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Naphtahlene and 2,3-dihydrobenzo[b][1,4]dioxin Derivatives With Extended Side Chains as New Scaffolds of CB_2 Selective Ligands

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Abstract

Herein, we report the synthesis of naphthalene, dihydrobenzodioxine and fluorene derivatives with extended side chains and their biological evaluation as ligands of CB_1 and CB_2 receptors. Compounds **6a** and **18b** showed K_i values in the sub-micromolar range and relatively high selectivity indices towards CB_2. 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 CB_2 ligands with enhanced potency and selectivity and the computational model can be applied for SAR interpretation &/or structure optimization of any other CB_2-selective ligand(s).

Keywords: CB_2 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.[1] 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.[2] Such effects have been found to be obtained by the modulation of two GPCR subtypes; CB_1 and CB_2. The CB_1 receptor is highly expressed in the brain and mediates many of the neurobehavioural and psychotropic effects of Δ^9-tetrahydrocannabinol (THC), the primary bioactive cannabinoid of Cannabis sativa.[3] However, the CB_2 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.[1,4] It is thus obvious that compounds with mixed CB_1/CB2 receptor activity elicit several therapeutic effects but produce unwanted psychotropic side effects resulting from central activation of the CB_1 receptor.[5] For this reason research activities are currently directed towards the development of CB_2-selective ligands for their diverse therapeutic utility without eliciting neurobehavioural side effects and potential for abuse.[6]

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 CB_2 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.[7-9] Ligands reported earlier were anandamide analogues obtained by modifications of the fatty acyl chain and/or the ethanolamide “tail”.

Since anandamide (AEA) is a very flexible molecule while tetrahydrocannabinol (THC) and cannabinoid analogues, including tricyclic and bicyclic cannabinoids, are rigid compounds[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_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_{max}=2.5 pmol/mg protein) and human recombinant CB_2 receptor (B_{max}=4.7 pmol/mg protein) were incubated with [^{3}H]-CP-55,940 (0.14 nM / K_d = 0.18 nM and 0.084nM / K_d = 0.31 nM,

respectively for CB_1 and CB_2 receptor) as the high affinity ligand and displaced with 10 µM of the newly synthesized compounds. IC_{50} 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 [^{3}H]-CP-55,940 for 90 minutes at 30 ^{o}C. K_i values were calculated by applying the Cheng-Prusoff equation to the IC_{50} values (obtained by GraghPad) for the displacement of the bound radioligand by increasing concentrations of the test compound. Data are means ±SEM of at least n=3 experiments[7], 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 CB_2 receptor since substitution with a bulkier fluorene ring (**5b**, **6b**, **7b**) dramatically diminished the ability to bind to CB_2 receptors. (b) As per the amidic "tail", it was found that the presence of an ethanolamide (**5a**) or a cyclopropylamide (**6a**) tail gave good CB_2 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 CB_2 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 CB_2 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 CB_2 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 CB_2 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 CB_2 binding affinities (**17b**, **18b**), independent of the tail type. (c) When the sulphonamide tail (**17b**) was replaced by was replaced by an ethyl urea moiety (**18b**), the CB_2 binding affinity was greatly enhanced producing a K_i 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 CB_2 ligands that were reported by our group.[7] Of these, compound **XI** (Figure 2**Figure 2**) ((*E*)-6-Naphthalen-2-yl-4-oxohex-5-enoic acid ethanolamide) showed a log (SI) of 1 where SI is calculated as K_i (CB_1)/K_i (CB_2) and K_i (CB_1) was >10,000 and K_i (CB_2) was 1000 nM.

Field templater 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).[12] 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 K_i (CB_1) is >10,000 nM and K_i (CB_2) is approximately 500 nM (almost half that of the previously discovered compound, XI).

Insert Figure 2

Conclusion

A new class of selective ligands to CB_2 receptors is reported. The ligands are made up of three parts aryl head, alkyl/alkene spacer and small alkyl/alicyclic 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.

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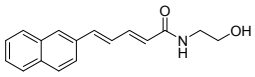
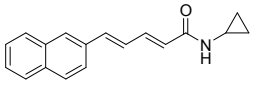
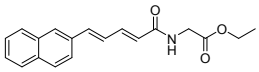
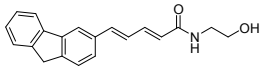
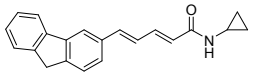
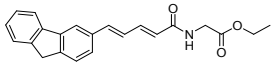
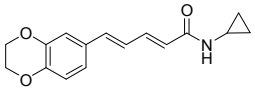
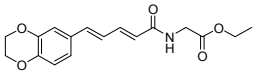
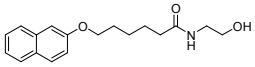
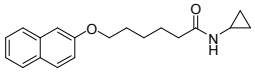
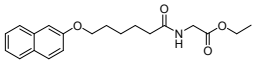
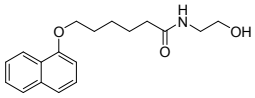
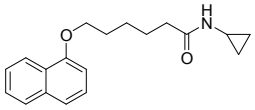
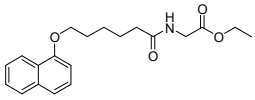
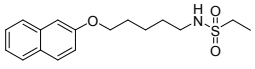
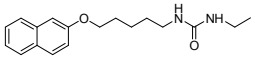
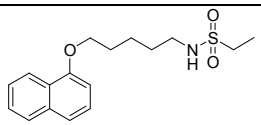
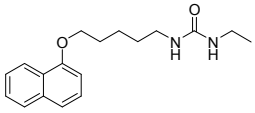
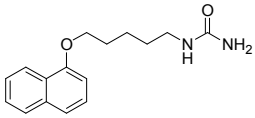
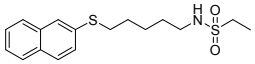
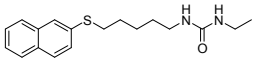
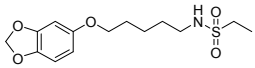
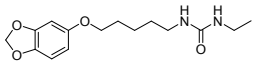
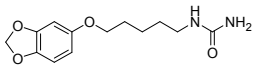
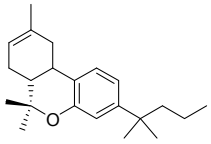
Table 1: Results of the radioligand binding assays of the synthesized compounds

Cpd #	Structure	Max Tested on CB_1(μM) (%displacement)	IC_{50} on CB_1 (μM)	K_i on CB1 (μM)	Max Tested on CB_2 (μM) (%displacement)	IC_{50} on CB_2 (μM)	K_i on CB_2 (μM)	Selectivity Index(K_i CB_1/K_i CB_2)
5a		10 (+4.91%)	>10	>10	10 (54.60%)	4.81	1.27	>7.87
6a		10 (18.54%)	>10	>10	10 (58.49%)	3.02	0.81	>12.19
7a		10 (+2.24%)	>10	>10	10 (48.49%)	>10	>10	_
5b		10 (0.00%)	>10	>10	10 (24.17%)	>10	>10	_
6b		10 (13.62%)	>10	>10	10 (36.11%)	>10	>10	_
7b		10 (+5.47%)	>10	>10	10 (39.06%)	>10	>10	_
6c		10 (+6.18%)	>10	>10	10 (26.47%)	>10	>10	_
7c		10 (4.77%)	>10	>10	10 (55.11%)	9.69	2.56	>3.90
10a		10 (3.65%)	>10	>10	10 (3.60%)	>10	>10	_
12a		10 (44.80%)	>10	>10	10 (31.37%)	>10	>10	_
13a		10 (+6,46%)	>10	>10	10 (25.47%)	>10	>10	_
10b		10 (+26.97%)	>10	>10	10 (14.78%)	>10	>10	_
12b		10 (24.72%)	>10	>10	10 (27.84%)	>10	>10	_
13b		10 (+27.67%)	>10	>10	10 (0.00%)	>10	>10	_
17a		10 (+11.94%)	>10	>10	10 (23.67%)	>10	>10	_
18a		10 (9.27%)	>10	>10	10 (27.05%)	>10	>10	_

17c		10 (+7.86%)	>10	>10	10 (30.86%)	>10	>10	_
18c		10 (18.54%)	>10	>10	10 (23.81%)	>10	>10	_
19c		10 (+6.88%)	>10	>10	10 (4.60%)	>10	>10	_
17b		10 (23.59%)	>10	>10	10 (63.31%)	5.02	1.32	>7.58
18b		10 (16.85%)	>10	>10	10 (68.20%)	2.11	0.56	>17.86
17d		10 (3.37%)	>10	>10	10 (29.50%)	>10	>10	_
18d		10 (3.38%)	>10	>10	10 (22.59%)	>10	>10	_
19d		10 (+2.39%)	>10	>10	10 (22.09%)	>10	>10	_
Ref.		10 (<50%)	>10	>10	10 (≥50%)	0.55	0.14	>71.43

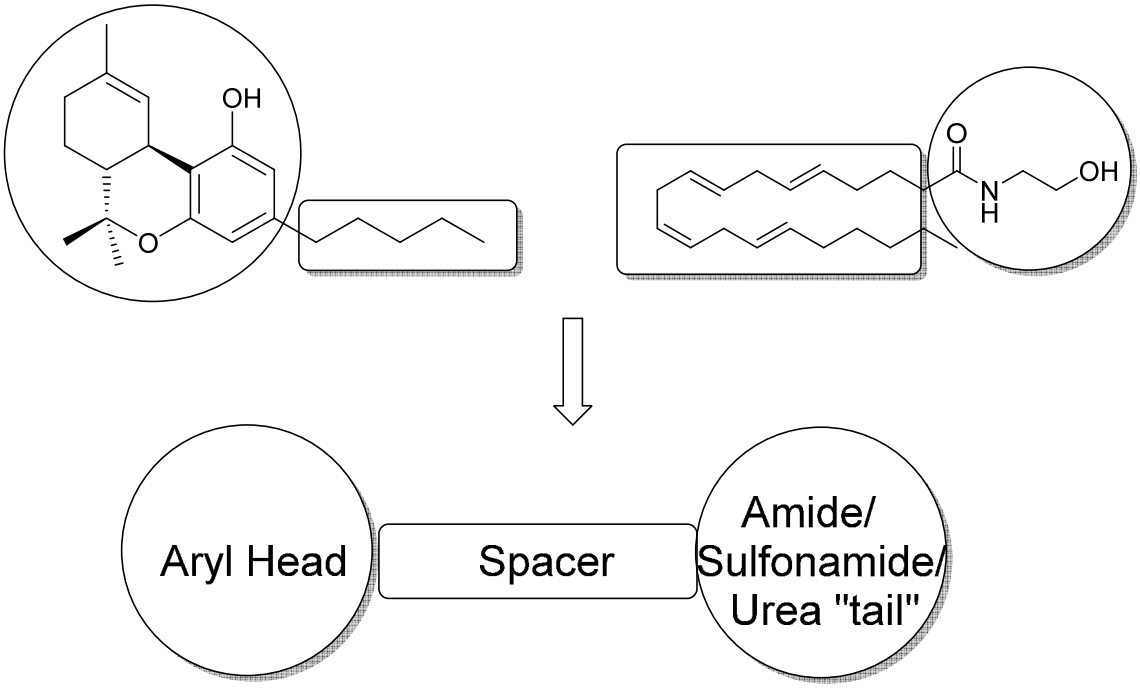


Figure 1: Chemical structures of the mixed CB_1/CB_2 ligands, viz AEA (upper left), THC (upper right) in comparison to the general skeleton of the synthesized compounds (lower).

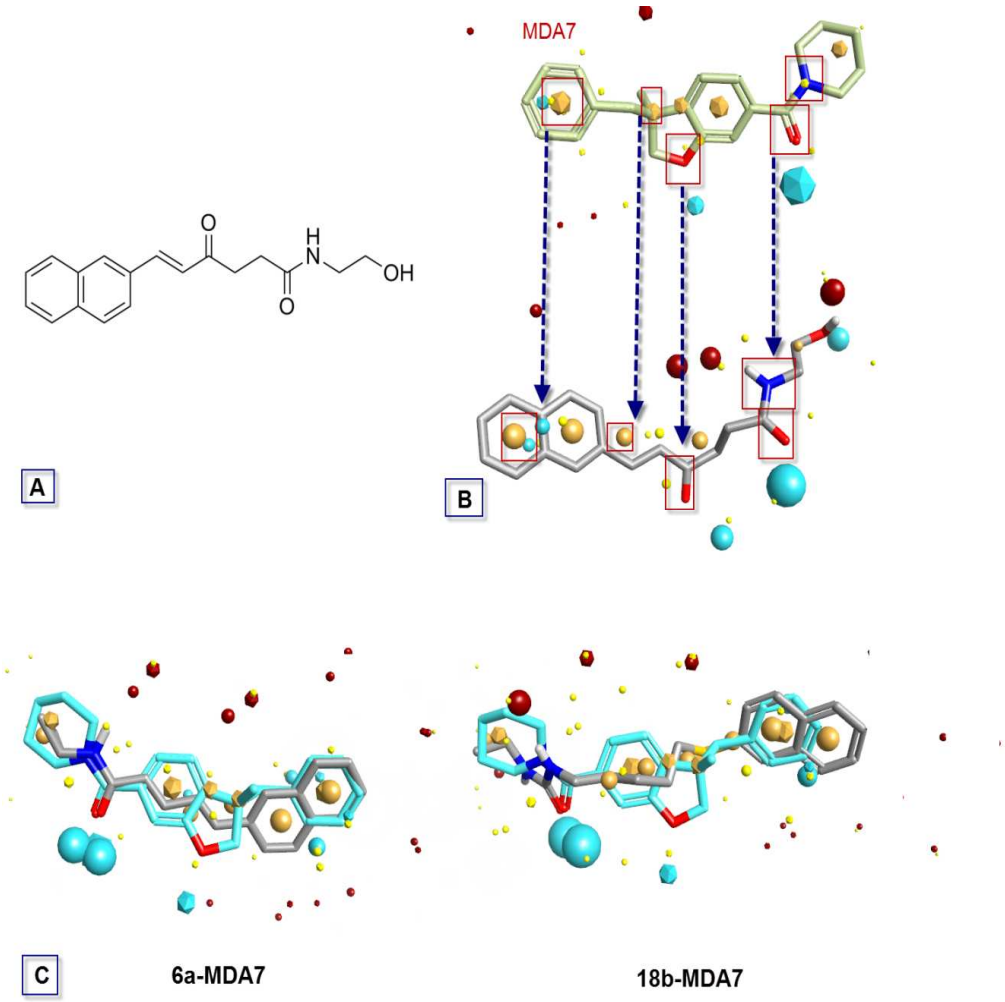
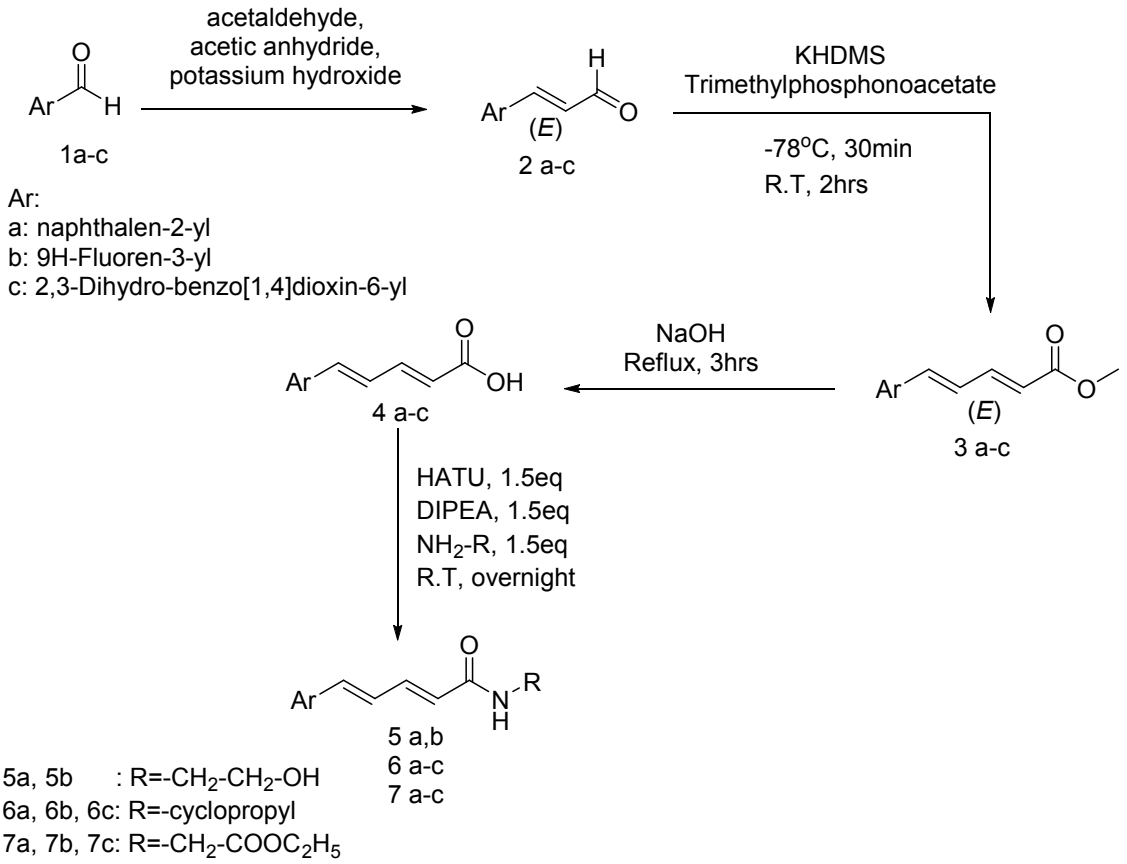


Figure 2: The application of molecular model in activity interpretation. A: our previously discovered CB_2 ligand, XI. B: The field similarity of XI to the CB_2 selective ligand MDA-7 (interpretation of the ligand activity). C: Activity of compounds 6a and 18b (in grey) is probably due to high field 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.

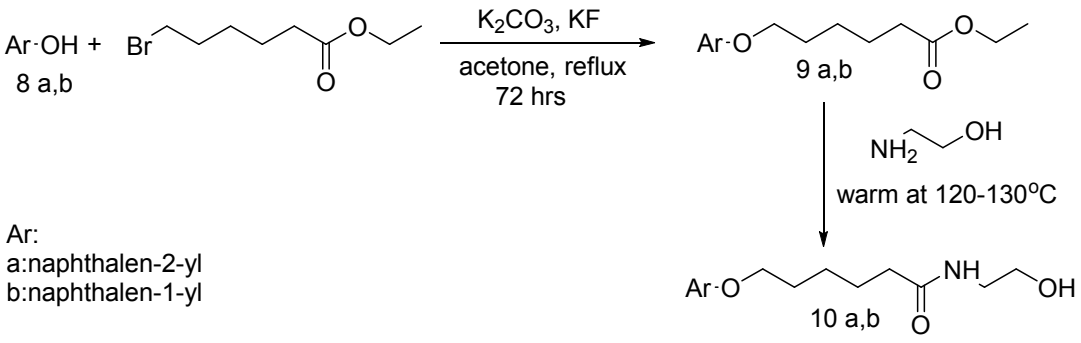
Scheme 1



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

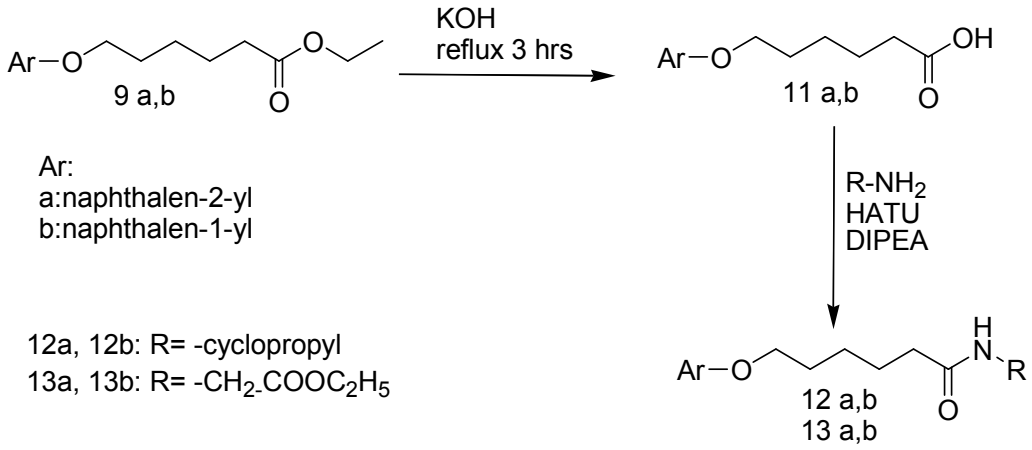
5a, 5b : R=-CH_2-CH_2-OH
6a, 6b, 6c: R=-cyclopropyl
7a, 7b, 7c: R=-CH_2-$COOC_2H_5$

Scheme 2



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

Scheme 3



Scheme 4