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Original article

Mastering tricyclic ring systems for desirable functional cannabinoid activity

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ABSTRACT

There is growing interest in using cannabinoid receptor 2 (CB2) agonists for the treatment of neuropathic pain and other indications. In continuation of our ongoing program aiming for the development of new small molecule cannabinoid ligands, we have synthesized a novel series of carbazole and γ-carboline derivatives. The affinities of the newly synthesized compounds were determined by a competitive radioligand displacement assay for human CB2 cannabinoid receptor and rat CB1 cannabinoid receptor. Functional activity and selectivity at human CB1 and CB2 receptors were characterized using receptor internalization and [^{35}S]GTP-γ-S assays. The structure–activity relationship and optimization studies of the carbazole series have led to the discovery of a non-selective CB1 and CB2 agonist, compound **4**. Our subsequent research efforts to increase CB2 selectivity of this lead compound have led to the discovery of CB2 selective compound **64**, which robustly internalized CB2 receptors. Compound **64** had potent inhibitory effects on pain hypersensitivity in a rat model of neuropathic pain. Other potent and CB2 receptor–selective compounds, including compounds **63** and **68**, and a selective CB1 agonist, compound **74** were also discovered. In addition, we identified the CB2 ligand **35** which failed to promote CB2 receptor internalization and inhibited compound CP55,940-induced CB2 internalization despite a high CB2 receptor affinity. The present study provides novel tricyclic series as a starting point for further investigations of CB2 pharmacology and pain treatment.

1. Introduction

Two cannabinoid receptors, CB1 and CB2, have been characterized and cloned [1,2]. The CB1 receptor is found predominantly in the brain [3]. Impairment of cognitive functions and psychoactivity induced by cannabinoids ligands are mediated by CB1 receptors activation [4]. Selective activation of CB2 receptors has been proposed as a strategy to curtail the negative central side effects seen with nonselective CB1/CB2 agonists. Several CB2-selective agonists have been described previously [5–11]. Although multiple preclinical studies suggest that the CB2 receptor is a viable target to decrease both acute and neuropathic pain responses [12,13], synthetic CB2 agonists have not advanced through clinical trials. In part, this is due to a lack of a thorough understanding of CB2-mediated analgesic mechanisms [14]. CB2 modulation is also implicated in immunomodulation and neuroprotection but the functional profile of the CB2 ligands inducing these effects has not been clearly defined [15]. Activation of CB2 receptor induces its coupling to the Gi/o class of G proteins. The dissociation of the α and βγ subunits resulting from the CB2 activation can influence multiple effector systems including adenylyl cyclase, p42/44 MAPK

Abbreviations: BSA, bovine serum albumin; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; EC_{50}, half maximal effective concentration; EDTA, ethylenediaminetetraacetic acid; GTP, guanosine-5′-triphosphate; *h*CB1, human CB1; *h*CB2, human CB2; IC_{50}, median inhibition concentration.

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(ERK1/2) or ion channels [16]. Currently, the diversity of cannabinoid agonists is very broad and continues to expand rapidly (Fig. 1). In our previous communications, the SARs of potent and selective CB2-receptor agonists [17] and antagonists [6] based on the isatin scaffold were described (MDA19 in Fig. 1).

Although the isatin core was identified as a good scaffold for designing CB2-selective cannabinoid agonists, we hypothesized that the intramolecular hydrogen bond pattern in the isatin scaffold can be replaced with a covalent C–C bond. Also considering the high pharmacological potential of carbazole-based natural products [18], we focused our attention on this scaffold for use of studying CB1/CB2 receptor pharmacology. Thus, we first identified compound **4** as a potent non-selective CB1/CB2 agonist [19]. We recently reported the cannabinoid and T-type calcium channel activities for three compounds from this novel series of cannabinoid ligands [7]. In this article, we optimized the CB2 selectivity of this series. We also explored the effect on CB2 activity of the introduction of quaternary ammonium moieties or introduction of heteroatoms increasing the polar surface area to help future identification of peripherally-restricted CB2 agonists in our tricyclic series. This research effort led to the discovery of a selective CB2 agonists with a polar surface area >70 Å^2 and expected to have a low blood–brain barrier (BBB) permeability. Functional activities of our most active compounds were determined using [^{35}S]GTP-γ-S assays and by characterizing their ability to internalize human CB1 and CB2 receptors. The ability of these compounds to interact with the orthosteric site on human CB2 receptor was assessed by determining their ability to block CP55940-induced hCB2 internalization. Moreover, the binding mode prediction through ligand-steered modeling highlighted a potential H-bond interaction between the alkylsulfonamide moiety in the N-1 position bored by compound **64** and N7.45 of the CB2 receptor. Similarly to what was previously observed within the isatin series, the presence of the OMe fragment in the carbazole scaffold turned out to be a key substituent responsible for the agonist-to-antagonist functionality switch.

2. Results and discussion

2.1. Chemistry

The pathway of achieving the synthesis of the first series of compounds (**4**–**11** and **14**–**23**) is outlined in Scheme 1. We found that desirable analogs **4**–**11** can be conveniently prepared from commercially available carbazole via consecutive substitution with *n*-pentyl bromide under alkaline conditions followed by electrophilic formylation using standard Vilsmeier–Haack conditions, oxidation, and, finally, amidation under standard peptide coupling conditions. Later, a more convenient method was developed for the synthesis of compound **4** that utilized a direct Friedel–Crafts reaction using piperidinecarbonyl chloride. While our conditions for the Friedel–Crafts reaction (compounds **4**, **18**, and **19**) were not expected to be optimal, this strategy did provide a rapid access to the desired compounds. Compound **19** was subjected to nucleophilic attack by the corresponding amines to give analogs **20**–**22**. Deprotection of the Boc protecting group in compounds **12** and **13** furnished compounds **14**–**15**. Analogs **16** and **17** were prepared by coupling of corresponding bromomethylpyridyl derivatives and acid **3** in the presence of TBAF under basic conditions [20]. The tertiary base **9** was converted to the quaternary form **23** by treatment with methyl iodide in diethyl ether at room temperature.

Next, we focused on modifications (Scheme 2) that led to a series of compounds in which the original 3-carbonyl group in the carbazole framework was either derivatized or entirely eliminated. Compounds **24**–**26**, **28** and **30** were synthesized following protocols as outlined in Scheme 2. Nitrile **24** was prepared by a one-pot solvent-free procedure from aldehyde **2** and hydroxylamine. Thioamide **25** was prepared by heating amide **4** with Lawesson's reagent under microwave conditions. Tertiary amine **26** was obtained upon treatment of **4** with LAH. The preparation of amines **28** and **30** was achieved by palladium-catalyzed coupling in analogy to known literature methods (e.g. Ref. [21]).

The pyrido[3,4-*b*]indole-based analogs **33**–**35** were prepared as depicted in Scheme 3. Commercially available ethyl 9*H*-pyrido[3,4-*b*]indole-3-carboxylate was alkylated with *n*-pentyl bromide under microwave conditions. Hydrolysis of ester **31** gave the corresponding carboxylic acid **31**. Activation of acid **32**, followed by coupling with the corresponding amines provided the corresponding target carboxamides **33**–**35**.

A series of *N*-substituted analogs were prepared according to Scheme 4. In an analogous fashion as previously described for compound **4**, *N*-alkylations of carbazole (compounds **36**–**39**) followed by Friedel–Crafts reaction using piperidinecarbonyl chloride onto the resultant substrates led to analogs **40**–**42**. *N*-Alkyl derivatives **43**–**44** were prepared according to the literature [22]. Intermediate **44** was prepared from carbazole and ethylene oxide

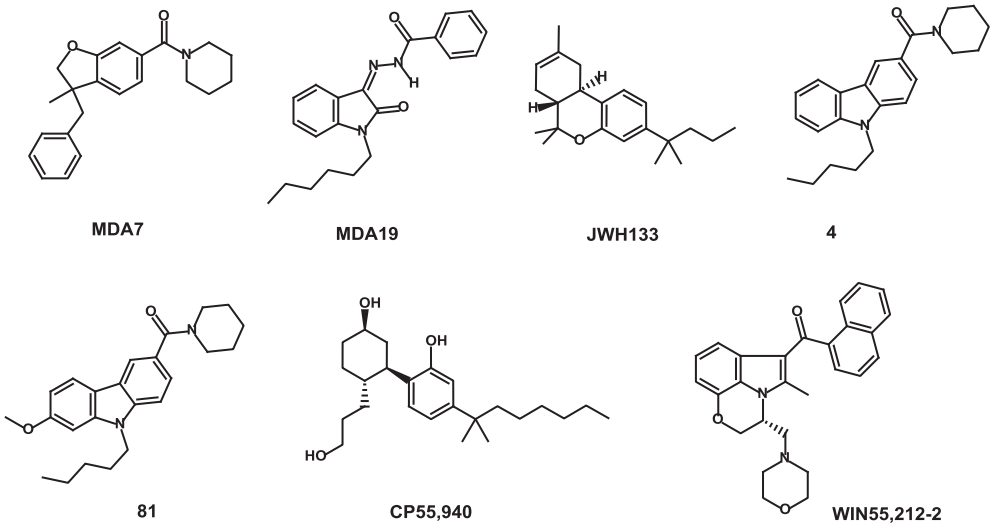

Fig. 1. Chemical structures of CB2 modulators (1–7). MDA7 [12]; MDA19 [17]; JWH133 [62]; (4) NMP7 [7]; (81) NMP4 [7]; CP55,940 [63]; WIN55,212-2 [64].