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# 2,4-Dicarboxy-pyrroles as selective non-competitive mGluR1 antagonists: an exploration of the role of the pyrrolic scaffold

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**Abstract**

Following the disclosure of 3-(1,2,2-trimethylpropyl) 4-[3,5-dimethyl-2-propyloxycarbonyl]pyrrolecarboxylate as a potent and selective mGluR1 non-competitive antagonist, the role and the importance of the pyrrole template were investigated. Different aromatic moieties were investigated as possible bio-isosteric replacement of the original scaffold and some of them were shown to be partially able to mimic the properties of the original pyrrole ring.
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*Keywords:* 2,4-Dicarboxy-pyrroles; Antagonists; Pyrrole scaffold

## 1. Introduction

Glutamate, the principal excitatory neurotransmitter in mammalian brain, is a key compound in a number of brain functions. Its actions are mediated through ionotropic (NMDA, AMPA and Kainate) [1–5] and metabotropic receptors (mGluRs) [6,7]. These latter receptors, characterised by a large amino-terminal domain, belong to the family C of G-protein coupled receptors (GPCRs), control the activity of membrane effectors and ion channels [8–12], and share very little homology with other cloned receptors [13–15].

To date, eight mGluRs subtypes have been identified and named mGluR1–8 according to the succession of the molecular cloning.

These receptors are divided into three main groups on the basis of sequence similarity, pharmacology and transduction mechanisms: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7 and mGluR8). Several splice variants for some of the different subtypes were also reported [16].

We have recently reported [17–19] that 3-(1,2,2-trimethylpropyl) 4-[3,5-dimethyl-2-propyloxycarbonyl]pyrrolecarboxylate (**1**, Fig. 1) is a potent and selective non-competitive mGluR1 antagonist endowed with an excellent in vitro and in vivo activity in different animal models of pain. The most potent molecules belonging to this class are characterised by nanomolar potency at the mGluR1 receptor and they are extremely selective vs. the other receptor subtypes ($\gg$500-fold). Moreover, an "opiate-like" profile was clearly shown for these derivatives in animal models of pain: actually they are active both on the early and the late phase of the formalin test in mice (a model of both nociception and inflammatory pain), in the carrageenan test in rat (a model of inflammatory pain) and in the chronic constriction injury in rats (a model of neuropathic pain). It was also demonstrated that the appropriate substitution of the ester moieties on the pyrrole scaffold led to an interesting modulation of both affinity and pharmacokinetic properties. Accordingly, a number of important tools endowed with different half-lives in rodents (from 5 min to $>$24 h) were identified.

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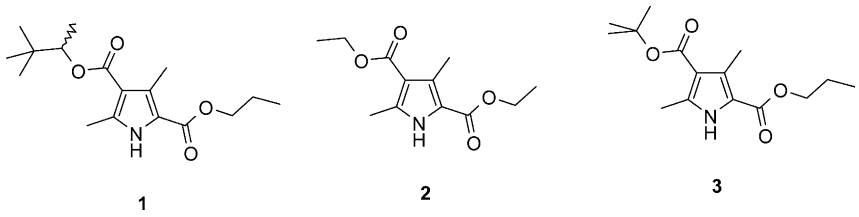


Fig. 1. The recently discovered pyrroles. Potent and selective non-competitive mGluR1 antagonists.

Due to the possible high value of these tools in a number of different physio-pathological animal models of unmet medical needs (stroke, cerebral ischemia and pain just to mention some of the main areas of interest), we decided to fully explore and understand the role of the pyrrole scaffold in these molecules, expanding its investigation and trying to replace it with properly selected templates.

Some results of these extensive investigations are reported in this manuscript.

## 2. Results and discussion

As described in a previous paper [19], also in this investigational work, computational techniques played a fundamental role in order to save time and resources. Accordingly, before starting any bench chemistry, we decided to screen in silico the corporate database (DB) and also commercial ones to retrieve templates showing a similar pattern of substitution with respect to the known pyrroles identified and tested in-house. An example of a generic query for such a task is reported in Fig. 2 (**4**). The direct comparison with the potency previously shown by the pyrrole templates was used, at this stage, as a filter to identify the templates worth of further exploration.

The lower limit of acceptable potency for the new templates identified in this task in order to be further progressed and explored was selected on the basis of the simpler selective pyrrole identified in the original paper [19].

Actually 2,4-dicarboxy-3,5-dimetyl pyrrole ethyl ester (**2**, Fig. 1) was shown to be endowed with a 16-μM potency on CHO cell lines expressing rat-mGluR1 and it also showed acceptable selectivity vs. mGluR Groups II and III.

As mentioned above, the query reported in Fig. 2 (**4**) was used to scan in silico both the corporate DB and the commercially available products. Such a simple query obviously produced a huge number of potential hits among the different heterocycles or aromatics. In silico physicochemical properties and "drug-like" filters (MW < 600; clog $P$ < 5; rotable bonds < 10; H-donors < 5; H-acceptors < 5) were subsequently used to reduce the number of possible candidates to be really screened in vitro.

Finally, a visual inspection of the results was performed and, accordingly, some templates were given priority for being tested on the basis of the possible future synthetic tractability.

After these filters and following the in silico screening, we moved to in vitro activities using the same protocols previously described [19]. Some of the results achieved are hence reported here.

The first series of derivatives selected for biological assays belonged to the iso-oxazole family. Some properly substituted derivatives, represented by compounds **5**, **6** and **7** (Fig. 2), were submitted to test on CHO cell lines expressing r-mGluR1 receptors [19]; unfortunately, none of these products showed interesting potency values when tested at a 10-μM concentration.

A similar pyrazole (**8**; Fig. 3) showed a weak $IC_{50}$ (53 μM), while the similar N-unsubstituted derivatives (**9**; Fig. 3) showed no activity at 10 μM. This puzzling result lightened our interest and led us to a limited investiga-

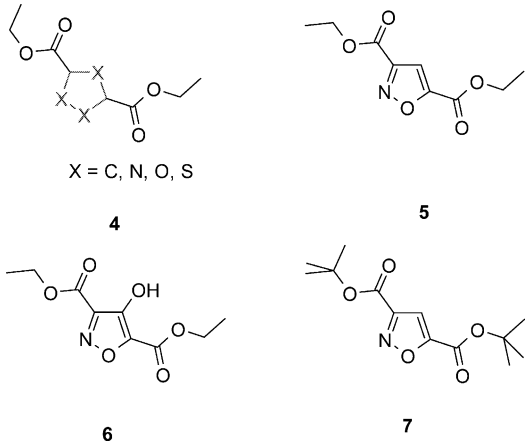


Fig. 2. The query (**4**) used for identifying alternative heterocycles and some of the hits coming from the virtual screen (**5**–**7**).

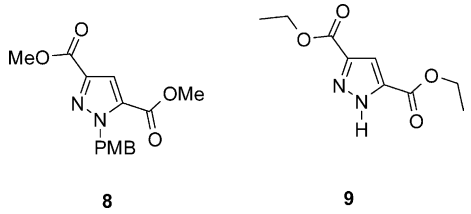
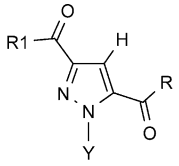


Fig. 3. The pyrazole analogues coming from the in silico screen. PMB, *p*-methoxybenzyl.

Table 1
$pIC_{50}$ values of pyrazole derivatives



| Entry | R | R' | Y | IC50 (μM) | pIC50 |
|---|---|---|---|---|---|
| 8 | OMe | OMe | PMB* | 53 | |
| 9 | OEt | OEt | H | N.A** | N.A. |
| 10 | n-OPr | n-OPr | Me | 14.1 | 4.85 |
| 11 | n-OPr |  | Me | 3.2 | 5.5 |
| 12 |  | OPr | Me | 3.2 | 5.5 |
| 13 |  |  | Me | 1.5 | 5.8 |
| 14 |  or OEt | OEt or  | H | 6.9 | 5.15 |
| 15 |  or n-OBu | n-OBu or  | H | 3.4 | 5.46 |
| 16 |  or n-OPr | n-OPr or  | H | 3.4 | 5.47 |

*PMB, *p*-methoxybenzyl.**N.A., not active up to 100 μM.

tion of this scaffold. The results of this exercise are reported in Table 1.

When the N-methylated pyrazole corresponding esters of derivative **1** were prepared (**11** and **12**; Table 1), the potency was much lower compared to the original pyrrole derivative (3.2 μM vs. 16 nM).

The synthesis of the corresponding N-demethylated derivative (theoretically much more similar to **1**) led to compound **16** (Table 1) whose structure was obviously in tautomeric equilibrium. Also, in this case, the potency was almost identical to derivative **11** and far below that of the original pyrrole, but not inactive as it happened for derivative **9**.

The possible explanation of these findings might be related either to the lack of the methyl groups which are missing on the pyrazole nucleus in comparison to the double methyl substitution present on the original pyrroles or to different electronic properties of the scaffold itself. Considering the poor electro-donating properties of the methyl group, it is unlikely that this is the main cause of this different activity, while the physicochemical properties of the molecules "per se" may play a more important role in this respect.

Considering that it was demonstrated that the pyrroles [17–19] bind in the region near the extracellular loops of the mGluR1 receptor, the overall lipophilicity of the scaffold might play a fundamental role in how the molecules reach their binding pockets.

Actually, the calculated log *P* (clog *P*) and molecular refractivity (MR) for derivative **1** are, respectively, 4.7 and 8.5 [20]. Also, considering derivatives **11/12** (i.e. the N-methylated derivatives of the pyrazole scaffold with respect to the more hydrophilic N–H derivative), these figures are much lower (3.8 and 7.8, respectively), and mainly one log *P* unit less.

These hypotheses are aligned with some experimental results previously reported [17,18] as the pyrrole nitrogen itself could be substituted by small alkyl derivatives (Me, Et, Pr, allyl) without a great loss of potency. Actually the real figures, depending obviously from the pattern of substitution of the esters, went from almost equal $IC_{50}$ (Me) to a 10-fold only loss in potency (Et, Pr, allyl). Bulkier groups (Bn, *p*-methoxybenzyl (PMB)) or EWG ones (e.g. BOC) led to almost complete inactivity.

Considering these findings on the nitrogen substitution (both on pyrroles and pyrazoles) the clog *P* hypothesis, and considering that some properly substituted thiophenes were also identified as hits from the in silico screening, we assayed in vitro some of these derivatives. As expected, all the derivatives tested showed $IC_{50}$ values greater than fixed 16 μM threshold, and therefore we decided to investigate the thiophene scaffold.

As reported in Table 2, a number of properly substituted products was prepared. SAR for the ester moieties was very similar to that already reported for the pyrrole scaffold. Accordingly, the most similar compound with respect to **1** was represented by derivative **22** (Table 2).

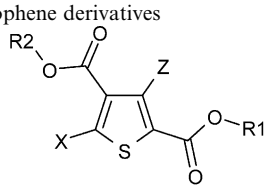
As can be clearly seen from this list, the replacement of the pyrrole with the thiophene template led to a very similar potency values on the mGluR1 receptor.

Further similarities were then identified as the non-competitive profile was confirmed by a number of experiments too. Also the selectivity pattern vs. mGluR Groups II and III resulted almost identical.

Very interestingly, the clog *P* and MR values calculated for derivative **22** were 5.8 and 8.2, respectively. It is interesting to note that the clog *P* value is, for this derivative, one unit higher than the original pyrrole **1**.

This is surely linked to the main drawback of this series, which was represented by the very poor aqueous solubility. This might also be the cause of some signs of cytotoxicity (observed at the microscope for some cells) shown by this product at the higher concentration tested

Table 2
$pIC_{50}$ values of thiophene derivatives




| Entry | X | R2 | Z | R1 | $IC_{50}$ (μM) | $pIC_{50}$ |
|---|---|---|---|---|---|---|
| **17** | $NH_2$ | Et | Me | Et | 8 | 5.1 |
| **18** | Me | Et | $NH_2$ | Et | 6 | 5.2 |
| **19** | H | Et | Me | Et | 4 | 5.4 |
| **20** | H | Bn | Me | Pr | 1.8 | 5.8 |
| **21** | H | t-Bu | Me | Pr | 0.16 | 6.8 |
| **22** | H |  | Me | Pr | 0.032 | 7.5 * |
| **23** | $NH_2$ |  | Me | Pr | 0.32 | 6.5 |

*Cytotoxicity observed at the higher concentrations.

during the generation of the concentration response curve (CRC).

Considering these interesting results, we reverted the "classical" bio-isoster paradigm and this time we moved from the thiophene to the benzene ring itself as reported in Table 3.

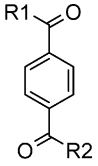
Also in this case, as the ester SAR was respected with respect to the pyrroles, the best derivative (**26**, Table 3) showed a very promising potency, a non-competitive profile and good selectivity vs. Groups II and III mGluRs. For derivative **26**, the clog *P* and MR values correspond to 5.6 and 8.2, respectively.

As foreseen from the calculated log *P* values, also in this case, the high lipophilicity (one log unit higher with respect to **1** as shown by **22**) led to very poor aqueous solubility and the same signs of cytotoxicity at the higher concentrations of CRC.

## 3. Conclusions

In this paper, we have clearly demonstrated that it is possible, in terms of potency and selectivity, to replace the pyrrole scaffold with other aromatics or heteroaromatic substituents endowed with appropriate stereoelectronic properties as SAR for the two ester moieties is very similar amongst the different templates explored.

Table 3
$pIC_{50}$ values of benzene derivatives




| Entry | R1 | R2 | $IC_{50}$ μM | $pIC_{50}$ |
|---|---|---|---|---|
| **24** | n-OPr | n-OPr | 4 | 5.4 |
| **25** | Ot-Bu | n-OPr | 0.4 | 6.4 |
| **26** |  | n-OPr | 0.063 | 7.2* |
| **27** |  |  | 0.026 | 7.6* |

*Cytotoxicity observed at the higher concentrations.

These important in vitro properties can be maintained selecting properly electron-rich templates. This was deduced according to the calculated log *P* values for a number of derivatives. If this figure is inferior to a defined threshold, a huge decrease in potency is observed. If this figure is maintained in an appropriate range, the potency is very similar. Nonetheless, when this figure is too high, a strong impact on the physicochemical properties of the series is reported.

It does seem, according to this preliminary report, that the pyrrole template itself may represent the best compromise between potency and physicochemical properties.

Further investigation on the role of the pyrrole itself and of its pattern of substitution is in progress and will be reported in due course.

## 4. General procedure for the synthesis of the esters

The appropriate carboxylic acid (1 mmol) was suspended in dry toluene (8 ml), trifluoroacetic anhydride (1.2 mmol) was added under a nitrogen atmosphere and the suspension stirred until complete solubilisation occurred at room temperature. The desired alcohol (1.2 mmol) was added, the solution was still stirred for 2 h at room temperature and then diluted with ethyl acetate (8 ml), washed with a 2-M NaOH solution (2 × 5 ml) and then with brine (2 × 5 ml). The organic phase

was dried over $Na_2SO_4$ and the solvent evaporated to obtain the crude compound, which was purified by column chromatography (generally cyclohexane/ethyl acetate, 9:1), to give the desired compound.

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