></td>
<td>10 (24.72%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (27.84%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>13b</td>
<td></td>
<td>10 (+27.67%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (0.00%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>17a</td>
<td></td>
<td>10 (+11.94%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (23.67%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td>18a</td>
<td></td>
<td>10 (9.27%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (27.05%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td></td>
<td>Formula</td>
<td>IC50 (μM)</td>
<td>pIC50</td>
<td>IC50 (μM)</td>
<td>pIC50</td>
<td>IC50 (μM)</td>
<td>pIC50</td>
<td>IC50 (μM)</td>
</tr>
<tr>
<td>---</td>
<td>---------</td>
<td>-----------</td>
<td>-------</td>
<td>-----------</td>
<td>-------</td>
<td>-----------</td>
<td>-------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>17c</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (+7.86%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (30.86%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td><strong>18c</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (18.54%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (23.81%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td><strong>19c</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (+6.88%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (4.60%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td><strong>17b</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (23.59%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (63.31%)</td>
<td>5.02</td>
<td>1.32</td>
<td>&gt;7.58</td>
</tr>
<tr>
<td><strong>18b</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (16.85%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (68.20%)</td>
<td>2.11</td>
<td>0.56</td>
<td>&gt;17.86</td>
</tr>
<tr>
<td><strong>17d</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (3.37%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (29.50%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td><strong>18d</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (3.38%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (22.59%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td><strong>19d</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (+2.39%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (22.09%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>_</td>
</tr>
<tr>
<td><strong>Ref.</strong></td>
<td><img src="image" alt="Formula" /></td>
<td>10 (&lt;50%)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>10 (≥50%)</td>
<td>0.55</td>
<td>0.14</td>
<td>&gt;71.43</td>
</tr>
</tbody>
</table>
Figure 1: Chemical structures of the mixed CB1/CB2 ligands, viz AEA (upper left), THC (upper right) in comparison to the general skeleton of the synthesized compounds (lower).
Figure 2: The application of molecular model in activity interpretation. A: our previously discovered CB₂ ligand, XI. B: The field similarity of XI to the CB₂ selective ligand MDA-7 (interpretation of the ligand activity). C: Activity of compounds 6a and 18b (in grey) is probably due to high field similarity to MDA-7 (in blue). Color codes used to designate field templates: Cyan ball: negative ionic fields; Red balls: positive ionic fields; yellow balls: hydrophobic fields. The size of the point indicates the potential strength of the interaction.
Scheme 1

Ar
H
O
Acetaldehyde, acetic anhydride, potassium hydroxide

Ar
H
O
Trimethylphosphonoacetate

(E)

-78°C, 30min
R.T, 2hrs

NaOH
Reflux, 3hrs

Ar

HATU, 1.5eq
DIPEA, 1.5eq
NH2-R, 1.5eq
R.T, overnight

Ar

Ar:
a: naphthalen-2-yl
b: 9H-Fluoren-3-yl
c: 2,3-Dihydro-benzo[1,4]dioxin-6-yl

5a, 5b : R=-CH2-CH2-OH
6a, 6b, 6c: R=cyclopropyl
7a, 7b, 7c: R=CH2-COOC2H5

Scheme 2

Ar
OH + Br
8
acetone, reflux
72 hrs

K2CO3, KF

Ar
O
9
NH2
OH
warm at 120-130°C

Ar:
a:naphthalen-2-yl
b:naphthalen-1-yl
Scheme 3

\[
\text{Ar-O-} + \text{Br-} \quad \xrightarrow{\text{KOH, reflux 3 hrs}} \quad \text{Ar-O-} \quad \xrightarrow{\text{R-NH$_2$, HATU, DIPEA}} \quad \text{Ar-NH$_2$}
\]

Ar:
- a: naphthalen-2-yl
- b: naphthalen-1-yl

12a, 12b: R = -cyclopropyl
13a, 13b: R = -CH$_2$COOC$_2$H$_5$

Scheme 4

\[
\text{Ar-XH + Br-} \quad \xrightarrow{\text{K$_2$CO$_3$, DMF, 80°C, 2 hrs}} \quad \text{Ar-X-} \quad \xrightarrow{\text{LiAlH}_4, \text{Et}_2\text{O}} \quad \text{Ar-NH$_2$}
\]

Ar-X:
- a: naphthalen-2-yloxy
- b: naphthalen-2-ylsulfanyl
- c: naphthalen-1-yloxy
- d: benzoc[1,3]dioxol-5-yloxy

16a-d

17a-d

18a-c

19c, d

4 hrs, R.T

R = -CH$_2$COOC$_2$H$_5$