Allosteric Modulators: A Side Door

Mary E. Abood*

Center for Substance Abuse Research, Lewis Katz School of Medicine at Temple University, 3500 N. Broad Street, Philadelphia, Pennsylvania 19140, United States

ABSTRACT: Allosteric modulators of the cannabinoid CB1 receptor were first discovered in 2005. Since then, although both negative and positive allosteric modulators have been uncovered, many questions remain about their site(s) of action, as well as the basis of their signaling. The described covalent probe with improved potency and efficacy will facilitate these studies.

Allosteric modulators are sought after as a means to avoid undesirable side effects of orthosteric ligands. In the case of the cannabinoid CB1 receptor, existing orthosteric agonists and antagonists both present a difficult side effect profile. Antagonists of the CB1 cannabinoid receptor were developed as antibesity agents; however, rimonabant (Accomplia) was withdrawn from the market due to depression, anxiety, and suicidal ideation, and further clinical development of this class of compounds was halted. In this issue, Kulkarni et al. report the first electrophilic and photoaffinity covalent probes for CB1 allosteric modulators. Furthermore, one of the reported compounds, GAT100 (20), was found to be the most potent negative allosteric modulator (NAM) and, further, did not exhibit inverse agonism.

The authors chose to target the two prototypical negative allosteric modulators for the CB1 receptor, 5-chloro-3-ethyl-N-(4-(piperidin-1-yl)phenethyl)-1H-indole-2-carboxamide (1, Org27569) and 1-(4-chlorophenyl)-3-(3-(6-(pyrrolidin-1-yl)-pyridine-2-yl)phenyl)urea (PSNCBAM-1). Regarding 1, it acts as an insurmountable antagonist/inverse agonist of CP55,940-stimulated [35S]GTPγS binding while exhibiting positive binding cooperativity with [3H]CP55,940, findings that were reproduced in this study. As inverse agonism is thought to be the property that was responsible for the side effects produced with rimonabant, one goal of the present study was to develop a neutral allosteric antagonist. They succeeded in producing a high affinity, neutral allosteric antagonist with 3-ethyl-5-isothiocyanato-N-(4-(piperidin-1-yl)phenethyl)-1H-indole-2-carboxamide (20) (Figure 1). The 5-chloro functionality which is critical for allosteric activity in 1 was replaced by the reactive (cysteine specific) isothiocyanate (-NCS) functionality. This structural change improved allosteric activity profile of the compound compared to 1; potency was improved in all assays described, and efficacy was improved in G-protein-dependent assays (cAMP inhibition and [35S]GTPγS binding). This change also reduced inverse agonist activity, as demonstrated by a lack of activity on its own in the [35S]GTPγS assay which is a direct measurement of G-protein signaling following receptor engagement.

The authors further characterized 20 with respect to its ability to covalently label the CB1 receptor. Preincubation of CB1-receptor expressing membranes with 20 for 60 min (followed by extensive washing) was sufficient to demonstrate more than a 2-fold increase in maximal binding of [3H]-CP55,940, an effect that was not observed with 1. Thus, this is the first allosteric probe that has been shown to label the CB1 allosteric site covalently.

In the present work, compounds, including 20, were examined using the PathHunter CB1 β-arrestin-2 recruitment assay. In this assay, all compounds antagonized CP55,940-dependent β-arrestin-2 recruitment. As 1 has been reported to recruit β-arrestin-1,5 future studies determining the effect of 20 on β-arrestin-1 interaction are of interest. Similarly, in the HitHunter cAMP assay, all compounds antagonized CP55,940 inhibition of cAMP. Two recent studies demonstrated that 1 attenuated cannabinoid agonists’ ability to inhibit forskolin stimulated cAMP production.6,7 However, the mechanism of 1’s effects on cannabinoid inhibition of forskolin stimulated cAMP production has been suggested to be through an increased rate of receptor desensitization and reduced internalization rather than through allosteric antagonism.7 Interestingly, the compounds in this manuscript demonstrated lower potencies in the cAMP assay as compared with β-arrestin-2 recruitment, suggesting signaling bias. Bias has also been reported with respect to potency for antagonizing different classes of CB1 agonists,6,7 this was not examined here.

Another important aspect of the present work was further structure–activity characterization of both 1 and PSNCBAM-1. The 5-chloro functionality and the terminal carbon of the C-3 side chain on 1 and the 4-chloro functionality on PSNCBAM-1 were replaced with electrophilic (isothiocyanate) or photoaffinity (azide or benzophenone) substituents. Modification of the C-3 side chain on 1 reduced functional activity, an effect that was more pronounced in the cAMP assay than with β-arrestin-2 recruitment. None of the modifications improved activity with PSNCBAM-1, although activity was retained with all analogs except for the benzophenone, which abrogated activity in the cAMP assay and greatly reduced β-arrestin-2 recruitment. Photoaffinity labeling was not demonstrated in this manuscript but should be possible with the new compounds.

In summary, new tools for exploring allosteric binding sites at the CB1 receptor have been discovered, along with the promise of translation to new treatments for metabolic syndromes and drug abuse disorders. It is hoped that this “side door” strategy...
will indeed avoid the side effects profile of CB1 receptor antagonists.

**AUTHOR INFORMATION**

*Corresponding Author*

*E-mail: mabood@temple.edu. Phone: 215-707-2638.*

**REFERENCES**


---

**Figure 1.** Structures of compounds 1 and 20. The 5-chloro functionality which is critical for allosteric activity in compound 1 was replaced by the reactive (cysteine specific) isothiocyanate (-NCS) functionality. This structural change improved allosteric activity profile of the compound compared to compound 1 and also reduced inverse agonist activity.