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From pyrroles to 1-oxo-2,3,4,9-tetrahydro-1*H*-β-carbolines: A new class of orally bioavailable mGluR1 antagonists

Romano Di Fabio,* Fabrizio Micheli, Giuseppe Alvaro, Paolo Cavanni, Daniele Donati, Tatiana Gagliardi, Gabriele Fontana, Riccardo Giovannini, Micaela Maffeis, Anna Mingardi, Maria Elvira Tranquillini and Giovanni Vitulli

GlaxoSmithkline Medicine Research Centre, Via Fleming 4, 37135 Verona, Italy

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Abstract—Exploiting the SAR of the known pyrrole derivatives, a new class of mGluR1 antagonists was designed by replacement of the pyrrole core with an indole scaffold and consequent cyclization of the C-2 position into a tricyclic β-carboline template. The appropriate exploration of the position C-6 with a combination of H-bond acceptor groups coupled with bulky/lipophilic moieties led to the discovery of a new series of mGluR1 antagonists. These compounds exhibited a non-competitive behavior, excellent pharmacokinetic properties, and good in vivo activity in animal models of acute and chronic pain, after oral administration.

Glutamate is the major excitatory neurotransmitter present in the mammalian brain and spinal cord. Its action mediates several key brain functions, including cognition and memory.[1] In addition, glutamate has been hypothesized to play a critical role in the pathophysiology of a variety of neurological and psychiatric diseases such as: acute stroke, traumatic brain injury, neuropathic pain, Huntington's disease, schizophrenia, depression, dependence, and addiction.[2] Two different families of glutamate receptors have been identified, namely ionotropic glutamate receptors[3,4] (NMDA, AMPA, and kainate) and metabotropic receptors[5,6] (mGluRs). Ionotropic receptors are ligand-gated cation channels responsible for fast excitatory neurotransmission and neuronal plasticity. Metabotropic receptors belong to the family of 7 TM class C G-protein coupled receptors (GPCRs) and control both glutamate release and post-synaptic excitability.

These receptors are characterized by the presence of a large amino-terminal domain containing a bilobal-structured Venus fly trap.[7] Recent molecular modeling studies, as well as receptor models based on crystallographic data, shed new light on the mechanism of interaction of these receptors with both agonists and antagonists.[8,9]

To date, eight different mGluR subtypes have been identified, named mGluR1–8. They can be subdivided into three groups based on their similarity sequence, pharmacology, and transduction mechanisms, namely group I (mGluR1 and mGluR5), group II (mGluR2 and mGluR3), and group III (mGluR4, mGluR6, mGluR7, and mGluR8). Our interest in this area was mainly devoted to the discovery of antagonists of the mGluR1 receptor subtype.[10] Pyrrole derivatives **1**–**4** (Fig. 1) have

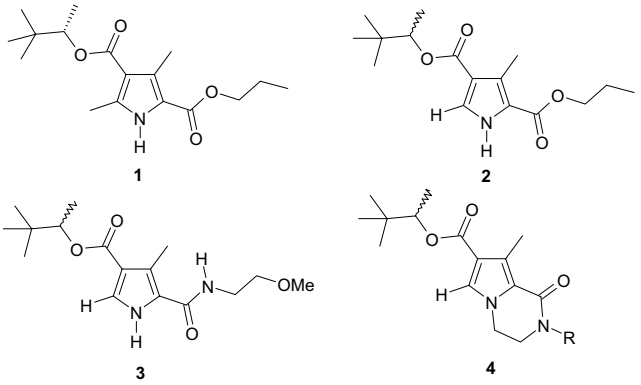

Figure 1. Series of pyrrole mGluR1 antagonists previously described.

Keywords: mGluR1 antagonists; Metabotropic receptors; Excitatory amino acids; Glutamate; Acute and chronic pain.
* Corresponding author. Tel.: +390458218879; fax: +390458218196; e-mail: rdf26781@gsk.com

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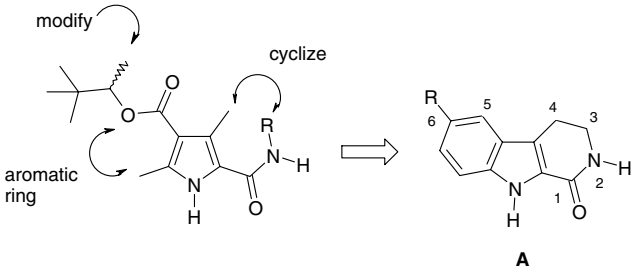


Figure 2. Proposed evolution of the pyrrole scaffold.

been discovered[11–16] as potent and selective compounds, active in different in vivo animal models of acute and chronic pain.[17]

As part of a broad chemical strategy aimed toward the discovery of new classes of orally bioavailable mGluR1 receptor antagonists, an attempt to modify the pyrrole scaffold was performed removing simultaneously the flexible/lipophilic side chain present in the top region of the pyrrole molecules. To this end, β-carboline derivatives of type **A**, shown in Figure 2, were designed as a potential structural evolution of the pyrrole compounds. In particular, this scaffold, as depicted in Figure 2, resulted from the replacement of the pyrrole nucleus by the corresponding indole, along with the cyclization of the C-2 amido moiety and the C-3 methyl group. Then, once identified the core template, we managed to maximize the in vitro activity varying the nature of the R group present at the position C-6. From the previous SAR elements gathered on the pyrrole series, it was known that the north-western region of these new molecules could have been highly sensitive to the effect of the substituents. In particular, in the pyrrole derivatives, the combination of a H-bond acceptor and bulky/lipophilic groups was essential to maximize their in vitro potency.

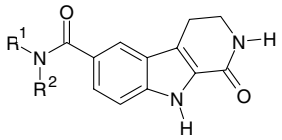
To explore this hypothesis two appropriate series of amides and a series of ether derivatives shown in Tables 1 and 2, respectively, were studied, targeting specifically the substitution at the position C-6.

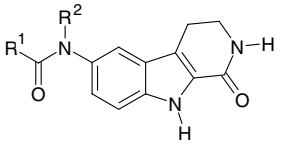
As far as the synthesis of amides **6**–**12** reported in Table 1 is concerned, they have been obtained in good yield as depicted in Scheme 1, from the known 6-carboxyethyl β-carboline intermediate **5a**[18] by sequential hydrolysis of the ethyl ester, activation of the carboxyl group as the pentafluorophenylester, and amidation reaction in good to excellent yield (41–100%) with either primary or secondary amines. Compounds **13** and **14** were prepared from the nitro derivative **5b**[19,20] in two steps, by reduction of the nitro group to the corresponding amine (CuCl, KBH_4, CH_3OH)[21] in 61% yield, followed by an amidation reaction with 1-adamantyl acyl chloride or formation of the corresponding urea in the presence of the 1-adamantyl isocyanate, respectively.

These compounds were characterized in terms of in vitro affinity on rat mGluR1a CHO cells, measuring intracellular $[Ca^{2+}]_i$ mobilization by a fluorescent imaging plate reader (FLIPR).[22] From the results summarized in Table 1 (entries **6**–**12**), it was evident that the 1,2,2-trimethylpropyl derivatives (entries **6** and **7**) were significantly less active than the corresponding pyrrole analogues,[11] suggesting a different SAR operating in this new series. In this case, only the tertiary amide **7** gave encouraging in vitro activity (IC_{50} = 120 nM), whereas compound **6** was found to be inactive (IC_{50} > 1 μM).

The 1-adamantyl **8** and the 2-adamantyl **9** derivatives were the most potent compounds identified (IC_{50} =

Table 1. Potency results on mGluR1 receptor

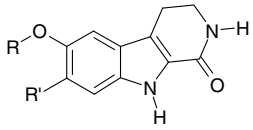




Entry	R^1	R^2	IC_{50}[a] (nM)
1	—	—	16
6	$(CH_3)_3CHCH_3$	H	na[b]
7	$(CH_3)_3CHCH_3$	CH_3	120
8	1-Adamantyl	H	71
9	2-Adamantyl	H	93
10	Bicycle(2.2.1)hept-2-yl[c]	H	226
11	CH_2-1-adamantyl	H	312
12	C_6H_5	H	1040
13	1-Adamantyl	H	71
14	NH-1-adamantyl	H	4660

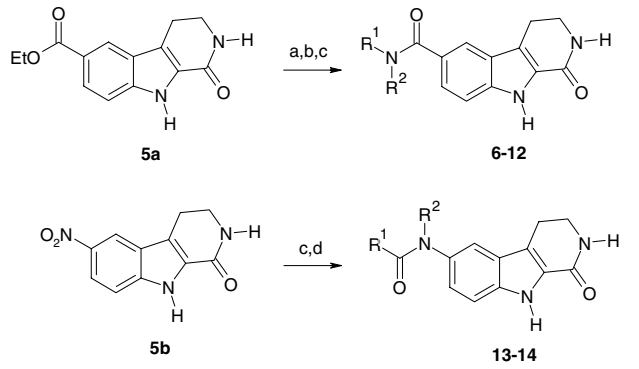
[a] Values are means of three experiments.
[b] na, activity > 10 μM.
[c] compound obtained as a mixture of *endo*/*exo* isomers.

Table 2. Potency results on mGluR1 receptor



Entry	R	R′	IC_{50}[a] (nM)
15	H	H	na
16	CH_3	H	na
17	$(CH_3)_3CHCH_3$	H	239
18	C_6H_5	H	4070
19	$CH_2C_6H_5$	H	104
20	$CH_2C_6H_{11}$	H	5560
21	$CH(CH_3)C_6H_5$[b]	H	380
22	$CH_2C_6H_5$	CH_3	167
23	—		2850
24	—		3980
25	—		1670
26	—		na
27	—		na
28	—		na
29	—		683
30	—	—	1020

[a] Values are means of three experiments; na, activity > 10 μM.
[b] Racemate.



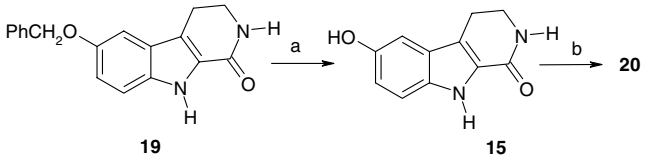
Scheme 1. Reagents and conditions: (a) LiOH, EtOH/H_2O 8:2, 60 °C, 3 h; (b) $C_6F_5OCOCF_3$, pyridine, DMF, rt, 2 h; (c) R^1R^2NH, DMF, 80 °C, 1–4 h; (d) CuCl, KBH_4 CH_3OH, rt, 30 min; (e) 1-adamantyl acyl chloride, pyridine, rt, 1 h or 1-adamantyl isocyanate, THF, rt, 12 h.

71 nM and 93 nM, respectively), confirming the need for the presence, similar to pyrroles, of a lipophilic/bulky substituent in the north-eastern region of the molecule to maximize the in vitro activity.[11] Notably, these compounds displayed the same non-competitive antagonism behavior already observed for the pyrrole derivatives.[11] The introduction of a methylene spacer (entry **11**) resulted in a significant drop in potency (IC_{50} = 312 nM) with respect to compound **8**. The presence of an aromatic substituent was less tolerated; the phenylamide derivative **12** was found in fact to be significantly less active (IC_{50} = 1040 nM) than the corresponding adamantyl analogues. Finally, the inverse adamantyl amide **13** exhibited the same in vitro potency as compound **8** (IC_{50} = 71 nM), whilst the corresponding urea derivative **14** was less active, probably due to the presence of an additional H-bond donor, a polar moiety not tolerated in this region of the molecule.

To enlarge the scope of this exploration, maintaining the presence of H-bond acceptor group, a series of C-6 substituted ether derivatives was prepared. To this end, compounds **15**–**22**, reported in Table 2, were synthesized.

In particular, **16**, **17**, **18**, **19**, **21**, and **22** were obtained in good yield following the classical synthetic approach for the preparation of substituted β-carboline compounds,[19] whereas analogue **20** was prepared, as shown in Scheme 2, by direct alkylation of the phenol derivative **15**, easily obtained by debenzylation reaction of compound **19**.

As expected, this alkylation reaction occurred in low yield, due to the intrinsic difficulty in controlling the parallel alkylation of the indole moiety.



Scheme 2. Reagents and conditions: (a) HCO_2NH_4, Pd/C 5%, MeOH, reflux, 1 h; (b) RX, K_2CO_3, CH_3CN, 80 °C, 24 h.

Compounds **15**–**22** were then evaluated in the in vitro assays. As shown in Table 2, both the unsubstituted phenol derivative **15** and the methoxy derivative **16** were found inactive, further reinforcing the hypothesis regarding the need for the presence of a lipophilic/bulky group in this region of the molecule. Conversely, the corresponding 1,2,2-trimethylpropyl ether **17** gave a positive sign of activity (IC_{50} = 239 nM), whereas the introduction of an aromatic ring directly linked to the oxygen atom led to a poorly active molecule (entry **18**, IC_{50} = 4070 nM). Notably, the benzyl derivative **19** exhibited the best in vitro potency (IC_{50} = 104 nM) as far as this subclass of ether compounds is concerned.

The replacement of the phenyl ring by the corresponding cyclohexyl group caused a significant reduction of the in vitro affinity (entries **20** and **19**: IC_{50} = 5560 nM and 104 nM, respectively). Moreover, the introduction of a methyl group at the benzylic position (entry **21**) gave an activity 3–4 times lower than the compound **19** (IC_{50} = 380 nM and 104 nM, respectively). Finally, the introduction of a methyl at the position C-7 can be considered almost neutral as far as the in vitro activity is concerned. (entries **22** and **19**: IC_{50} = 167 nM and 104 nM, respectively).

Following these interesting findings, a wider synthetic program was undertaken to explore in more detail the SAR associated to the β-carboline scaffold. To this end, the series of modified analogues **23**–**30**, shown in Figure 3, were synthesized. As depicted in Scheme 3, compound **25** was smoothly prepared from derivative **19** by reduction with Mg in methanol at reflux for 1 h.[23] The amino analogues **26** and **27** were obtained as a 1:1 mixture by simple reduction of compound **19** with BH_3·THF, followed by smooth separation by flash chromatography (AcOEt/CH_3OH/NH_4OH 80:19:1). The five-membered ring derivative **29** was synthesized in three steps, in good total yield, from the known aldehyde derivative **31**,[24] by sequential reductive amination, hydrolysis of the ethyl ester, and final cyclization reaction. Compound **30** was synthesized as previously reported.[24]

As shown in Table 2, a significant reduction in potency was observed for both the C-5 and C-7 benzyloxy derivatives **23** and **24** with respect to C-6 analogue **19**. In addition, both the amino derivative **25** and **26** were inactive. The five-membered ring **29** and compound **30** exhibited reduced in vitro potency compared to compound **19** (IC_{50} = 683 nM and 1020 nM, respectively vs 104 nM). Finally, compounds **26**, **27** and **28** were found to be inactive (IC_{50} > 10 μM).

In summary, from the exploration performed, it seems that the following pharmacophoric features should be present to maximize the in vitro potency of this class of mGluR1 antagonists:[25]

(a) a N-unsubstituted δ-lactam moiety acting as H-bond acceptor and/or donor;

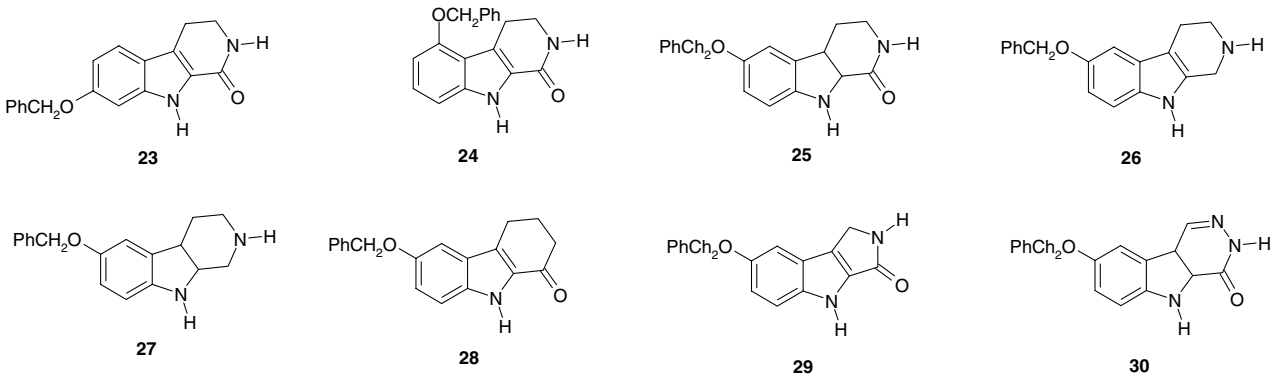
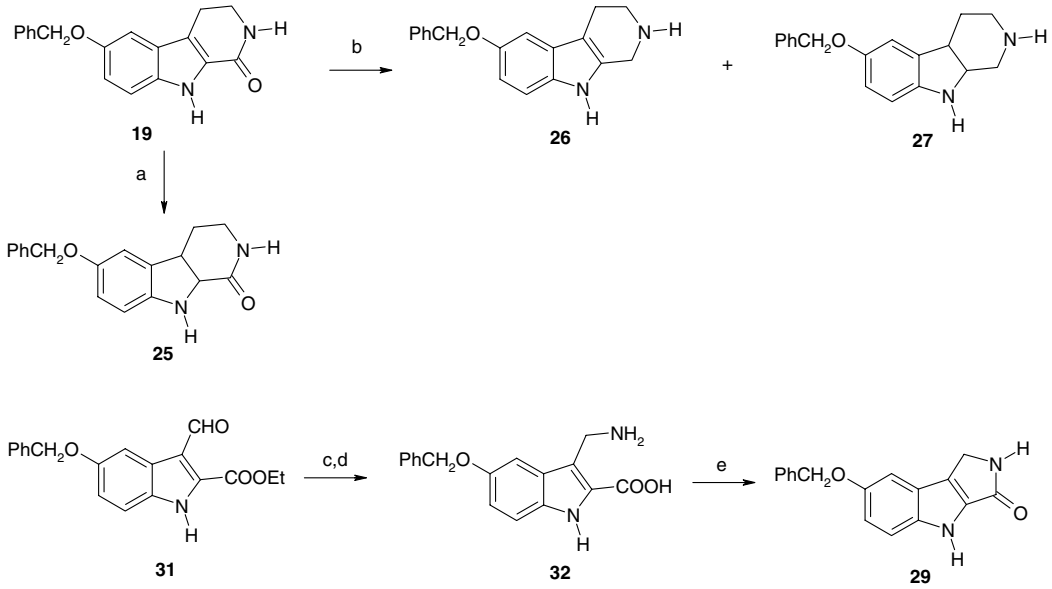


Figure 3. Structural modifications of the β-carboline core.



Scheme 3. Reagents and conditions: (a) Mg, CH_3OH, reflux, 1 h; (b) BH_3. THF, reflux, 4 h; (c) $CH_3CO_2NH_4$, $(CH_3O)_3BHNa$, dichoroethane, rt, 24 h; (d) LiOH, THF/H_2O 1:1, reflux, 8 h; (e) EDC, DMAP, *N*-methyl morpholine, rt, 36 h.

(b) a H-bond acceptor group (ether or amide moiety) at the position C-6;
(c) an appropriate bulky/lipophilic group in the north-eastern region of the molecule.

Based on these results, the most potent compounds[26] identified **8**, **13**, and **19** were characterized in terms of in vivo pharmacokinetic in rats. As shown in Table 3, compound **8** showed encouraging signals of oral bioavailability ($F = 12\%$). Conversely, **13** exhibited a poor in vivo pharmacokinetics profile despite the comparable Clp (52 ml/min/kg and 54 ml/min/kg for compounds **13** and **8**, respectively), accounting for a solubility-limited absorption process. As far as compound **19** is concerned, the oral bioavailability was variable and highly dependent on the oral dose used.[27] Conversely, the substituted analogue **22**, a compound specifically synthesized to shield the metabolically labile benzyl position,[28] exhibited a considerable improvement of the pharmacokinetic profile both in terms of oral bioavailability ($F = 36\%$) and maximum exposure in plasma (C_{max} = 264 ng/ml).

Table 3. Comparison of the PK profiles

Compound	**8**	**13**	**19**	**22**
$F\%$	12	—	9	36
C_{max}[a]	37	—	145	264

[a] Maximum concentration in plasma after oral administration (ng/ml).

Compound **22** also showed an excellent level of brain penetration ($B/P = 6.1$), assessed 1 h after dosing at 3 mg/kg iv.

According to these results, compound **22** was characterized in vivo in the formalin test in mice, a model of sustained inflammatory pain.[29] As shown in Figure 4, when **22** was given po at 10 mg/kg and 30 mg/kg, 30 min before the injection of formalin into the left hind paw, a significant reduction[30] of the nociceptive behavior was observed only in the LP of the study. Conversely, at a dose 30 mg/kg of **22**, a significant suppression[30] of the nociception in both the EP and LP was observed,[31,32] further confirming the broad spectrum analgesic potential associated with the mGluR1 antagonists.[33]

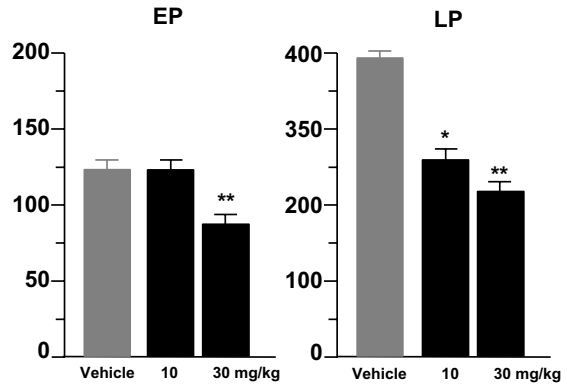


Figure 4. Formalin test in mice. EP (early phase): 0–5 min after 20 μl 1% formalin; LP (late phase): 20–60 min after 20 μl 1% formalin; $^{*}p < 0.05$, $^{**}p < 0.01$; $n = 8$–19.

Therefore, despite the limited in vitro potency, compound **22** showed good in vivo activity, due to the combination of the good oral bioavailability, excellent brain penetration, and relatively high fraction unbound in plasma.[34]

In conclusion, C-6 substituted β-carboline derivatives were identified as new mGluR1 antagonists. These compounds were rationally designed based on the known pharmacophore model available for the pyrrole derivatives previously explored in house. In particular, the appropriate exploration of the C-6 position of this class of β-carboline derivatives enabled the design of compounds exhibiting good in vitro potency and similar non-competitive antagonism behavior as the pyrrole derivatives. Among the different compounds prepared, the benzyloxy derivative **22** was identified as the most balanced compound in terms of in vitro activity and pharmacokinetic properties. According to these positive characteristics the compound was tested in vivo and a good analgesic activity both in the EP and LP of the formalin test in mice was observed, further confirming the therapeutic potential of the mGluR1 antagonists in acute and chronic pain.

Acknowledgments

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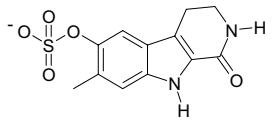
25. From the exploration performed to date, it is evident that the presence of the ether group present at the C-6 position, probably acting as H-bond acceptor, is critical for

maximizing the in vitro potency of this subclass of β-carboline derivatives. To prove this hypothesis, a limited series of C-6 carbon linked derivatives (aryl heterocycle, hetero–aryl, alkyl–aryl, alkenyl–aryl, and alkynil–aryl derivatives) was prepared by cross-coupling reaction starting from the 6-Br and/or 6-OTf β-carboline derivatives (R. Di Fabio and R. Giovannini, unpublished results). These compounds showed a significant drop of the in vitro potency versus compound **19**. The only one exhibiting residual sign of activity was the (*E*)-2-(4-fluorophenyl)ethenyl derivative (IC_{50} = 549 nM), in which the double bound is able to mimic somehow the electron content of the ether moiety. Final results of this exploration will be reported in due course.

26. Compound **8**: ^{1}H NMR (300 MHz): DMSO δ 11.77 (s, 1H); 8.10 (s, 1H); 7.66–7.46 (m, 3H); 7.34 (d, 1H); 3.51 (m, 2H); 2.94 (t, 2H); 2.08–2.05 (m, 9H); 1.65 (m, 6H).Compound **13**: ^{1}H NMR (300 MHz): DMSO δ 11.47 (s, 1H); 8.99 (s, 1H); 7.86 (d, 1H); 7.50 (s, 1H); 7.36 (dd, 1H); 7.26 (d, 1H); 3.48 (td, 2H); 2.85 (t, 2H); 2.0 (s, 3H); 1.90 (s, 6H); 1.69 (s, 6H).Compound **19**: ^{1}H NMR (300 MHz): DMSO δ 11.41 (s, 1H); 7.49 (s, 1H); 7.46 (d, 1H); 7.38 (t, 2H); 7.31 (t, 1H); 7.27 (d, 1H); 7.16 (d, 1H); 6.93 (dd, 1H); 5.09 (s, 2H); 3.48 (m, 2H); 2.86 (t, 2H).Compound **22**: ^{1}H-NMR (300 MHz): DMSO δ 11.29 (s, 1H); 7.48 (d, 2H); 7.39 (m, 3H); 7.31 (t, 1H); 7.16–7.11 (ds, 2H); 5.10 (s, 2H); 3.46 (m, 2H); 2.85 (t, 2H); 2.28 (s, 3H).
27. The oral bioavilability was 0% to 9% at 1 mg/kg po and 5 mg/kg po, respectively. At the latter dose an encouraging concentration of compound in plasma was observed (C_{max} = 145 ng/ml). This result was explained in terms of progressive saturation of the first-pass metabolism at the higher dose.
28. As expected, the oxidation of the benzyl position was the main route of metabolism of this subclass of compounds. Compound **33** was in fact the most abundant metabolite excreted in urine in the 0–4 h interval after oral dosing, roughly accounting for the 40% of the amount of parent compound **22** administered.



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29. For a detailed description of the experimental procedure used for the in vivo in the formalin model in mice, see: Quartaroli, M.; Carignani, C.; Dal Forno, G.; Mugnaini, M.; Ugolini, A.; Arban, R.; Bettelini, L.; Maraia, G.; Belardetti, F.; Reggiani, A.; Trist, D. G.; Ratti, E.; Di Fabio, R.; Corsi, M. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 158, and references herein enclosed.
30. EP: vehicle mean = 121.7 s, SD = 20.7 s, SE = 4.7 s; 10 mg/kg mean = 114.4 s, SD = 40.2 s, SE = 10.1 s; 30 mg/kg mean = 83.5 s, SD = 13.6 s, SE = 4.8 s. LP: vehicle mean = 415.6 s, SD = 167.0 s, SE = 38.3 s; 10 mg/kg mean = 299.7 s, SD = 139.7 s, SE = 34.9 s; 30 mg/kg mean = 227.6 s, SD = 96.3 s, SE = 34.0 s.
31. The exposure in plasma of compound **22** in mice was measured at the end of the formalin test, 2 h after the oral administration of the compound at 10 mg/kg dose. The average maximum concentration observed was 495 ng/ml, confirming that the animals were successfully exposed to the compound during the in vivo test.
32. Compound **22** displayed the same non-competitive antagonism behavior seen for the corresponding amide derivatives previously described, along with good receptor selectivity over the mGluR5 receptor.
33. This research complied with national legislation and with company policy on the Care and Use of Animals and with related codes of practice.
34. The free fraction in rat plasma was 3.4%. For a recent paper on the importance of the free fraction in plasma/brain, see: Liu, X.; Smith, B. J.; Chen, C.; Callegari, E.; Becker, S. L.; Chen, X.; Cianfrogna, J.; Doran, A. C.; Doran, S. D.; Gibbs, J. P.; Hosea, N.; Liu, J.; Nelson, F. R.; Szewc, M. A.; Van Deusen, J. *Drug Metab. Dispos.* **2006**, *34*, 1443, and references herein enclosed.