Bioorganic & Medicinal Chemistry Letters 22 (2012) 1619–1624

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

γ-Carbolines: A novel class of cannabinoid agonists with high aqueous solubility and restricted CNS penetration

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ARTICLE INFO

Article history:
Received 1 November 2011
Revised 22 December 2011
Accepted 27 December 2011
Available online 4 January 2012

Keywords:
Cannabinoid
Carbolines
Chronic pain

ABSTRACT

An oral, peripherally restricted CB1/CB2 agonist could provide an interesting approach to treat chronic pain by harnessing the analgesic properties of cannabinoids but without the well-known central side effects. γ-Carbolines are a novel class of potent mixed CB1/CB2 agonists characterized by attractive physicochemical properties including high aqueous solubility. Optimization of the series has led to the discovery of **29**, which has oral activity in a rat inflammatory pain model and limited brain exposure at analgesic doses, consistent with a lower risk of CNS-mediated tolerability issues.

The plant derived cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC, **1**) and its synthetic analogues act as agonists at both cannabinoid receptor subtype 1 (CB1) and subtype 2 (CB2) (Fig. 1).[1]. CB1 receptors are widely expressed in the central nervous system (CNS), and in the peripheral nervous system, especially in sensory nerve fibers.[2]. CB2 receptors, on the other hand, are mainly located in immune tissues and cells.[2,3] Recently, it has been shown that CB2 is also expressed in the CNS.[4] Cannabinoid receptors and ligands have been implicated in pain transduction and perception[5] as well as in neuroinflammation.[6] There are published studies demonstrating that cannabinoids, CB1/CB2 mixed agonists, are potent analgesics not only in a variety of pre-clinical pain models but also in the clinic.[7–9] Unfortunately, in the clinic these agents also show undesirable CNS side effects such as euphoria and drowsiness, which are believed to be mediated through the activation of CB1 receptors in the brain.[10] There is a substantial pre-clinical literature supporting a peripherally-mediated nociceptive action of cannabinoids, suggesting that cannabinoid-mediated analgesia without the well known central side effects is achievable.[11–16]

One strategy to dissociate the analgesic effect of cannabinoid agonists from CNS side effects would be to develop ligands with a low potential to cross the blood brain barrier. Ajulemic acid (**2**), a THC derivative possessing a carboxylic acid moiety, has been reported active in phase II clinical trial for neuropathic pain with no major adverse effect[17] and Ajulemic acid has reduced CNS penetration (**2**, rat Cbrain/Cplasma ~0.3–0.4) compared to Δ9-THC (**1**, rat Cbrain/Cplasma 1).[18] More recently, the group of Dziadulewicz reported naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone (**3**) to be a CB1/CB2 mixed agonist with limited brain penetration (rat Cbrain/Cplasma 0.07), which displays good oral bioavailability and antihyperalgesic activity in animal pain models without obvious side-effects.[19] This anti-nociceptive effect was blocked by pretreatment with a selective CB1 antagonist, but not by a CB2 selective antagonist. Recently, we reported that AZ11713908 (**4**), a molecule from a different chemical series, which is both a potent rat CB1 agonist and rat CB2 inverse agonist, exhibited analgesic effects in inflammatory and neuropathic pain models.[20] AZ11713908 showed a low potential to cross the blood brain barrier. Systemic administration of AZ11713908, produced robust analgesia in rodent pain models via peripheral CB1 receptors. A peripheral CB1 receptor mechanism of action proved to be sufficient to reverse hypersensitivity in rodent inflammatory pain models. These results support a strategy aimed at developing CB1 or CB1/CB2 mixed agonists for the treatment of pain via activation of peripheral CB1 receptors while limiting CNS exposure so as to minimize potential side effects.

Despite the considerable number of cannabinoid ligands reported thus far[7,21] there is a scarcity of potent CB1 or CB1/CB2 agonists which combine low CNS penetration together with good

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0960-894X/$ - see front matter © 2012 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2011.12.124

physicochemical and pharmacokinetic (PK) properties. Compounds **3** and **4** have a high binding affinity for human CB1/CB2 receptors and are potent human CB1 receptor agonist with low CNS penetration. However, they both possess high lipophilicity (*C*log*P* = 7.8 for **3** and *C*log*P* = 6.5 for **4**), and poor aqueous solubility (<0.01 mg/mL).[19] In the present study we describe the γ-carbolines, a new class of potent CB1/CB2 mixed agonists, and the identification of compound **29**, which shows oral bioavailability, high aqueous solubility, robust analgesic activity in a rat inflammatory pain model, and limited brain penetration.

The γ-carboline series was identified by scaffold-hopping from the bicyclic benzimidazole core.[22] Compound **5**, a representative early analogue of the carboline series, displayed a good binding affinity at both human CB1 (K_i 143 nM) and CB2 (K_i 14 nM), as well as CB1 agonist activity in rat brain tissue (rat CB1 EC_{50} 1440 nM, E_{max} 47%). Interestingly, unlike most known cannabinoid ligands, the carboline series demonstrates good aqueous solubility. For example, the solubility of **5** at pH 7.4 is >3 mg/mL. Furthermore, even though compound **5** has a low polar surface area (PSA) of 21, it shows moderate CNS penetration with a brain to plasma ratio

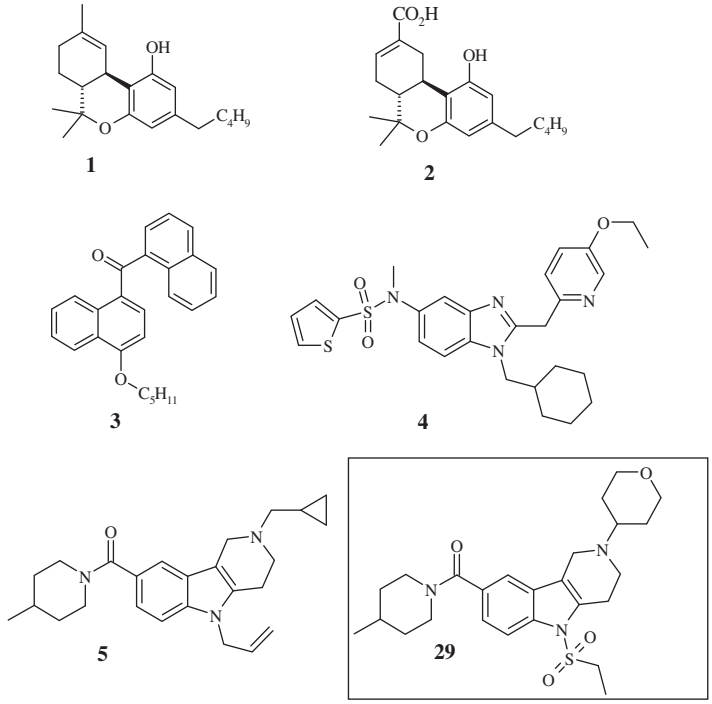
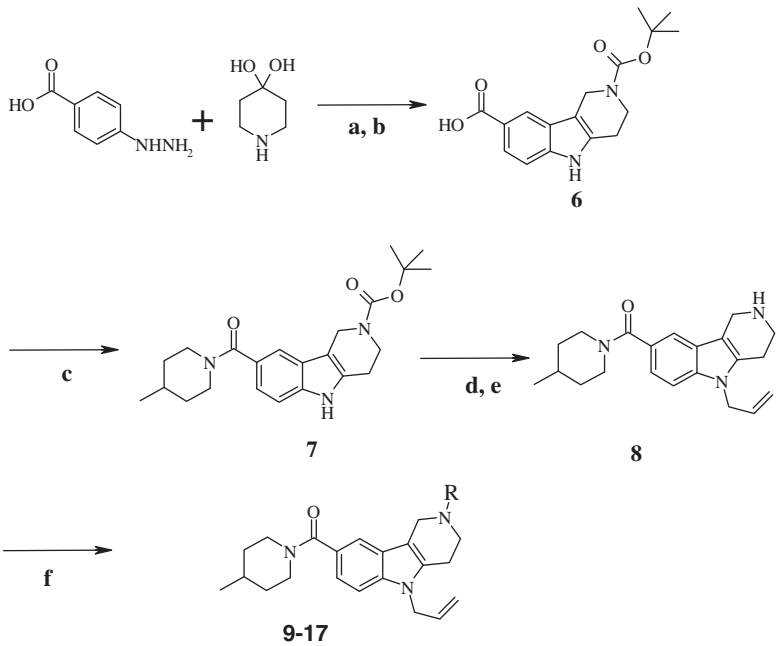


Figure 1. Several cannabinoid ligands.



Scheme 1. Reagents and conditions: (a) 1 N HCl, dioxane/H_2O, reflux; (b) Boc_2O, 5 N NaOH; (c) methylpiperidine, HATU, Hunig base; (d) NaH, DMF, allyl bromide; (e) TFA, DCM; (f) appropriate aldehyde or ketone, $NaBH(OAc)_3$, DCM.

of 0.74.[23,24] One strategy undertaken to decrease CNS penetration in γ-carbolines was via increasing the PSA (vide infra).

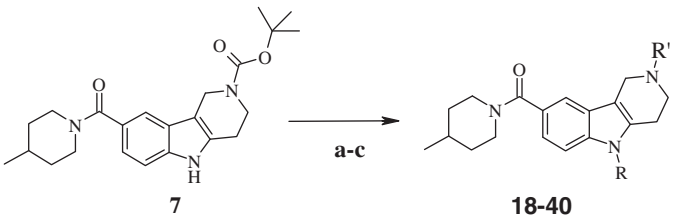
Compounds **9**–**40** were all derived from common intermediate **6** as shown in Schemes 1 and 2.

Our SAR investigation started with modifications to the right-hand side, the basic region of the γ-carboline scaffold. The human CB1 agonist activity and in vitro human liver microsome metabolic stability (HLM) of selected examples are summarized in Table 1. Substitution on the tetrahydropyridinyl N-atom with cyclic alkyl and heterocyclic groups led to improved hCB1 activity (**9**–**14**). The introduction of polar fragments such as pyridinylmethyl groups was detrimental to CB1 functional activity (**15**–**17** vs **9**). Substitution by tetrahydrothiophenyl ring (**12**) afforded a very potent agonist although the metabolic stability was lost. The best results came from cyclopentyl and 4-tetrahydropyran (4-THP) groups, as **11** and **14** achieved a >10 fold gain in agonist activity and maintained reasonable metabolic stability. Overall, 4-THP and cyclopentyl moieties were considered as preferred groups for the right hand side.

We then turned to building SAR on the southern portion, the indole N-atom (Table 2). Compounds **18**–**37** were prepared according to the general method described in Scheme 2. In order to increase the likelihood of identifying compounds with limited CNS penetration, we adopted a design strategy which included increasing polar surface area of γ-carboline analogues. Mahar Doan et al. analyzed the physical chemical properties of CNS and non-CNS drugs on the market and found that non-CNS drugs have higher PSA values (mean PSA value of 56) than CNS drugs (mean PSA value of 40).[25] To this end, a variety of functional groups were introduced at the indole nitrogen region to increase the overall polarity. Gratifyingly, with few exceptions, modifications to this southern region of the carboline were well tolerated. Alkyl, amide, sulfonamide (**18**–**31**), and sulfamide (**32**,**33**) groups all exhibited good CB1 affinity. Insertion of a carbonyl or sulfonyl group between the nitrogen and propyl group maintained the CB1 potency (**19** vs **23**,**24**) and, in addition, afforded a substantial increase in polarity (**19** PSA = 30 vs **24** PSA = 67). Although amide **23** and sulfonamide **24** were both equipotent in CB1 receptor binding, amide **23** was a partial CB1 agonist in the [^{35}S]GTPγS functional assay with only 17% efficacy, while sulfonamide **24** was a full agonist (hCB1 EC_{50} 129 nM and E_{max} 100%). Encouraged by the results of sulfonamides **24** and **25**, additional sulfonamides (**26**–**31**) were investigated. The ethylsulfonyl analogue, compound **29**, was found to be a potent agonist on hCB1 (EC_{50} of 49 nM, E_{max} 120%) and in rat brain tissue (rat CB1 EC_{50} 85 nM, E_{max} 156%). The in vitro selectivity of **29** was also examined in 52 diverse targets including a number of pain targets, and no significant activity was observed.[26]

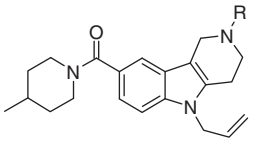
Further characterization of **29** alongside the starting γ-carboline hit, **5**, are shown in Table 3. Compound **29** is a potent dual CB1/CB2 agonist which combines restricted CNS penetration in rodents (brain to plasma ratio 0.07 in rat and 0.05 in mice)[27] together with good Caco-2 permeability. Although compound **29** and two reference compounds **3** and **4** display similar low levels of CNS penetration, their physicochemical properties and plasma protein binding profiles are very different (Table 3). Compounds **3** and **4** are characterized by high lipophilicity (*C*log*P* >6), high plasma protein binding (<1% free)[19,28] and low solubility. In contrast, compound **29** shows 13% free in rat plasma protein binding, *C*log*P* of 2.4, and aqueous solubility of 0.48 mg/mL. Furthermore, with an efflux ratio of 1.5 in MDR1/MDCK assay, compound **29** has a low potential for being a P-gp substrate in man.

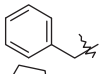
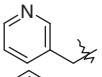
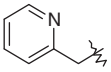
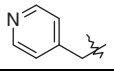
The rat pharmacokinetic profile for **29** is shown in Table 4. Compound **29** exhibited moderate plasma clearance (28 mL/min/kg corresponding to 40% liver blood flow in rat) that is consistent with predicted hepatic clearance from the in vitro metabolic stability in rat liver microsomes (28 mL/min/kg while taking plasma protein binding into account). The oral bioavailability of **29** in rats is 16%,



Scheme 2. Reagents and conditions: (a) NaH, DMF, RX; (b) TFA, DCM; (c) cyclopentoanone or 4-tetrahydropyranone, $NABH(OAC)_3$, DCM.

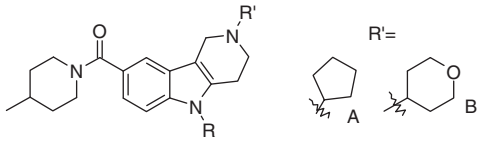
Table 1
The hCB1 agonist activity and in vitro metabolic stability data of **5** and **9**–**17**

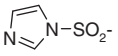


Compound	R	hCB1[a] EC_{50} (nM)	hCB1[a] E_{max} (%)	HLM (μl/min/mg)	Compound	R	hCB1[a] EC_{50} (nM)	hCB1[a] E_{max} (%)	HLM (μl/min/mg)
5		768	71	66	**13**		60	57	135
9		103	94	>300	**14**		60	57	42
10		115	63	>300	**15**		>14000		>300
11		33	65	93	**16**		5423	66	ND
12		5	58	>300	**17**		1360	76	>300

[a] Mean value of at least 2 independent measurements. EC_{50} were measured by GTPγ^{35}S binding on membranes of Human Embryonic Kidney (HEK) 293 EBNA stable cell line transfected with the cloned human CB1 receptor using Win55,212-2 as the reference agonist for E_{max} calculations.

Table 2
hCB1 binding affinity, functional activity and PSA of compounds **11**, **13** and **18–37**



Compound	R	R′	PSA	hCB1[a] K_i (nM)	hCB1[a] EC_{50} (nM)	hCB1[a] E_{max} (%)	Compound	R	R′	PSA	hCB1[a] K_i (nM)	hCB1[a] EC_{50} (nM)	hCB1[a] E_{max} (%)
11	Allyl	A	21	9	33	65	**27**	$MethylSO_2$-	B	67	239	473	103
13	Allyl	B	30	56	60	57	**28**	$EthylSO_2$-	A	58	17	11	122
18	*n*-Propyl	A	21	15	45	85	**29**	$EthylSO_2$-	B	67	120	49	120
19	*n*-Propyl	B	30	58	103	72	**30**	*i*-$PrSO_2$-	B	67	170	150	96
20	H	A	35	264	4253	115	**31**	$PhSO_2$-	B	67	31	59	46
21	Methyl	A	21	32	77	133	**32**	Me_2NSO_2-	A	60	22	26	114
22	Ethyl	A	21	34	63	118	**33**	Me_2NSO_2-	B	69	110	71	112
23	PropylCO-	B	48	102	117	17	**34**	Me_2NCO-	B	49	2380	ND	ND
24	$PropylSO_2$-	B	67	115	129	100	**35**	*p*-$EtOPhCH_2$-	B	39	51	53	113
25	$PropylSO_2$-	A	58	22	22	97	**36**	$EtNHSO_2$-	B	83	424	ND	ND
26	$MethylSO_2$-	A	58	36	50	109	**37**		B	78	592	ND	ND

[a] Mean value of at least 2 independent measurements. K_i values were measured by displacement of the agonist ^{3}H-CP55,940 binding on membranes of Human Embryonic Kidney (HEK) 293s stable cell lines transfected with the cloned human CB1 receptor. EC_{50} were measured by GTPγ^{35}S binding on membranes of Human Embryonic Kidney (HEK) 293 EBNA stable cell line transfected with the cloned human CB1 receptor using Win55,212-2 as the reference agonist for E_{max} calculations.

Table 3
Characterization of selected carboline compounds and reference compounds

Compound	Compound class	hCB1[a] K_i (nM)	hCB1[a] EC_{50} (nM)	hCB1[a] E_{max} (%)	hCB2[a] K_i (nM)	HLM (μl/min/mg)	RLM(μl/min/mg)	Br/Pl ratio (rat)	Caco-2 Papp ($\times10^{-6}$ cm/s)	PSA	ClogP	Solubility @pH 7.4 (mg/mL)
29	Carboline	120	49	120	9	52	202	0.07	13	67	2.4	0.48
5	Hit carboline	143	768	71	14	66	>300	0.74	3	21	4.8	3.3
1	Δ9-THC	10	17	20	24			1.00[18]		32	7.2	<0.01
3	Napthyl	35	72	111	19			0.04		27	7.8	<0.01
4	Benzimidazole	3	6	115	0.56	>300	>300	0.07	0.6	69	6.5	<0.01

[a] Mean value of at least 2 independent measurements. K_i values were measured by displacement of the agonist ^{3}H-CP55,940 binding on membranes of Human Embryonic Kidney (HEK) 293s stable cell lines transfected with the cloned human CB1 receptor. EC_{50} were measured by GTPγ^{35}S binding on membranes of Human Embryonic Kidney (HEK) 293 EBNA stable cell line transfected with the cloned human CB1 receptor using Win55,212-2 as the reference agonist for E_{max} calculations.

Table 4
Rat pharmacokinetic profile of compound **29**

Compound	Administration route	Parameters	Mean
29	IV	Cl (mL/min/kg)	28
		$T_{1/2}$ (h)	0.63
	Oral	V_{ss} (l/kg)	1.2
		F%	16

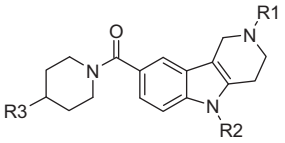
which suggests that absorption may be incomplete. The CYP inhibition potential is low for compound **29** as IC_{50} values for CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 are greater than 20 μM. Compound **29** shows moderate hERG activity with an IC_{50} of 3.1 μM using voltage ion flux electrophysiology assay.

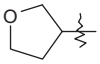
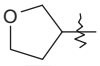
Strategies that have been developed to reduce hERG activity include modulating pK_a, *C*log*P*, and making discreet structural changes to the scaffolds to introduce hydrophilic groups in key positions.[29] In the case of the γ-carbolines, changes in pK_a appear to strongly influence the hERG activity (Table 5). Compound **39** with a THF moiety on N-1 is 1 log unit less basic than compound **29**, and **39** is sixfold less active on hERG (IC_{50} of 3.1 μM for **29** and 25 μM for **39**). Similarly compound **38**, the corresponding THF analogue of **24**, has a lower potential to interact with hERG channel than **24** (2 μM for **24** and 13 μM for **38**). The hERG activity is also found to be sensitive to changes in the amide region. For example, there is only one methyl group difference between **40** (*C*log*P* 2.6) and **41** (*C*log*P* 2.0), and the hERG channel activity drops sixfold (IC_{50} of 2.6 μM for **40** and 18 μM for **41**).

Before investigation of the in vivo efficacy of **29**, it was tested in the field-stimulated mouse vas deferens (MVD), which is a more physiological CB1 receptor agonism assay than membrane [^{35}S]GTPγS binding assay. Thus, the purpose of assessing **29** in MVD was to confirm qualitatively its agonist activity rather than to interpret in vivo activity; the MVD is a mouse model, whereas we used a pain model in rat, and species differences could affect the absolute potency of compounds. The agonist activity of **29** was confirmed in the MVD assay, in which it displayed an IC_{50} of 1.9 nM (data not shown). Thus, compound **29** showed CB1 agonist activity not only in membranes of cloned rat and human CB1 receptor in heterologous system, but also in a physiologically intact tissue containing a native receptor.

Compound **29** was then tested in vivo in rat Carrageenan inflammatory pain model dosed subcutaneously at 0.35, 0.70, and 1.4 mg/Kg. These doses resulted in total plasma concentrations of 126–213 nM and between 10 and 13 nM in brain and exhibited dose dependent reversal of heat hyperalgesia.[30,31] No severe side effect associated with CB1 receptor at central nervous system was observed at these doses (Fig. 2).[32] This is in contrast to known CNS acting compounds, such as WIN55,212-2, which shows no margin between analgesic effect and CNS side effect.[20] Although

Table 5
Reducing activity at hERG: **29**→**39**, **24**→**38** and **40**→**41**



Compound	R1	R2	R3	hERG IC_{50}(μM)	pK_a (measured)	*C*log*P*	hCB_1[a] K_i (nM)
29		EthylSO_2-	Methyl	3.1	6.9	2.4	120
39		EthylSO_2-	Methyl	25	5.9	2.5	197
24		PropylSO_2-	Methyl	2	7.3	3	115
38		PropylSO_2-	Methyl	13	5.8	3.1	320
40		Methyl	Methyl	2.6	—	2.6	76
41		Methyl	H	18	—	2.0	226

[a] Mean value of at least 2 independent measurements. K_i values were measured by displacement of the agonist ^{3}H-CP55,940 binding on membranes of Human Embryonic Kidney (HEK) 293s stable cell lines transfected with the cloned human CB1 receptor.

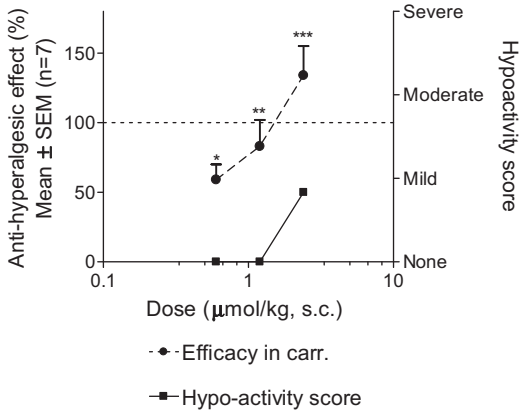


Figure 2. Anti-hyperalgesic effects and hypoactivity score of **29** (sc) in rat carrageenan model (method see References 20,32). Data were shown as mean ± SEM ($n = 7$). $^*p$ <0.05, $^{**}p$ <0.01 and $^{***}p$ <0.001 versus vehicle group by one way ANOVA followed by Holm-Sidak test. Slightly hypo-activity was observed at rats treated with 2.4 μmol/kg dose.

we can not rule out contribution of central CB1 activation to the analgesic activity, the fact remains that the more peripherally restricted compound **29** shows considerably improved safety margin compared to a CNS active CB agonist, WIN55,212-2.[20]

In summary, we have identified γ-carbolines as a new structural class of potent mixed CB1/CB2 agonists, and we have demonstrated that solubility and potency can be achieved together for a cannabinoid ligand. Carbolines are a class of cannabinoid agonists with good physicochemical properties and low CNS penetration, and compound **29** demonstrates significant anti-hyperalgesia in a rat inflammatory pain model with significant margin versus CNS side effects. Further investigation of this promising chemical series is under way and more detail accounts will be reported in due course.

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23. All the animal uses were approved by AstraZeneca R&D Montreal Animal Care Committee in accordance with Canadian Council on Animal Care guidelines. Unless specified otherwise, the in vivo PK study and CNS experiments were conducted in Sprague–Dawley male rat.
24. For the measurement of brain to plasma ratio of compound **5**, the compound was administered at 1 mg/kg via subcutaneous route (sc) in rats. The blood and brain samples were collected at 30 min after compound administration.
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26. Compound **29** shows less than 50% activity (10 μM) against the following targets: hACE, hCOX-1, hMAO-A, mERK2, hPKCα, rα1, hα2a, hβ1, hD2L, hERα, rGABAa, hH1, hM2, hM3, n-AChR, 5-HT2a, hNET, LTCC, NaV site 2, h5HT6, hA2a, hAT1, hBRS-3, hCCR1, hCCR2b, hCCR5, hCGRP, hCRF1, hCX3CR1, hCXCR2, hCXCR6, hETA, hFPR1, hGHS-R, hMC4, hMCH1, hM4, hNK3, hGLP-1, hGLP-2, hMtl, hSST4, hV1B, rA3, rGPR103, rPK2r, mGPR73, hFPRL-1, and mUT II. Compound **29** has an IC_{50} >10 μM against 3 opioid receptors hDOR, hMOR and hKOR receptors.
27. Animals were treated with **29** (sc and po) and samples were collected at 30 or 60 min after drug administration. The animals are sacrificed by decapitation, the brain collected immediately and after removing the pons, cut in two to separate the right and left hemispheres. Visible blood clots are removed. The right hemisphere is snap-frozen by immersion in liquid nitrogen and stored frozen at −80 °C. They are then pulverized with the cryogenic system. Pulverized samples are then transferred into glass tubes and weighing the samples, the appropriate amount of buffer is added and samples are homogenized with the Covaris proce.
28. Unpublished results from this laboratory.
29. For recent reviews on strategies to mitigate hERG activity, see (a) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *49*, 5029; (b) Durdagi, S.; Subbotina, J.; Lees-Miller, J.; Guo, J.; Duff, H. J.; Noskov, S. Y. *Curr. Med. Chem.* **2010**, *17*, 3514.
30. The determination of the total plasma or brain concentration of **29** was performed by protein precipitation (after homogenization of brain samples), followed by reversed-phase liquid chromatography and electrospray mass spectrometry. Twenty microliter of biological samples from CNS experiments (plasma and brain) and blank matix (ces) are added manually into a 96 well plate. All the in vivo samples are precipitated by adding 60 μl of ACN+. The plate is carefully capped, vortexed and centrifuged at 9000*g* for 30 min at 4 °C. The samples are then ready to be analyzed by LC–MS/MS. After preparing an analytical standard in ACN+, serial dilutions are carried out in order to prepare a calibration curve. The range of the calibration curve is 1.22 to 1000.
31. Brain samples were corrected for 2% blood contamination according to Heisey SR. Brain and choroid plexus blood volumes in vertebrates. Comp. Biochem. Physiol. 1968, Aug; 26(2):489.
32. Method to score hypo-activity: hypo-activity was visually observed and scored by the experimenters. Rats were observed for the following behaviors: sniffing, grooming, scratching, vocalization, stereotypy (excessive repetition or lack of variation in movements), rearing, and body posture. Animals were also examined for signs of ptosis (drooping of the upper eyelid), exophthalmos (abnormal protrusion of the eyeball). Hypoactivity was rated 0, absent; 1, mild; 2, moderate; and 3, severe.