Rapid Combinatorial Access to a Library of 1,5-Disubstituted-3-indole-*N*-alkylacetamides as CB2 Receptor Ligands

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The indole ring system represents a "privileged structure" in drug discovery. The number of bioactive compounds containing this ring system is so vast that the complete range of their biological activities can be hardly classified.[1]

Cannabinoid receptor ligands possess interesting therapeutic potential in a number of pathological conditions, such as pain, immunosuppression, peripheral vascular disease, appetite regulation, and locomotor disorders.[2] Currently, cannabinoid type 1 receptor (CB1) antagonists/inverse agonists are evaluated for obesity, metabolic disorders, smoking cessation, and alcohol abuse.[3,4] Rimonabant (Acomplia) is a potent and selective antagonist for the CB1 receptor that was launched in Europe in 2006 by Sanofi-Aventis for the treatment of obesity and associated risk factors,[5,6] although the suspension of its marketing authorization in October 2008 has stressed once again the need for more effective and safer drugs targeting the endocannabinoid system. Selective CB2 ligands may be used to treat pain, inflammation, osteoporosis, CB2-expressing malignant gliomas, tumors of immune origin, immunological disorders such as multiple sclerosis, and neurodegenerative disorders such as Alzheimer's disease.[7] Some representatives of CB2 agonists are, for example, compounds **1** (AM1241), **2** (GW405833), and **3** (JWH-015), that possess an indole structure,[7,8] while **4** (AM630) is a cannabinoid receptor inverse agonist (Figure 1).

The intense and growing medicinal chemistry effort in the area of CB2 ligands resulted in a great number of compounds characterized by a wide chemical diversity and different biochemical profile. Even modest modifications of the ligand structure may alter its receptor binding affinity or selectivity, as well as shift its functional activity from agonist to inverse agonist/antagonist. That may explain why highly selective full agonists at CB2 receptor are still in development and are generally not widely available. To this aim, while optimization of known CB2 agonists in terms of efficacy and selectivity toward the different receptor subtypes is an important task that needs to be addressed, on the other hand the identification of new CB2 ligands may open the avenue to the discovery of highly selective CB2 agonists.

In our research program on new cannabinoid receptor modulators,[9–11] we decided to investigate the possibility to identify novel CB2 ligands starting from commercially available 5-hydroxyindole-3-acetic acid (Figure 2). This scaffold offers three points (COOH, OH, NH groups) to enhance chemical diversity through chemical manipulations in an easy, rapid, and profitable way. In particular, we envisioned the possibility to generate a small library of compounds by converting the carboxylic acid functionality into a secondary or tertiary amide group and performing selective alkylation reactions of the OH and NH moieties using benzyl or allyl halides.

A careful analysis of the literature revealed that a limited number of examples regarding *N*,*O*-dialkylated 5-hydroxyindole derivatives are known, all involving the *N*-alkylation of the previously isolated *O*-alkylated product.[12]

Therefore, we became interested in developing an efficient one-pot, two-step *N*,*O*-dialkylation protocol which, in principle, could allow the rapid generation of a library of 1,5-difunction-

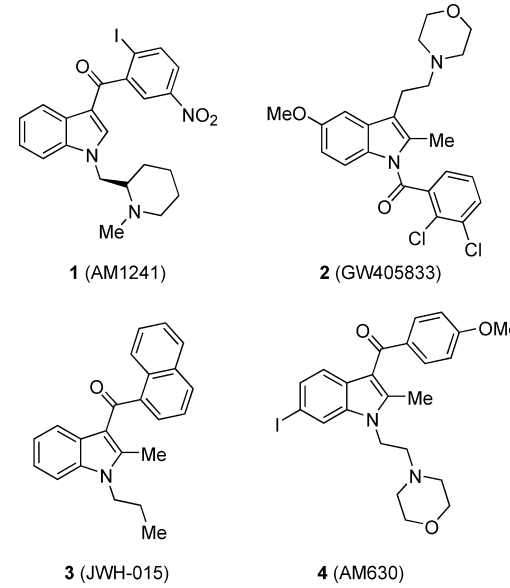

Figure 1. CB2 ligands characterized by an indole core.

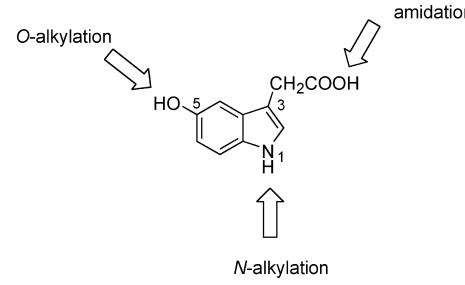

Figure 2. 5-Hydroxyindole-3-acetic acid: points for enhancing chemical diversity.

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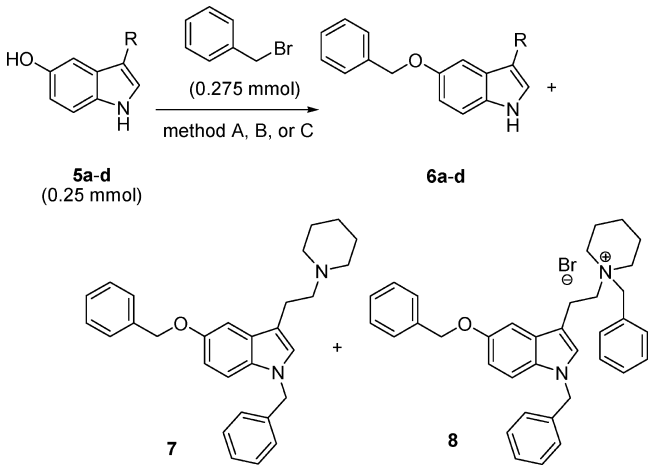
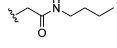
Scheme 1[a]



[a] Reagents and conditions. Method A: Bu_4NBr (0.125 mmol), DCM, 20% aq. NaOH, rt. Method B: K_2CO_3 (0.5 mmol), acetone, reflux. Method C: NaH (0.3 mmol), DMF, rt.

Table 1. Optimization of the Selective *O*-Alkylation Reaction

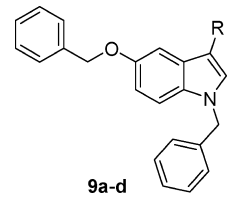
Entry	Substrate	R	Method[a]	Reaction time (h)	Product (yield, %)	Starting material/ byproduct (yield, %)
1	**5a**[13]	H	A	2	**6a** (90)	-
			B	48	**6a** (85)	-
			C	4	**6a** (65)	-
2	**5b**[14]		A	1	**6b** (80)	-
			B	48	**6b** (75)	**5b** (19)
			C	24	**6b** (72)	**5b** (16)
3	**5c**[14]		A	0.5	**6c** (88)	-
			B	48	**6c** (77)	**5c** (19)
			C	24[b]	**6c** (52)	**5c** (17)
4	**5d**[15]		A	0.5	**6d** (53)	**7** (3)
			B	48	-	**8** (33)
			C	24	-	**7** (25) and **8** (35)

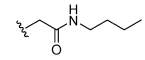
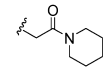
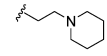
[a] Method A: Bu_4NBr (0.125 mmol), DCM, 20% aq. NaOH, rt. Method B: K_2CO_3 (0.5 mmol), acetone, reflux. Method C: NaH (0.3 mmol), DMF, rt. [b] 22 h at rt, then 2 h at 80 °C.

alized indole derivatives. To set up a selective *O*-alkylation procedure, we first explored the OH group alkylation of four different 5-hydroxyindole derivatives (**5a**-**d**)[13–15] using benzyl bromide and three different alkylating approaches (Scheme 1): Bu_4NBr in 20% aq NaOH/DCM at room temperature (method A), K_2CO_3/acetone at reflux temperature (method B), and NaH/DMF at room temperature (method C). The results are summarized in Table 1.

The *O*-alkylation of **5a**−**c** occurred smoothly in short times and high yield when method A was followed, whereas when method B or C was used, reaction times were longer and yields were lower. Moreover, no complete conversion of compounds **5b** and **5c** through method B or C was achieved, leading to the recovery of the starting material in moderate yield (entry 2 and 3). The presence of unreacted starting material represents a problem when a second alkylation step has to be carried out without isolating the first reaction product, as in this case the first alkylation reaction should be quantitative. On the other hand, the conversion of **5d** to **6d** was obtained only through method A (entry 4). Indeed,

Table 2. Optimization of the Dialkylation Reaction



Entry	Substrate	R	Product	Yield (%)
1	**5a**	H	**9a**	73
2	**5b**		**9b**	67
3	**5c**		**9c**	63
4	**5d**		**9d**	17

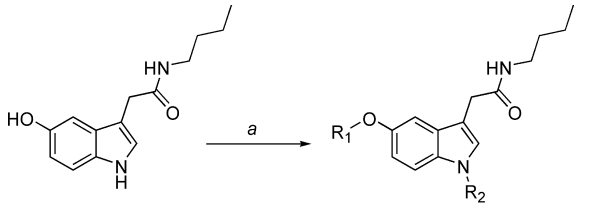
following either method B or C, the exclusive formation of the dialkylated compound **7** and the ammonium salt **8** was observed (Scheme 1). As a result, method A proved to be the optimal *O*-alkylation protocol in terms of time, yield, and compounds purity.

In the next step, we investigated the ability of this phase transfer catalyzed reaction to alkylate the indole nitrogen regioselectively, without affecting the amide NH and amino groups in the side chain. Accordingly, derivatives **5a**−**d**, used as test compounds, were treated with 0.25 mmol of benzyl bromide according to method A; when reaction was complete (TLC control using authentic samples of **5a**-**d**), further 0.275 mmol of benzyl bromide (for sake of simplicity the same alkylating agent was used) were added and the reaction was carried on for 18 h. Compounds **9a**−**c** were obtained in good yield (63−73%), while **9d** was obtained in only 17% yield accompanied by many unidentified degradation products (Table 2). On the basis of these findings, both secondary and tertiary amides proved to be suitable substrates for regioselective *N,O*-dialkylation reaction.

Accordingly, we extended this one-pot, two-step alkylation methodology to the synthesis of compounds **10a**−**c** characterized by different *N,O*-dialkylation patterns in order to verify its applicability to a combinatorial approach. To this aim, compound **5b** was selected as test substrate and was subjected to three different *N,O*-dialkylation reactions (Table 3) using allyl bromide, 4-chlorobenzyl chloride, and 4-methoxybenzyl chloride as the alkylating agents in step 1, and 4-bromobenzyl bromide, allyl bromide, and 3-chlorobenzyl bromide as alkylating agents in step 2, respectively. In this way, compounds **10a**−**c** were obtained in acceptable to good yield, thus confirming the feasibility of our approach. Once the methodology was set up, it was applied to the synthesis of the target compounds **11**−**13** (Scheme 2, Table 4−6).

On the basis of our previous findings on amide derivatives as cannabinoid receptors ligands,[9,10] morpholine, 1-aminoadamantane, and cyclohexylamine were selected for the amidation reaction on 5-hydroxyindole-3-acetic acid. Allyl bromide, 4-bromobenzyl bromide, 3-methoxybenzyl chloride, and 4-(bromomethyl)pyridine were chosen as *N,O*-alkylating

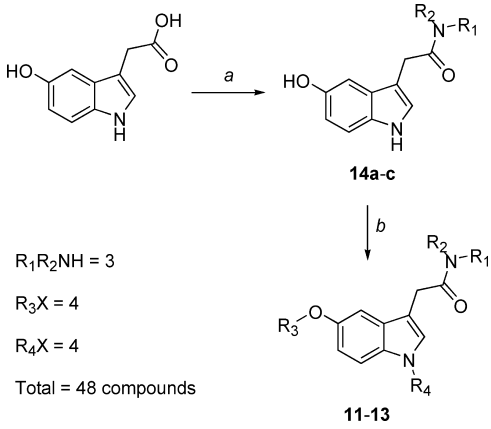
Table 3. One-Pot, Two-Step *N,O*-Dialkylation of Compound **5b**[a]



entry	product	R_1	R_2	yield (%)
1	**10a**	Allyl	*p*-Br-benzyl	66
2	**10b**	*p*-Cl-benzyl	allyl	63
3	**10c**	*p*-CH_3O-benzyl	*m*-Cl-benzyl	55

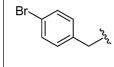
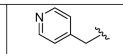
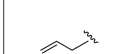
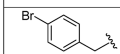
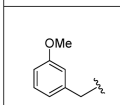
[a] Reagents and conditions: (a) 1) Bu_4NBr, 20% NaOH/DCM, R_1X (1 equiv); 2) R_2X (1.1 equiv).

Scheme 2[a]



[a] Reagents and conditions: (a) R_1R_2NH, EDC, HOBt, DCM; (b) (1) Bu_4NBr, 20% NaOH/DCM, R_3X (1 equiv), (2) R_4X (1.1 equiv). R_1R_2NH: morpholine, 1-aminoadamantane, cyclohexylamine. R_3X and R_4X: 4-(bromomethyl)pyridine, allyl bromide, 4-bromobenzyl bromide, 3-methoxybenzyl chloride.

Table 4. Structure and CB1/CB2 Binding Data (K_i, μM) of Compounds **11**

		R_4			
11					
R_3		**11a**	**11b**	**11c**	**11d**
		**11e**	**11f**	**11g** CB1 = 2.04 CB2 = 1.34	**11h**
		**11i**	**11j**	**11k**	**11l**
		**11m**	**11n** CB1 = 0.080 CB2 = 1.44 SI = 0.05	**11o**	**11p**

agents because of their high chemical reactivity and with the aim of introducing further π-systems into the structure of the final compounds.

A parallel solution-phase synthesis was exploited for the amidation reaction, which was used to introduce the first point of diversity into the indole scaffold. 5-Hydroxyindole-3-acetic acid was portioned into three reaction vessels of a Büchi Syncore synthesizer and reacted with three different amines (morpholine, 1-aminoadamantane, and cyclohexylamine, respectively) in the presence of EDC and HOBt. Excess reagents and the reaction byproduct were removed from the reaction mixture by the use of appropriate scavengers, such as morpholinomethyl-polystyrene and polymer bound *p*-toluenesulfonic acid for acidic and basic substances, respectively; amides **14a**−**c** were obtained as pure compounds by filtration, followed by crystallization. This approach allowed for a rapid access to the target compounds avoiding either aqueous workup, which proved to be troublesome due to partial precipitation of the compounds, or chromatographic purification.

To extend the chemical diversity beyond the C3 position, amides **14a**−**c** were submitted to the one-pot, two-step *N,O*-dialkylation reaction following the synthetic protocol previously developed. Being the reaction outcome for phase transfer catalyzed reactions strictly dependent on the efficiency of stirring, the reactions were carried out using Carousel reaction stations which could ensure the appropriate mixing of the two phases. Each amide was partitioned into sixteen reaction vessels and was dialkylated combining four different alkylating agents; accordingly, a library of 48 compounds was obtained containing the three desired point of diversity at positions 1, 3, and 5 of the indole system with a total yield, after chromatographic purification, ranging from 42 to 74%.

A selection of compounds **11**−**13** were submitted to preliminary biological tests in order to evaluate their ability to bind to cannabinoid receptors. Since in our experience morpholine derivatives, such as **11**, are scarcely effective CB2 ligands, we focused our attention mainly on compounds **12** and **13**. The binding affinities (K_i values) for human recombinant CB1 and CB2 receptors are reported in Table 4−6.

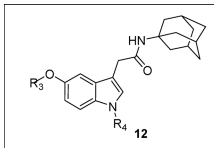
Most of the tested compounds did not show significant binding affinity toward CB2 receptor subtype, eliciting K_i values >10 μM. However, compounds **12b**, **12f**, **12h**, **12n** (Table 5), and **13n** (Table 6) proved to be selective CB2 ligands with K_i values in the range 0.70−0.16 μM and selectivity indices (SI = K_iCB1/K_iCB2) >13.9.

As far as the CB1 affinity is concerned, only one of the tested compounds displayed significant activity for this receptor subtype: compound **11n** elicited K_iCB1 = 0.08 μM and SI = 0.05 proving to be the highest affinity derivative.

It is interesting to note that, with the exception of compound **12b**, regardless of the type of the amide substituent, all the active derivatives are characterized by the presence of the allyl and 3-methoxybenzyl moieties. In particular, three (**11n**, **12f** and **13n**) out of five present the allyl and 3-methoxybenzyl substituents at the position 1 and 5, respectively.

Results reported herein derived from a preliminary biological screening regarding the CB2 receptor affinity. More

Table 5. Structure and CB1/CB2 Binding Data (K_i, μM) of Compounds **12**



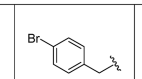
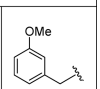
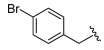
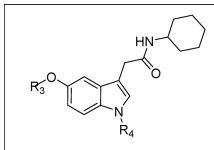
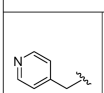
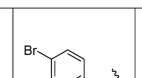
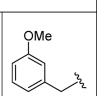
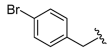
R_3 \ R_4				
	**12a**	**12b** CB1 = 2.42 CB2 = 0.16 SI = 15.1	**12c**	**12d**
	**12e** CB1 > 1.00 CB2 = 1.01	**12f** CB1 = 4.25 CB2 = 0.17 SI = 25.0	**12g** CB1 >10.00 CB2 = 1.48	**12h** CB1 >10.00 CB2 = 0.63 SI > 15.87
	**12i**	**12j** CB1 >10.00 CB2 = 3.37	**12k** CB1 >10.00 CB2 >10.00	**12l** CB1 >10.00 CB2 >10.00
	**12m**	**12n** CB1 >10.00 CB2 =0.72 SI > 13.88	**12o** CB1 >10.00 CB2 >10.00	**12p** CB1 >10.00 CB2 >10.00

Table 6. Structure and CB1/CB2 Binding data (K_i, μM) of Compounds **13**



R_3 \ R_4				
	**13a** CB1 >10.00 CB2 >10.00	**13b** CB1 = 4.63 CB2 = 4.21	**13c** CB1 >10.00 CB2 >10.00	**13d**
	**13e** CB1 >10.00 CB2 = 2.30	**13f** CB1 >10.00 CB2 >10.00	**13g** CB1 = 1.50 CB2 = 1.10	**13h**
	**13i**	**13j** CB1 >10.00 CB2 = 7.52	**13k** CB1 >10.00 CB2 >10.00	**13l**
	**13m** CB1 = 1.66 CB2 = 2.16	**13n** CB1 >10.00 CB2 = 0.40 SI >25.0	**13o** CB1 >10.00 CB2 >10.00	**13p**

extensive studies on the whole set of compounds and on the functional activity of the most significant derivatives are ongoing.

In conclusion, starting from 5-hydroxyindole-3-acetic acid we have described the rapid combinatorial synthesis of a library of 48 *N*,*O*-dialkylated-5-hydroxy-3-indole-*N*-alkylacetamides as potential cannabinoid ligands. After the amidation step, the development of an efficient and selective methodology for the one-pot, two-step alkylation allowed the facile functionalization of the molecule at position 1 and 5, leading to compounds characterized by different *N*,*O*-dialkylation patterns. Preliminary biological investigation on selected compounds highlighted the combination allyl/3-methoxybenzyl moieties as the most profitable one for the receptor affinity of the molecules.

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Supporting Information Available. Synthesis and characterization of all the new compounds and methods for their biological analysis. This information is available free of charge via the Internet at http://pubs.acs.org.

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