# Design, Synthesis, and Pharmacological Characterization of Indol-3ylacetamides, Indol-3-yloxoacetamides, and Indol-3-ylcarboxamides: Potent and Selective CB2 Cannabinoid Receptor Inverse Agonists

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#### **(5)** Supporting Information

**ABSTRACT:** In our search for new cannabinoid receptor modulators, we describe herein the design and synthesis of three sets of indole-based ligands characterized by an acetamide, oxalylamide, or carboxamide chain, respectively. Most of the compounds showed affinity for CB2 receptors in the nanomolar range, with  $K_i$  values spanning 3 orders of magnitude (377–0.37 nM), and moderate to good selectivity over CB1 receptors. Their in vitro functional activity as inverse agonists was confirmed in vivo in the formalin test of acute peripheral and inflammatory pain in mice, in which



compounds 10a and 11e proved to be able to reverse the effect of the CB2 selective agonist COR167.

# INTRODUCTION

Cannabinoid receptors 1 and 2 (CB1 and CB2) are G proteincoupled receptors that were characterized and cloned in the early 1990s.<sup>1,2</sup> While CB1 receptors are expressed at high levels in the central nervous system (CNS), CB2 receptors are primarily found in the immune system, in tonsils, spleen, macrophages, and lymphocytes (B-cells and natural killer cells),<sup>3</sup> although there is some evidence of their presence also in the CNS.<sup>4</sup>

Agonists of both cannabinoid receptor types produce strong antinociceptive effects in animal models of chronic, neuropathic, and inflammatory pain and are intensively investigated as potential new antihyperalgesic and antiinflammatory agents.<sup>5</sup> Activation of central CB1 receptors, rather than peripheral CB1 or CB2 receptors, is thought to mediate the psychotropic effects associated with nonselective agonists such as  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC).<sup>6</sup> The occurrence of these adverse effects limits the therapeutic usefulness of mixed cannabinoid agonists that show high affinity for CB1 receptors. Differences in receptor distribution and signal transduction mechanisms<sup>7,8</sup> are likely to account for the relative absence of CNS side effects induced by CB2 agonists, which, in addition to pain, are also being actively investigated for use in a multitude of disparate diseases and pathological conditions,<sup>9</sup> ranging from atherosclerosis,<sup>10</sup> myocardial infarction,<sup>11</sup> stroke,<sup>12</sup> gastrointestinal inflammatory,<sup>13</sup>and autoimmune<sup>14</sup> and neurodegenerative disorders<sup>15</sup> to hepatic ischemia/reperfusion injury,<sup>16</sup> inflammation<sup>17</sup> and fibrosis,<sup>18</sup> kidney<sup>19</sup> and bone<sup>20</sup> disorders, and cancer.<sup>21</sup>

The indole nucleus represents a "privileged structure" in medicinal chemistry<sup>22</sup> and a well-established scaffold for the development of CB2 receptor ligands.<sup>23</sup> Pioneering work at the Sterling-Winthrop Research Institute, leading to the potent but nonselective ligand  $1^{24}$  (Chart 1), was followed by the development of CB2-selective ligands 2 by Huffmann et al.<sup>25</sup> and 3 by Makriyannis and co-workers<sup>26</sup>. CB2-Selective agonists as well as inverse agonists (exemplified by  $4^{27}$  and 5,<sup>28</sup> respectively) were obtained by fine chemical modulation of the prototypical structures, while a ground-breaking work by Frost et al.<sup>23</sup> demonstrated that indol-3-ylcycloalkyl ketones, such as 6, lacking an aromatic acyl group, are new high-affinity CB2 ligands.

In our research program on new cannabinoid receptor modulators,  $^{29}$  we have described the rapid combinatorial

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#### Chart 1. Representative Indole-Based CB2 Ligands



synthesis, starting from 5-hydroxyindole-3-acetic acid, of a library of N,O-dialkylated 5-hydroxy-3-indole-*N*-alkylacetamides, some of which (e.g., 7, Chart 2) proved to be weak

Chart 2. Prototypical Structures (7, 8) and Novel 5-(Hetero)arylindole-Based Acetamides (9), Oxoacetamides (10), and Carboxamides (11)



but selective CB2 ligands.<sup>30</sup> Here we describe our investigations aimed at identifying new indole-based compounds with enhanced affinity and/or selectivity for the CB2 receptor.

# RESULTS AND DISCUSSION

**Compound Design and Selectivity Prediction.** Structural modifications were planned to reduce the conformational freedom of lead compound 7, thereby modifying receptor selectivity and affinity according to a popular tactic in medicinal chemistry.<sup>31</sup> On the other hand, the choice of the substituents to decorate the indole scaffold was suggested by results obtained in our previous studies on quinolone cannabinoid ligands.

Accordingly, the new compounds were designed through an approach that, acknowledging the structure–affinity relationship developed for quinolone-3-carboxamide CB2 ligands<sup>29a,f,i</sup> (such as 8), entailed (i) the removal of the linker between the aromatic rings of 7 in order to obtain 5-(hetero)arylsubstituted indoles 9–11, more closely mimicking 8, and (ii) either preserving the acetamide moiety (9) or replacing it with oxalylamide (10) and carboxamide (11) side chains. We have recently developed a pharmacophore based three-dimensional quantitative structure-selectivity relationship model (3D-QSSR) able to predict in a semiquantitative manner the selectivity index (SI) of novel CB2 receptor ligands belonging to several structural classes.<sup>29h</sup> Herein, we report the prediction of the SI of three molecules, taken as representative samples of structural classes 9, 10, and 11, by application of our selectivity model. The outcome of this prediction was expected to possibly support the approach we had chosen to design CB2-selective 5-(hetero)arylsubstituted indoles and to provide a theoretical grounding to rationalize the subsequent pharmacological results.

The SI of prototypes 9i, 10b, and 11d (Table 1) was predicted by application of our 3D-QSSR model after a conformational search was performed on these derivatives and their conformers were aligned onto the CB2 pharmacophore CB2PHAM.<sup>29h</sup> The results of the in silico selectivity prediction are shown in Table 1. All three derivatives 9j, 10b, and 11d were predicted to be selective, even if at a different extent. Compounds 10b and 11d were estimated to possess similar selectivities (corresponding to an activity difference between CB1 and CB2 receptors of at least 2 orders of magnitude) and to be roughly 10 times more selective than 9i. Accordingly, compounds 9j, 10b, and 11d were synthesized and tested. Comparison between calculated and experimental SI values for the three compounds confirmed the prediction ability of our model and hence all the designed derivatives 9-11 (as well as compounds 13 and 14) were checked by theoretical estimation of their SI. As can be seen in Table 1, for all these compounds the calculated SI values were larger than 10. On the basis of these results, the preparation of the whole set of compounds 9-11 was confidently undertaken.

**Chemistry.** The preparation of indole derivatives **9** was accomplished by the route shown in Scheme 1, following synthetic procedures already set up for 3-indole-*N*-alkylacetamide<sup>30</sup> and quinolone-3-carboxamide analogues.<sup>29a,fi</sup> 5-Bromo-1*H*-indole-3-acetic acid was converted into the corresponding amides **12a** and **12b** 

(method A), which then underwent N-alkylation with the appropriate alkyl halide under phase-transfer catalysis (PTC) conditions (method B) to yield compounds 13a and 13b,c, respectively. Suzuki coupling (method C) with furan-2-ylboronic acid provided the final compounds 9a-c. With the aim to enhance chemical

| $compd^b$      | CB1 <sup>c,e</sup>            | CB2 <sup>d,e</sup>  |                 |                  |                   | $\operatorname{compd}^{b}$ | CB1 <sup>c,e</sup>            | $\mathrm{CB2}^{d,e}$ |                 |                  |                   |
|----------------|-------------------------------|---------------------|-----------------|------------------|-------------------|----------------------------|-------------------------------|----------------------|-----------------|------------------|-------------------|
|                | $K_i^f(\mathbf{n}\mathbf{M})$ | K <sub>i</sub> (nM) | SI <sup>g</sup> | log SI<br>(exp.) | log SI<br>(calc.) |                            | $K_i^f(\mathbf{n}\mathbf{M})$ | K <sub>i</sub> (nM)  | $\mathrm{SI}^g$ | log SI<br>(exp.) | log SI<br>(calc.) |
| Pa<br>9a       | )<br>>10000                   | 377                 | > 26            | 1.42             | 1.16              | 10a                        | 344.9                         | 0.37                 | 932.2           | 2.97             | 1.96              |
| 9b             | 560                           | 50                  | 11.2            | 1.05             | 1.26              | 10b                        | > 10000                       | 12.7                 | > 787           | 2.90             | 2.05              |
| 9c             | 1660                          | 110                 | 15.1            | 1.18             | 1.63              |                            | 3617                          | 10.3                 | 351.2           | 2.55             | 2.00              |
| Pd             | 488                           | 98                  | 5.0             | 0.70             | 1.35              | IOd                        | 3915                          | 25.4                 | 154.1           | 2.19             | 1.80              |
| Ad<br>De<br>De | 698                           | 67                  | 10.4            | 1.02             | 1.28              |                            | > 10000                       | 11.2                 | > 892           | 2.95             | 2.06              |
| 9f             | 1508                          | 11.8                | 127.8           | 2.11             | 1.28              |                            | 48.2                          | 1.82                 | 26.5            | 1.42             | 1.72              |
| A C C C C N Ad | 530                           | 50                  | 10.6            | 1.03             | 1.26              |                            | > 10000                       | 22.2                 | > 450           | 2.65             | 1.86              |
| 9g             | > 10000                       | 20.5                | > 487           | 2.69             | 1.15              |                            | 2794                          | 5.73                 | 487.6           | 2.69             | 2.00              |
| 9h             | 1827                          | 17.8                | 102.6           | 2.01             | 1.14              | 11d                        | 1052                          | 11.6                 | 90.7            | 1.96             | 2.08              |
| 9i             | 2380                          | 70                  | 34              | 1.53             | 1.38              |                            | 341.6                         | 2.48                 | 137.7           | 2.14             | 2.30              |
| 9j             | > 10000                       | 189                 | > 52            | 1.72             | 1.98              |                            | 3621                          | 6.37                 | 568.4           | 2.75             | 2.13              |

С

# Table 1. CB1 and CB2 Receptor Affinity Values for Compounds 9a-k, 10a-e, 11a-g, 13b,c, and 14<sup>a</sup>

| Table 1. continued |                            |                      |                 |                  |                   |                                       |                               |                      |                 |                  |                   |
|--------------------|----------------------------|----------------------|-----------------|------------------|-------------------|---------------------------------------|-------------------------------|----------------------|-----------------|------------------|-------------------|
| $compd^b$          | $\operatorname{CB1}^{c,e}$ | $\mathrm{CB2}^{d,e}$ |                 |                  |                   | $compd^b$                             | CB1 <sup>c,e</sup>            | $\mathrm{CB2}^{d,e}$ |                 |                  |                   |
| _                  | $K_i^f(\mathrm{nM})$       | K <sub>i</sub> (nM)  | $\mathrm{SI}^g$ | log SI<br>(exp.) | log SI<br>(calc.) |                                       | $K_i^f(\mathbf{n}\mathbf{M})$ | $K_i(nM)$            | $\mathrm{SI}^g$ | log SI<br>(exp.) | log SI<br>(calc.) |
| Ilg                | 2.03                       | 3.52                 | 0.6             | -0.23            | 2.06              | Br CI N H Ad                          | 5510                          | 170                  | 32.4            | 1.51             | 1.58              |
| Br C N Ad          | > 10000                    | 1090                 | > 9             | 0.96             | 1.56              | H <sup>Ad</sup>                       | > 10000                       | 70.8                 | > 141           | 2.15             | 1.79              |
| 130                |                            |                      |                 |                  |                   | SR144528 <sup><i>h</i>,<i>i</i></sup> | >2820                         | 5.4                  | >522            | 2.72             | N.C               |
|                    |                            |                      |                 |                  |                   | Rimonabant <sup>h,j</sup>             | 12.0                          | 790                  | 0.015           | -1.82            | N.C               |

<sup>*a*</sup>Data represent mean values for at least three separate experiments performed in duplicate and are expressed as  $K_i$  (nanomolar). <sup>*b*</sup>Ad, adamantan-1-yl. <sup>*c*</sup>CB1, human cannabinoid type 1 receptor. <sup>*d*</sup>CB2, human cannabinoid type 2 receptor. <sup>*c*</sup>For both receptor binding assays, the new compounds were tested by use of membranes from HEK cells transfected with either the CB1 or CB2 receptor and [<sup>3</sup>H]-(-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol ([<sup>3</sup>H]CP-55,940). <sup>*f*</sup>K<sub>i</sub> = "Eeuilibrium dissociation constant", that is, the concentration of the competing ligand that will bind to half the binding sites at equilibrium, in the absence of radioligand or other competitors. <sup>*g*</sup>SI = selectivity index for CB2, calculated as  $K_i(CB1)/K_i(CB2)$  ratio. <sup>*h*</sup>Binding affinities of reference compounds were evaluated in parallel with the test compounds under the same conditions. <sup>*i*</sup>CB2 reference compound.

Scheme 1. Synthesis of 2-(1H-Indol-3-yl)acetamides  $9a-k^{a}$ 



<sup>a</sup>Reagents and conditions: (a) Method A: appropriate amine, EDC, HOBt, DCM, room temp. (b) Method B: alkyl halide, TBAB, 20% NaOH/ DCM, room temp. (c) Method C: arylboronic acid, Pd(OAc)<sub>2</sub>, PPh<sub>3</sub>, 2 M Na<sub>2</sub>CO<sub>3</sub>, DME, EtOH, MW, 150 °C, 10 min.

diversity at N1 position, amide **12b** was first transformed with phenylboronic acid to **14**, which was then subjected to N-alkylation

with several alkyl halides to provide a set of 1-substituted 2-(5-bromo-1H-indol-3-yl)-N-(adamantan-1-yl)acetamides (9d-k).

Scheme 2. Synthesis of 2-(1H-Indol-3-yl)-2-oxoacetamides  $10a-d^a$ 



"Reagents and conditions: (a) Method D: appropriate amine, TEA, DCM, room temp. (b) Method B: alkyl halide, TBAB, 20% NaOH/DCM, room temp. (c) Method C: arylboronic acid, Pd(OAc)<sub>2</sub>, PPh<sub>3</sub>, 2 M Na<sub>2</sub>CO<sub>3</sub>, DME, EtOH, MW, 150 °C, 10 min.

Starting from 2-(5-bromo-1*H*-indol-3-yl)-2-oxoacetyl chloride<sup>32</sup> (Scheme 2), the final oxalylamides **10a**–**d** were synthesized via amidation (method D) to **15**, alkylation to derivatives **16a,b**, and final cross-coupling reaction to install the 5-(hetero)aryl substituent. To test a different synthetic route, compound **10e** (Scheme 3) was prepared by Suzuki arylation of **16c**, in turn obtained through amidation of the N-alkylated indol-3-oxalyl chloride **17**, which could be prepared by treatment of **18** with oxalyl chloride.

5-Bromo-1*H*-indol-3-carboxylic acid and its 6-bromo isomer (Scheme 4), prepared according to a literature protocol,<sup>33</sup> were converted into the amides **19a,b** (method E), which were then subjected to alkylation (to **20a,b,c**), followed by Pd-catalyzed arylation to afford the indol-3-yl-carboxamides **11a–g**.

In Vitro Pharmacology and SAR. All the newly synthesized indole derivatives were screened, in a competitive binding experiment, for their affinity and selectivity toward the human recombinant CB1 and CB2 receptors. The tested compounds were evaluated in parallel with SR144528<sup>34</sup> and rimonabant<sup>35</sup> as CB2 and CB1 reference ligands, respectively, as previously described.<sup>29</sup> The results, in terms of binding affinities for the two receptors ( $K_i$  values), are reported in Table 1.

With the exception of compound 13b, which proved to be devoid of affinity at both cannabinoid receptor types, all the tested compounds showed affinity at CB2 receptor in the nanomolar range, with  $K_i$  values spanning 3 orders of magnitude (from 377 for 9a to 0.37 for 10a; Table 1).

The structural optimization strategy, based on conformational restriction of the aryl substituent at position 5 and amide side chain at position 3 of the prototypical compound 7, led to a significant improvement in the binding profile of the new Scheme 3. Synthesis of 2-(1*H*-Indol-3-yl)-2-oxoacetamide  $10e^{a}$ 



<sup>*a*</sup>Reagents and conditions: (a) Method B: pentyl iodide, TBAB, 20% NaOH/DCM, room temp. (b) Oxalyl chloride, Et<sub>2</sub>O, 0 °C. (c) Method D: cyclohexylamine, TEA, DCM, room temp. (d) Method C: phenylboronic acid, Pd(OAc)<sub>2</sub>, PPh<sub>3</sub>, 2 M Na<sub>2</sub>CO<sub>3</sub>, DME, EtOH, MW, 150 °C, 10 min.

indole derivatives. Thus, the direct connection of an aryl group to the indole moiety, by removing the methylene bridge at position 5 of 7 while retaining the acetamide chain at position 3, positively affected CB2 affinity, as compounds 9 and 14 showed  $K_i$  values 3–30 times lower than that of 7. Further conformational restriction at the level of the amide side chain, as in compounds 10 and 11, characterized by a carbonyl group directly linked to the indole nucleus, caused a 16–1000-fold enhancement in CB2 receptor affinity compared to the prototype 7.

Our computational approach not only demonstrated predictive significance but also allowed us to rationalize the structure-affinity and structure-selectivity relationships through its microscopic interpretation. In Figure 1 the superposition of the 3D-QSSR model and compounds **9***j*, **10b**, and

# Scheme 4. Synthesis of (1H-Indol-3-yl)carboxamides 11a-g<sup>a</sup>



"Reagents and conditions: (a) Method E: 1-aminoadamantane, CDI, DMF, room temp to 120 °C. (b) Method B: alkyl halide, TBAB, 20% NaOH/ DCM, room temp. (c) Method C: arylboronic acid, Pd(OAc)<sub>2</sub>, PPh<sub>3</sub>, 2 M Na<sub>2</sub>CO<sub>3</sub>, DME, EtOH, MW, 150 °C, 10 min.



Figure 1. (Crossed stereoview) Superposition of the 3D-QSSR model with compounds 9j (yellow carbon atoms), 10b (green carbon atoms), and 11d (white carbon atoms).

**11d** (the three chosen representatives) is shown. The different matching of both the CB2 pharmacophore and the QSSR model by the three compounds is quite evident. The acetamide moiety of **9***j*, in fact, causes a different orientation of the indole moiety with respect to that of **10b** and **11d**, thereby markedly affecting the orientation of the phenyl substituent at position 5 of the indole nucleus. The different three-dimensional arrangement of crucial substituents of **9***j*, **10b**, and **11d** is likely to account for the difference observed in their receptor affinity. Moreover, the three sets of compounds elicited the following SI values: **9**,  $5 \le SI \le 487$ ; **10**,  $154 \le SI \le 932$ ; **11**,  $26 \le SI \le 568$ . Therefore, compounds **9** are the least selective while compounds **10** are the most selective for CB2 receptor. Inspection of Figure 1 can explain this selectivity trend. With respect to **11d**, in fact, compound **10b** is able to locate its

amide carbonyl in the blue area representing increased CB2 selectivity, whereas **9j** occupies the red area of decreased CB2 selectivity with the methylene group of the amide side chain.

Compounds bearing the acetamide chain on the indole ring (general structures 9, 13, and 14) exhibited moderate to good CB2 affinity. In this series, the replacement of the 1-adamantyl group (as in 9b) by the cycloheptyl residue (compound 9a) had a detrimental effect on the binding profile. The substituent on the amide nitrogen matches a hydrophobic feature of our CB2 pharmacophore model, and hence it is reasonable that CB2 affinity may be modulated by the lipophilicity of this substituent. This finding is in line with previous observations on quinolone-3-carboxamide derivatives, where replacement of the adamantyl group by less bulky/lipophilic moieties markedly reduced CB2 receptor affinity, <sup>29a,t</sup> as a result of decreased

| Table 2. Effects of Compounds | 9g, 10a-c, and | l 11b,e,f on cAMF | Production in hCB2 | CHO Cells in Com | parison with Reference |
|-------------------------------|----------------|-------------------|--------------------|------------------|------------------------|
| Compound JWH133 <sup>a</sup>  |                |                   |                    |                  |                        |

|   | increase in cAMP production (%) |              |            |        |        |              |              |             |  |  |
|---|---------------------------------|--------------|------------|--------|--------|--------------|--------------|-------------|--|--|
| concn   | 9g                              | 10a          | 10b        | 10c    | 11b    | 11e          | 11f          | JWH133      |  |  |
| $1 \ \mu M$   | $67 \pm 6$                      | $138 \pm 12$ | 55 ± 4     | 62 ± 5 | 51 ± 5 | 93 ± 9       | $106 \pm 10$ | $-87 \pm 8$ |  |  |
| $10 \ \mu M$  | 88 ± 9                          | $163 \pm 15$ | $70 \pm 6$ | 75 ± 7 | 68 ± 6 | $110 \pm 11$ | $135 \pm 14$ | $-94 \pm 9$ |  |  |
| <sup>a</sup> Data represent mean values for at least four independent experiments performed in duplicate. |                                 |              |            |        |        |              |              |             |  |  |

hydrophobic interactions with lipophilic residues.<sup>36</sup> The insertion of an aromatic or heteroaromatic substituent at the 5 position of the indole ring improved receptor affinity (compare **13b,c** with compounds **9**), although no substantial difference was noticed among compounds bearing a phenyl or a 2-furyl group.

On the other hand, the N1 side chain exerts a modulatory effect on affinity, depending on its length, the highest affinity being demonstrated by compounds with chains of 3-4 carbon atoms (9f–I).

All the compounds in the oxalamide series (10a-e) are highaffinity CB2 ligands, also endowed with remarkable selectivity over CB1 receptor. Comparison between corresponding acetamide and oxalamide derivatives 9j/10b, 9b/10c, and 9g/ 10d clearly shows that higher CB2 selectivity of compounds 10, with respect to derivatives of 9, is due to both greater affinity for CB2 receptor and lower affinity for CB1 receptor. Such an in vitro profile was determined by the replacement of a methylene group by a carbonyl group at position 3 of the indole ring, that in turn influences the steric and electronic properties of the amide side chain. Thus, the different relative orientation of the amide group and indole ring may help discriminate between cannabinoid receptor subtypes, while a more profitable H-bond networking between ligands 10 and CB2 receptor is likely to account for enhanced CB2 affinity. In fact, although all the tested compounds are able to match the H-bond donor feature with a carbonyl oxygen when superimposed to the 3D-QSSR model, compounds of structure 10 possess another carbonyl group that may be involved in further H-bonding interactions within the CB2 receptor binding pocket, so as to enhance receptor affinity. Compound 10a, in particular, showed  $K_i(CB2) = 0.37$  nM. Although no definitive explanation for such a notable affinity can be given, this resulted in the most active compound among the 26 indole derivatives assayed. Contrary to what was observed in the acetamide series, substitution of a cyclohexyl group for the 1-adamantyl one did not alter the binding properties (compare 10b with 10e).

Also, the compounds belonging to the carboxamide series (11a-g) displayed very high affinity for CB2 receptor, with  $K_i$ values ranging from 22.2 nM (11b) to 1.82 nM (11a), and in most cases well compare with the CB2 reference standard SR144528 ( $K_i = 5.4$  nM). By comparison with the corresponding derivatives 10, it can be seen that differences in  $K_i(CB2)$  values are within 1 order of magnitude. In particular, the ratio between  $K_i(CB2)$  values for the couples 11a/10d, 11c/10c, 11d/10b, and 11e/10a are 0.07, 0.6, 0.9, and 6.7, respectively. As a result, compounds 11 are substantially equivalent to derivatives 10 in terms of affinity, though endowed with somewhat lower receptor selectivity. It is noteworthy that moving the aromatic substituent from the 5-position (11d) to the 6-position (11g) gave rise to a dramatic increase in CB1 receptor affinity, leading to a compound possessing very high affinity at both CB1 and CB2 receptors and inverted receptor selectivity (SI < 1). Although this finding is not completely surprising on the basis of what described in the arylquinolone-3-carboxamide series, where CB2 selectivity was lost by moving the aryl substituent from position 6 to position 7, nevertheless it cannot be rationalized by resorting to our selectivity model.

Functional Activity at CB2 Receptors in Vitro. The effect of compounds 9g, 10a–c, and 11b,e,f on forskolininduced elevation of cyclic adenosine monophosphate (cAMP) levels in Chinese hamster ovary (CHO) cells transfected with human CB2 receptor was also assessed. Table 2 reports the effect of the examined compounds at concentrations of 1 and 10  $\mu$ M, expressed as percentage of the cAMP increase.

In our experimental conditions, a well-known CB2 agonist, JWH-133, was able to inhibit cAMP production, whereas the novel CB2 ligands were able to increase cAMP production with a typical inverse agonist behavior. This finding was not completely unexpected, because 6-arylquinolone-3-carboxamides behave similarly. Thus, if one takes into consideration the structural analogy between 6-arylquinolones and 5-arylindoles and grants that both these heterocyclic systems simply act as scaffolds able to correctly orient their substituents in the 3D space, it is not surprising that compounds belonging to either class may exert the same functional effects.

In Vivo Pharmacology. Finally, the in vivo activity of 10a and 11e, that is, the most potent and CB2-selective indole derivatives, was evaluated in the formalin test of acute peripheral and inflammatory pain in mice. Formalin injection induces a biphasic stereotypical nocifensive behavior. Nociceptive responses are divided into an early, short-lasting first phase (0-7 min), caused by a primary afferent discharge produced by the stimulus, followed by a quiescent period and then a second, prolonged phase (15-60 min) of tonic pain. Fifteen minutes before injection of formalin, mice received intraperitoneal (ip) administration of vehicle or either of the two compounds (1 or 3 mg/kg), alone or in combination with the selective CB2 agonist N-(adamantan-1-yl)-6-isopropyl-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide<sup>29a<sup>1</sup></sup> (hereafter referred to as COR167) (1 mg/kg, ip), administered 5 min before the compound.<sup>29e</sup> The results are presented in Figures 2 and 3.

When assayed in the formalin test, **10a** and **11e** exhibited a behavior we have shown to be typical of CB2 inverse agonists in several previous studies,<sup>29e,f,i</sup> with no effect on the first phase of formalin nocifensive behavior and a weak but dose-related effect on the second, inflammatory phase. Accordingly, compound **10a**, the least potent of the two compounds, at a dose inactive per se (1 mg/kg), completely reversed the effect of the CB2 agonist COR167; and compoind **11e**, despite having being tested at a dose still exerting some effect per se (1 mg/kg), still counteracted the effect of COR167.

In summary, three different series of 5-arylindole derivatives namely, indol-3-ylacetamides 9, indol-3-yloxalamides 10, and indol-3-ylcarboxamides 11—were studied as CB2 receptor ligands. Most of these compounds, particularly those belonging to the second and third groups, elicited high affinity at CB2



**Figure 2.** Effect of compound **10a** (1-3 mg/kg, ip), (top) alone or (bottom) in combination, at the dose of 1 mg/kg, with COR167 (1 mg/kg, ip) in the formalin test in mice. The total time of the nociceptive response was measured every 5 min. Results are mean  $\pm$  SEM (n = 8-10 for each group). ( $\blacktriangle$ , top) Statistically significant differences vs formalin; ( $\blacklozenge$ , bottom) statistically significant differences (P < 0.05) vs formalin; ( $\blacksquare$ , bottom) statistically significant differences (P < 0.05) vs COR167. One-way analysis of variance followed by a Tukey–Kramer multiple comparisons test was used for data analysis.



**Figure 3.** Effect of compound **11e** (1-3 mg/kg, ip), (top) alone or (bottom) in combination, at the dose of 1 mg/kg, with COR167 (1 mg/kg, ip) in the formalin test in mice. The total time of the nociceptive response was measured every 5 min. Results are mean  $\pm$  SEM (n = 8-10 for each group). ( $\oplus$ ,  $\blacktriangle$ , top) Statistically significant differences (P < 0.05) vs formalin; ( $\spadesuit$ ,  $\bigoplus$ , bottom) statistically significant differences (P < 0.05) vs formalin; ( $\blacksquare$ ) statistically significant differences (P < 0.05) vs COR167. One-way analysis of variance followed by a Tukey–Kramer multiple comparisons test was used for data analysis.

receptor and moderate to good selectivity over CB1 receptor. As a whole, the new indole-based CB2 ligands favorably

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of the CB2 selective agonist COR167. These results are reminiscent of those obtained with 6-(hetero)arylquinolone-3carboxamides (like 8) and seem to support our initial idea that the quinolone and indole rings may act as interchangeable scaffolds, which, though not identical in terms of physicochemical properties, are able to orient in a similar way some crucial pharmacophoric groups, thereby yielding compounds that exert similar pharmacodynamic effects.

A number of reports have suggested that selective cannabinoid CB2 inverse agonists may serve as novel immunomodulatory agents in the treatment of a variety of acute and chronic inflammatory disorders.<sup>29e,37,38</sup> With this background, the new compounds here described might represent an interesting starting point for the development of potential antiinflammatory agents with an innovative mechanism of action.

#### EXPERIMENTAL SECTION

Chemistry. Reagents were purchased from commercial suppliers and used without further purification. Anhydrous reactions were run under positive pressure of dry N2. Merck silica gel 60 was used for flash chromatography (23-400 mesh). IR spectra were recorded on a Perkin-Elmer BX Fourier transform infrared (FT-IR) system with CHCl<sub>3</sub> as the solvent or a Nujol dispersion. <sup>1</sup>H NMR and <sup>13</sup>C NMR were recorded at 200 and 50 MHz, respectively, on a Bruker AC200F spectrometer and at 400 and 100 MHz on a Bruker Advance DPX400. Chemical shifts are reported relative to that of tetramethylsilane at 0.00 ppm. Mass spectral (MS) data were obtained on an Agilent 1100 LC/MSD VL system (G1946C) with a 0.4 mL/min flow rate by use of a binary solvent system of 95:5 methanol/water. UV detection was monitored at 254 nm. Mass spectra were acquired in either positive or negative mode scanning over the mass range 105-1500. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Microwave irradiations were conducted with a CEM Discover synthesis unit (CEM Corp., Matthews, NC). Elemental analyses were performed on a Perkin-Elmer PE 2004 elemental analyzer, and the data for C, H, and N are within 0.4% of the theoretical values. The chemical purity of the target compounds was determined under the following conditions: zorbax eclipse C8, MeOH/H2O (MeOH 80-95, t = 20 min), 0.8 mL/min. The purity of each compound was >96% in either analysis.

Synthesis of Amides 12a,b by Method A: General Procedure. To a solution of 5-bromoindoleacetic acid (254 mg, 1 mmol) in dichloromethane (DCM; 15 mL) were added successively HOBt (135 mg, 1 mmol), EDC (383 mg, 2 mmol), and the appropriate amine (2 mmol). After the mixture was stirred at room temperature for 2 h, solvent was evaporated and the residue was flash-chromatographed on silica gel eluted with DCM/MeOH (95:5) to give the pure product.

*Example:* 2-(5-Bromo-1H-indol-3-yl)-N-(adamantan-1-yl)acetamide (**12b**). Obtained in quantitative yield. White solid, mp 93–94 °C. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  10.94 (br s, 1H), 7.68 (s, 1H), 7.39 (br s, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.11–7.06 (m, 2H), 3.23 (s, 2H), 1.90 (s, 3H), 1.83 (s, 6H), 1.52 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  170.3, 135.1, 128.8, 125.2, 124.8, 121.4, 113.0, 109.2, 51.9, 41.5, 36.3, 34.6, 29.4. IR (Nujol) 1654 cm<sup>-1</sup>. MS (ESI) *m/z* 388 [M + H]<sup>+</sup>, 410 [M + Na]<sup>+</sup>. Anal. (C<sub>20</sub>H<sub>23</sub>BrN<sub>2</sub>O) C, H, N.

Synthesis of Compounds 9d–k, 13a–c, 16a,b, 18, and 20a– c by Method B: General Procedure. A mixture of the appropriate starting compound (0.9 mmol), alkyl halide (1.1 mmol), tetrabutylammonium bromide (TBAB; 0.5 mmol), 20% aqueous sodium hydroxide (10 mL), and DCM (15 mL) was stirred at room temperature overnight. The reaction mixture was diluted with water and the organic layer was extracted with DCM ( $3 \times 10$  mL). The combined organic solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude residue was purified by flash chromatography on silica gel, eluting with hexanes/ethyl acetate (2:1) to provide the title compound.

*Example:* 2-[[5-Bromo-1-(prop-2-en-1-yl)-1H-indol]-3-yl]-2-oxo-N-(adamantan-1-yl)acetamide (**16b**). Prepared in 80% yield from **15**. Yellow solid, mp 150–152 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  8.97 (d, *J* = 4.0 Hz, 1H), 8.55 (s, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.29 (s, 1H), 7.18 (d, *J* = 8.0 Hz, 1H), 6.00–5.91 (m, 1H), 5.28 (d, *J* = 12.0 Hz, 1H), 5.16 (d, *J* = 20.0 Hz, 1H), 4.72 (s, 2H), 2.10 (s, 9H), 1.72 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  181.1, 161.2, 141.6, 134.9, 131.3, 129.5, 126.7, 125.3, 119.2, 117.1, 111.7, 111.5 51.8, 49.8, 41.2, 36.3, 29.4. IR (Nujol) 1679, 1639 cm<sup>-1</sup>. MS (ESI) *m/z* 442 [M + H]<sup>+</sup>, 464 [M + Na]<sup>+</sup>. Anal. (C<sub>23</sub>H<sub>25</sub>BrN<sub>2</sub>O<sub>2</sub>) C, H, N.

Synthesis of Compounds 9a–c, 10a–e, 11a–g, and 14 by Method C: General Procedure. A 5-mL process vial was charged with the appropriate bromoindole derivative (1 mmol), the appropriate boronic acid (5 mmol), Pd(OAc)<sub>2</sub> (22.5 mg, 0.1 mmol), PPh<sub>3</sub> (78.6 mg, 0.3 mmol), 2 M Na<sub>2</sub>CO<sub>3</sub> (2 mL, 4 mmol), EtOH (1 mL), and 1,2-dimethoxyethane (DME) (4 mL). The vessel was sealed under air and exposed to microwave heating for 10 min at 150 °C. The reaction mixture was thereafter cooled down to room temperature, diluted with AcOEt, and filtered through a short plug of Celite. The solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was purified by flash chromatography on silica gel, eluted with DCM/MeOH (95:5).

Example: 2-[[5-Phenyl-1-(prop-2-en-1-yl)-1H-indol]-3-yl]-2-oxo-N-(adamantan-1-yl)acetamide (**10d**). Prepared from **16b** in 62% yield. White solid, mp 151–152 °C. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  9.06 (s, 1H), 8.70, (s, 1H), 7.71 (m, 2H), 7.57 (d, J = 8.0 Hz, 1H), 7.48–7.33 (m, 5H), 6.07–5.98 (s, 1H), 5.32 (d, J = 8.0 Hz, 1H), 5.23 (d, J = 16.0 Hz, 1H), 4.78 (d, J = 4.0 Hz, 2H), 2.14 (s, 9H), 1.75 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  181.0, 161.7, 141.9, 141.9, 137.1, 135.9, 131.8, 128.9, 128.8, 127.8, 127.0, 123.6, 121.4, 119.3, 112.4, 110.8, 52.0, 50.1, 41.4, 36.5, 29.6. IR (Nujol) 1676, 1636 cm<sup>-1</sup>. MS (ESI) m/z 439 [M + H]<sup>+</sup>, 461 [M + Na]<sup>+</sup>. Anal. (C<sub>29</sub>H<sub>30</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

Synthesis of Oxalylamides 15 and 16c by Method D: General Procedure. A solution of the appropriate 2-(5-bromo-1*H*indol-3-yl)-2-oxoacetyl chloride (1.8 mmol), primary amine (2.1 mmol), and triethylamine (TEA) (2.6 mmol) in dry DCM (25 mL) was stirred at 0 °C for 30 min and then at room temperature overnight. The solution was washed with 1 N HCl, a saturated solution of sodium bicarbonate, and brine. After the solution was dried over anhydrous sodium sulfate, volatiles were removed in vacuo and the residue was recrystallized from EtOH.

Example: 2-(5-Bromo-1H-indol-3-yl)-2-oxo-N-(adamantan-1-yl)-acetamide (**15**). Yield 82%. White solid, mp >300 °C. <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ ) δ 12.37 (br s, 1H), 8.67 (s, 1H), 8.31 (s, 1H), 7.83 (s, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 2.49 (s, 3H), 2.05 (s, 6H), 1.65 (s, 6H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 222.6, 205.3, 138.4, 127.1, 125.2, 51.6, 41.2, 36.3, 29.4. IR (Nujol) 1684, 1622 cm<sup>-1</sup>. MS (ESI) m/z 402 [M + H]<sup>+</sup>. Anal. (C<sub>20</sub>H<sub>21</sub>BrN<sub>2</sub>O<sub>2</sub>) C, H, N.

Synthesis of Carboxamides 19a,b by Method E: General Procedure. A solution of the appropriate carboxylic acid (6.1 mmol) and CDI (7.3 mmol) in *N*,*N*-dimethylformamide (DMF; 7 mL) was stirred at room temperature for 45 min. To this yellowish solution, 1-aminoadamantane (12.2 mmol) dissolved in DMF (10 mL) was added and the reaction mixture was heated at 120 °C for 7 h. After cooling, the solution was diluted with ethyl acetate and a saturated solution of sodium bicarbonate. The organic layer was washed with brine (4 × 30 mL), and the precipitate that formed was filtered off. The organic solution was evaporated in vacuo and the crude solid residue was triturated with ethyl acetate and hexanes.

*Example:* (5-Bromo-1H-indol-3-yl)-N-(adamantan-1-yl)carboxamide (**19a**). Yield 76%. White solid, mp > 280 °C (decomp). <sup>1</sup>H NMR (400 MHz, DMSO- $d_6$ )  $\delta$  11.62 (br s, 1H), 8.26 (s, 1H), 8.12 (s, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 8.0 Hz, 1H), 7.10 (s, 1H), 2.49 (s, 6H), 2.08 (s, 9H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$ 164.3, 135.2, 129.4, 128.8, 124.6, 123.9, 114.1, 113.4, 111.6, 51.5, 43.7, 41.9, 41.6, 36.7, 36.4, 36.0, 29.5, 29.2. IR (Nujol) 1621 cm<sup>-1</sup>. MS (ESI) m/z 372 [M – H]<sup>-</sup>. Anal. (C<sub>19</sub>H<sub>21</sub>BrN<sub>2</sub>O) C, H, N. **2-(5-Bromo-1-pentyl-1***H***-indol-3-yl)-2-oxoacetyl chloride (17).** Oxalyl chloride (197  $\mu$ L, 2.25 mmol) was added at 0 °C to a solution of 5-bromo-1-pentyl-1*H*-indole (500 mg, 1.88 mmol) in dry Et<sub>2</sub>O. After the solution was stirred at room temperature for 1 h, a further amount of oxalyl chloride (164  $\mu$ L, 1.88 mmol) was added and the solution was maintained at room temperature for a further 20 min. The solution was evaporated in vacuo and the yellow-orange residue was washed with hexanes to give 402 mg (60%) of the title compound, which was used in the next step without further purification.

Molecular Modeling. Three-dimensional structure modeling and matching onto the previously developed atom-based 3D-QSSR model were carried out on an IBM workstation with Linux operating system running Maestro 8.0, MacroModel 9.5 and phase 2.5 programs (Schrödinger, LLC, New York). The use of phase, implemented in the Maestro modeling package, to generate a pharmacophore and a 3D-QSSR model for cannabinoid receptor CB2 has been already described elsewhere.<sup>29h</sup> The 3D structure of all the derivatives modeled in this study was built in Maestro. Conformers of each molecule were generated in MacroModel by use of the OPLS 2005 force field, GB/SA water, and no cutoff for nonbonded interactions. Molecular energy minimizations were performed via the PRCG method with 5000 maximum iterations and a numerical value of 0.001 as gradient convergence threshold. The conformational searches were carried out by application of the MCMM torsional sampling method, performing automatic setup with 20 kJ/mol in the energy window for saving structure and a 0.5 Å cutoff distance for redundant conformers. Each SI value was predicted by application of our 3D-QSSR model after alignment of the corresponding molecule to the CB2 pharmacophore.

In Vitro Functional Assay. CHO cells transfected with human CB2 receptors (Perkin-Elmer Life and Analytical Sciences, Boston, MA) were washed with phosphate-buffered saline and diluted trypsin and centrifuged for 10 min at 200g. The pellet containing CHO cells  $(1 \times 10^6 \text{ cells/assay})$  was suspended in 0.5 mL of incubation mixture: NaCl 150 mM, KCl 2.7 mM, NaH<sub>2</sub>PO<sub>4</sub> 0.37 mM, MgSO<sub>4</sub> 1 mM, CaCl<sub>2</sub> 1 mM, Hepes 5 mM, MgCl<sub>2</sub> 10 mM, and glucose 5 mM, pH 7.4 at 37 °C. Then 0.5 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as phosphodiesterase inhibitor was added and preincubated for 10 min in a shaking bath at 37 °C. The effects of test compounds (9g, 10a-c, and 11b,e,f) and reference compound JWH-133 were studied in the presence of forskolin (1  $\mu$ M). The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000g for 10 min at 4 °C and the supernatant was extracted four times with water-saturated diethyl ether. The final aqueous solution was tested for cAMP levels by a competition protein binding assay. Samples of cAMP standard (0-10 pmol) were added to each test tube containing the incubation buffer (Trizma base 0.1 M, aminophylline 8.0 mM, and 2 mercaptoethanol 6.0 mM, pH 7.4) and [<sup>3</sup>H]cAMP (specific activity 26 Ci/mmol; Perkin-Elmer Life and Analytical Sciences, Boston, MA). The binding protein, previously prepared from beef adrenal glands, was added to the samples previously incubated at 4 °C for 150 min, and after the addition of charcoal thes samples were centrifuged at 2000g for 10 min. The clear supernatant was counted by using a Tri Carb Packard 2810 TR scintillation counter.

In Vivo Antinociceptive Assay (Formalin Test). The experimental procedures applied in the formalin test were approved by the Animal Ethics Committee of the Second University of Naples. Animal care was in compliance with the IASP and European Community guidelines on the use and protection of animals in experimental research (E.C. L358/1 18/12/86). All efforts were made to minimize animal suffering and to reduce the number of animals used. Formalin injection induces a biphasic stereotypical nocifensive behavior.<sup>3</sup> Nociceptive responses are divided into an early, short-lasting first phase (0-7 min) caused by a primary afferent discharge produced by the stimulus, followed by a quiescent period and then a second, prolonged phase (15–60 min) of tonic pain. Mice received formalin (1.25% in saline, 30  $\mu$ L) in the dorsal surface of one side of the hindpaw. Each mouse was randomly assigned to one of the experimental groups (n = 8-10) and placed in a Plexiglas cage and allowed to move freely for 15-20 min. A mirror was placed at a 45° angle under the

cage to allow full view of the hind-paws. Lifting, favoring, licking, shaking, and flinching of the injected paw were recorded as nociceptive responses. The duration of those mentioned noxious behaviors was monitored by an observer blind to the experimental treatment for periods of 0-10 min (early phase) and 20-60 min (late phase) after formalin administration. Results are expressed as means ± SEM. Significant differences between groups were evaluated by using analysis of variance followed by Dunnett's test. The version of the formalin test we applied is based on the fact that a correlational analysis showed that no single behavioral measure can be a strong predictor of formalin or drug concentrations on spontaneous behaviors.<sup>40</sup> Consistently, we considered that a simple sum of time spent licking plus elevating the paw, or the weighted pain score, is in fact superior to any single (lifting, favoring, licking, shaking, and flinching) measure (r ranging from 0.75 to 0.86).41 Groups of 8-10 animals per treatment were used, with each animal being used for one treatment only. Mice received intraperitoneal vehicle (20% dimethyl sulfoxide, DMSO, in 0.9% NaCl) or different doses of before-mentioned compounds.

#### ASSOCIATED CONTENT

#### **S** Supporting Information

Analytical data for compounds 9a-k, 10a-c, 10e, 11a-g, 12a, 13a-c, 14, 16a, 16c, 18, 19a, 19b, and 20a-c. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interest.

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# ABBREVIATIONS

CB1, cannabinoid type 1; CB2, cannabinoid type 2; CDI, 1,1'carbonyldiimidazole; COR167, *N*-(adamantan-1-yl)-6-isopropyl-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide; DME, 1,2-dimethoxyethane; EDC, *N*-(3-dimethylaminopropyl)-*N*'ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole; MW, microwaves; SI, selectivity index; TBAB, tetrabutylammonium bromide; TEA, triethylamine

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