Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 2113–2118

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2,4-Dicarboxy-pyrroles as Selective Non-Competitive mGluR1 Antagonists: Further Characterization of 3,5-Dimethyl Pyrrole-2,4-dicarboxylic Acid 2-Propyl Ester 4-(1,2,2-Trimethyl-propyl) Ester and Structure–Activity Relationships

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Received 28 February 2003; revised 27 March 2003; accepted 10 April 2003

Abstract—Following the disclosure of 3,5-dimethyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester [3,5-dimethyl PPP] as a potent and selective mGluR1 non-competitive antagonist, we report here further in vivo characterization of this important tool and disclose the investigation of the C-5 position, which led to very potent compounds.

Glutamate, the principal excitatory neurotransmitter in mammalian brain, is a key mediator for a number of brain functions and acts through modulation of ionotropic (NMDA, AMPA and Kainate)[1–5] and metabotropic receptors (mGluRs).[6,7]

The metabotropic receptors are characterized by a large amino-terminal domain, which includes the Glu binding site, and they belong to the family C group of G-protein coupled receptors (GPCR). Through different effectors and second messengers, they control key functions within the cell and ion channels.[8–12] Finally, they share very little homology with other GPCR's.[13,14]

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 have also been reported and competitive and non-competitive antagonists are available.[15,16]

A number of computational studies were therefore aimed at modelling the function of these receptors and very interesting results were recently shown by Pellicciari[17] and by Pin and Acher in agreement with X-ray structures.[18–20]

We have recently reported[21–24] that 3,5-dimethyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester (3,5-dimethyl PPP—**1**, Fig. 1) is a potent and selective non-competitive mGluR1 antagonist endowed with excellent in vitro (15.8 nM) and in vivo activity in different animal models of pain. A clear 'opiate-like' antinociceptive profile was show for these derivatives in animal models of pain together with the more widely recognized activity of the mGluR1 antagonists in chronic/inflammatory models.[23]

The most potent molecules belonging to this newly reported class are endowed with nanomolar potency at the mGluR1 receptor, and they are extremely selective

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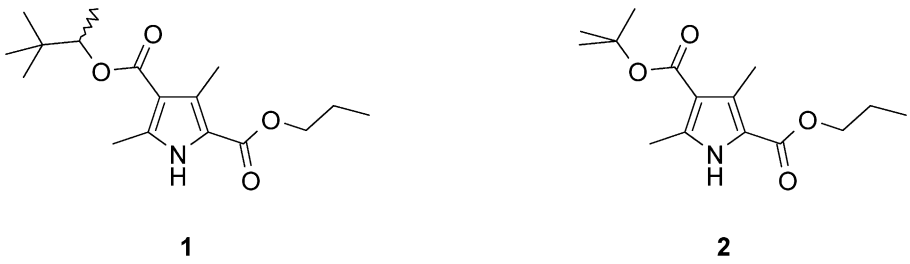
doi:10.1016/S0960-894X(03)00396-2



1 2

Figure 1. The recently discovered pyrrole class.

versus the other receptor subtypes both in the same group I and groups II and III (>>500-fold).

In electrophysiological studies, these compounds showed a very potent activity in the 'wind-up' experiment in vitro on baby rat spinal cord[23] strongly suggesting that mGluR1 antagonists could be useful in blocking the central sensitization process.

Clearly, such a result would have a much stronger impact if a similar effect was also recorded in vivo. Accordingly, electrophysiological recordings were made of nociceptive responses of wide dynamic range (WDR) neurons in the ventroposterolateral (VPL) nucleus of the thalamus of urethane anesthetized rats,[25] as recently reported for the mGluR5 antagonist MPEP.[26,27]

Non-nociceptive mechanosensitive neurons were also studied in VPL nucleus to determine whether the effects of 3,5-dimethyl PPP **1** were selective to nociceptive transmission. These experiments demonstrated that **1** did not suppress firing evoked by non-noxious stimulation.

As it can be seen from Figure 2A, 3,5-dimethyl PPP **1**, dosed at 2 mg/kg ip in urethane anesthetized animals, inhibited the stimulus-evoked activity of nociceptive responses in VPL neuron of the rat. The effect was potent, and selective for noxious stimulation because it did not alter the firing of non-nociceptive neurons responding to touch, as reported in Figure 2B. Moreover, as it can be observed in Figure 3, the analgesic effect is long lasting and similar to the one obtained after morphine iv injection.

These very exciting results, together with the ones already reported[23] strengthen our confidence in the pyrrole class of mGluR1 antagonists and committed us to further SAR explorations.

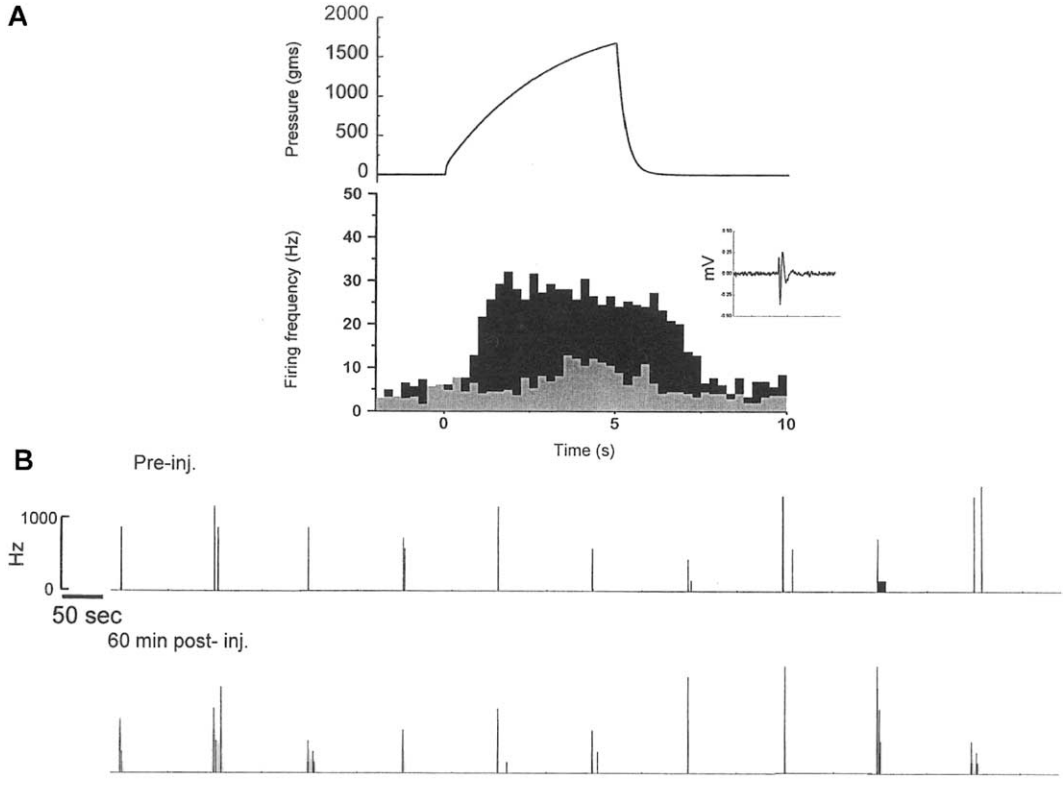


Figure 2. (A) Evoked firing of a representative neuron before and after administration of 3,5-dimethyl PPP **1** (2 mg/kg, ip). Top, level of applied increasing pressure to the rat's paw in register with the post-stimulus histogram below. Bottom, average of 10 trials of firing rate histograms pre-drug (in black) compared to post-drug (in gray). (B) Activity of a representative non-nociceptive neuron before and after administration of 3,5-dimethyl PPP **1** (2 mg/kg, ip). The drug did not reduce response to light brush.

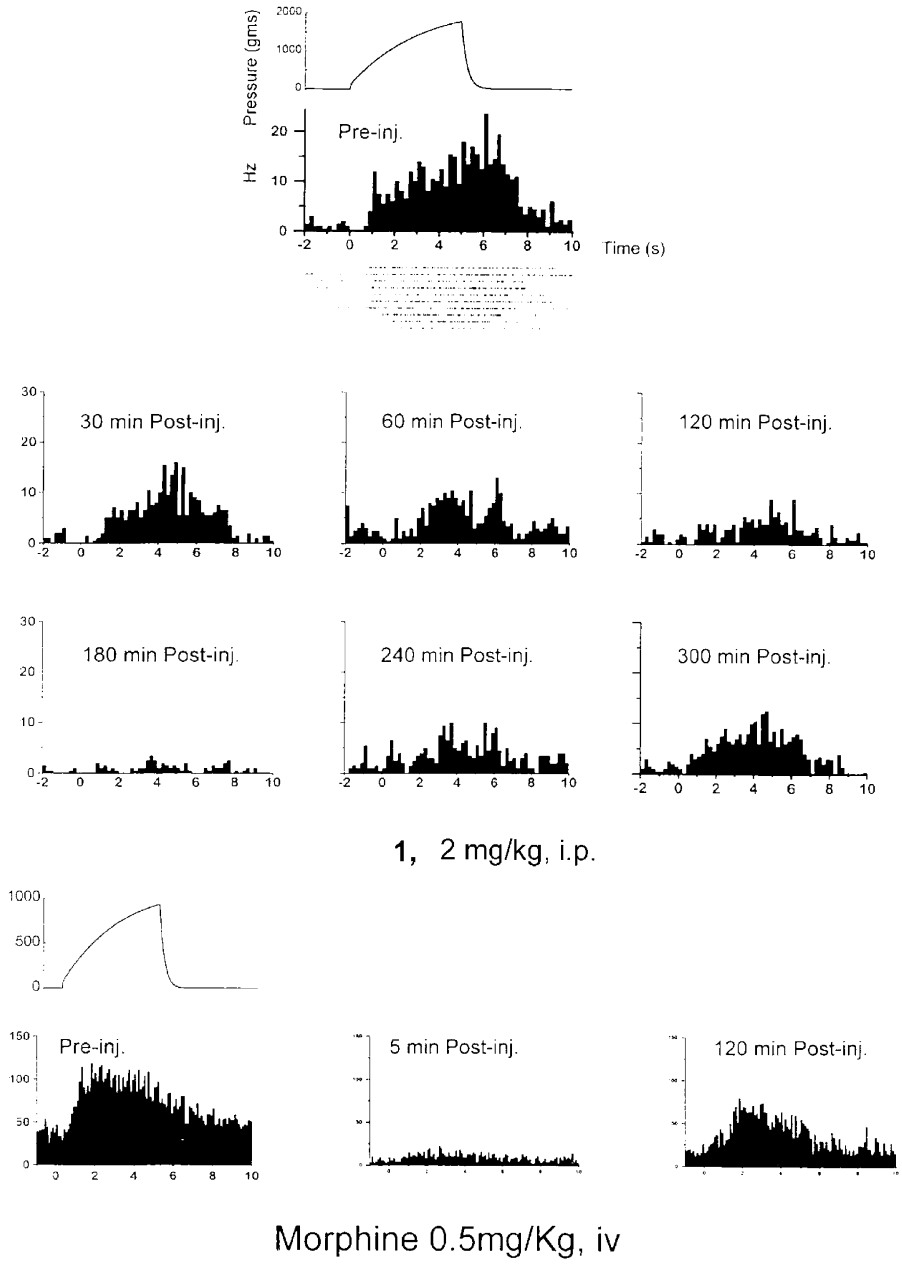


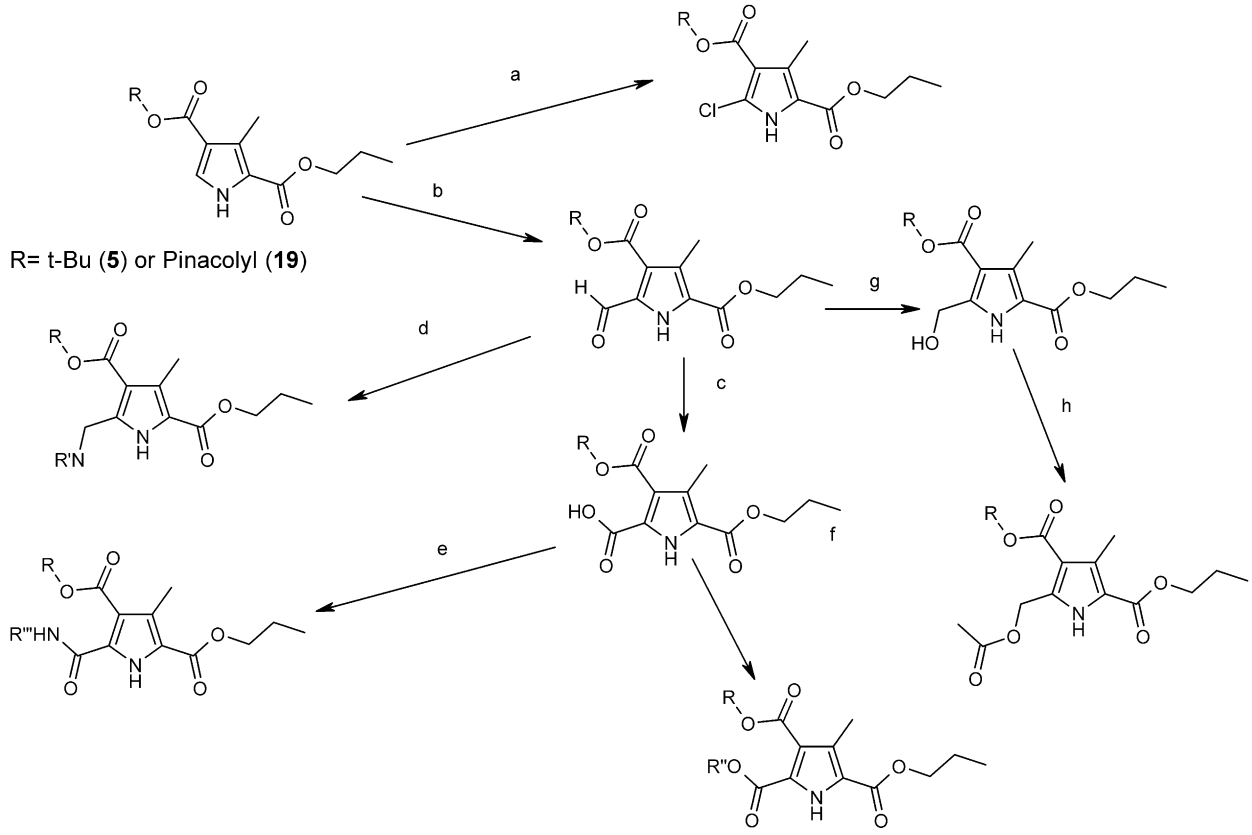
Figure 3. Effects of 3,5-dimethyl PPP **1** (2 mg/kg ip) and morphine (0.5 mg/kg, iv) on nociceptive VPL neurons in the anesthetized rat. Top: Mean of 10 trials of firing rate histograms before and after 3,5-dimethyl PPP **1** administration recorded at different times. Level of applied pressure to the rat's paw is in register with the response. At 60 min post-injection, neuron firing to nociceptive stimulation is evidently decreased. At 300 min post-injection time, neuron firing is starting to recover. Bottom: Firing rate histograms before and after morphine injection. The effect is very rapid because the injection was iv and the response recovered completely about 2 h post-morphine administration.

More precisely, we were interested in a better understanding of the role of the C-5 position and to enlarge our exploration of the pyrrole nitrogen.

A number of compounds were prepared according to the synthetic routes described in Scheme 1 starting from derivative **5**[28] and according to the references there reported.

As shown in Table 1, we started our exploration from derivative **2** (Fig. 1), retaining the C-3 methyl group and the C-4 *t*-Butyl ester, and replacing the C-5 methyl group with a number of different functionalities. Subsequently, in order to have a better understanding of the structure–activity of our template, we also replaced the *t*-Bu group with the pinacolyl ester present in 3,5-dimethyl PPP **1**.

As already reported, these pyrrole derivatives are completely inactive when the C-2 and C-4 carboxylic acids are left unprotected. These original findings were further confirmed by the introduction of another carboxylic group in position C-5, with the free acid being completely inactive (**9**, Table 1), whereas its methyl ester (**10**) retained an acceptable potency. The introduction of an oxygenated function (**6**, **7**, **8**) was well tolerated, while the presence of a free amine (**12**, **13**) led to complete inactivity.



Scheme 1. Reaction conditions and references: (a) NCS, 1.1 equiv, DMF, 0 °C; 30% yield (**23**); (b) $POCl_3$, DMF, CH_2Cl_2, from 0 °C to rt; 50% yield. (**8**); (c) NaOClO, CH_3CN, H_2O, rt, 70% yield (**9**); (d) $NaCNBH_3$, $R'NH_2$, THF, 0 °C; 30% yield (**12**); H_2, Pd/C, 1 atm 95% yield (**13**); (e) $R''NH_2$, DCC, THF, rt; 80% yield (**11**); (f) $(CF_3CO)_2O$, rt, $R'''OH$, THF; 80% yield (**10**); (g) $NaBH_4$, 1.1 equiv, MeOH, from 0 °C to rt; 95% yield (**6**); (h) CH_3COCl, Py, THF, rt; 95% yield (**7**).

While the introduction of an electronwithdrawing group (**23**) lead to a 10-fold decrease in potency, to our great pleasure, the replacement of C-5 methyl group with a hydrogen atom led to an increased potency with respect to the parent derivatives (**15**, **19**).

To increase the water solubility of our derivatives, having gained further potency with the C-5 des-methylated pyrrole, we also incorporated esters carrying a basic moiety on the position C-2. As previously reported,[21,22] this substituents were poorly tolerated, but in this case some activity was retained (**22**, **24**, **25**). It is also worth noting that derivative **21**, the lipophylic pentafluoro phenyl ester intermediate to an array of amides, still retained acceptable potency.

As far as the pattern of substitution of the pyrrole nitrogen is concerned, a number of derivatives were simply prepared alkylating the anion of derivative **2** (NaH, 1.1 equiv, DMF, 0 °C) with the appropriate haloderivative (Bromo or Chloro) in DMF at room temperature. As already observed in previous exploration,[21–23] small alkyl group substituents (**33**, Table 2) were tolerated and still showed a certain degree of potency, while generally, the introduction of bulkier group carrying basic amines led to complete inactivity.

Nonetheless, the presence of free hydroxy groups on linear alkylic chains (**37**) or esteric derivatives (**28**) was tolerated.

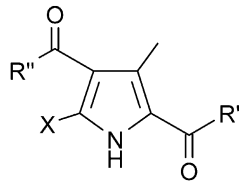
This, in agreement with what already shown for the position C-5, seems to point out that the region where these derivatives bind in the receptor might be rich in lipophilic residues, which clearly tolerate hydrogen bond acceptors, but tend to repel protonated/charged basic groups.

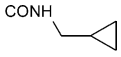
All the active compounds reported in Tables 1 and 2 were tested for their selectivity versus mGluR5, mGluR2 and mGluR4. As for the derivatives previously reported,[23] all these compounds were endowed with a selectivity greater than 100-fold with respect to the above mentioned receptors.

The exploitation of our best tool, 3,5 dimethyl PPP **1**, led to further in vivo confirmation of the importance of the mGluR1 in nociceptive transmission. In particular, we demonstrated a clear analgesic effect with no influence on non-nociceptive transmission and devoid of sedative effect, and therefore suggesting that mGluR1 antagonists are able to interfere with the nociceptive pathway without altering the normal, tactile transmission.

The further exploration of the pyrrole template confirmed the poor tolerance of the binding site to both

Table 1. Potency values of the pyrrole derivatives on r-mGluR1a-CHO cells using the CDP-DAG accumulation method[23]



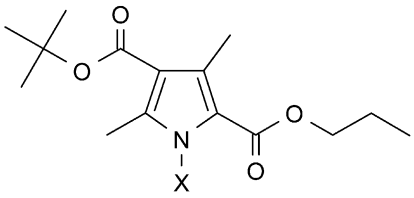
X	R′	R″	Entry	IC_{50} (μM)
H	OEt	*t*-BuO	**3**	0.34
H	OH	*t*-BuO	**4**	N.A.
H	*n*-OPr	*t*-BuO	**5**	0.075
CH_2OH	*n*-OPr	*t*-BuO	**6**	7.9
CH_2Oac	*n*-OPr	*t*-BuO	**7**	2.7
CHO	*n*-OPr	*t*-BuO	**8**	2
COOH	*n*-OPr	*t*-BuO	**9**	N.A.
COOMe	*n*-OPr	*t*-BuO	**10**	3.2
	*n*-OPr	*t*-BuO	**11**	2.5
CH_2NHBn	*n*-OPr	*t*-BuO	**12**	N.A.
CH_2NH_2	*n*-OPr	*t*-BuO	**13**	N.A.
COSPy	*n*-OPr	*t*-BuO	**14**	3.2
H	*t*-BuO	*t*-BuO	**15**	0.1
H		*t*-BuO	**16**	1.2
H	SPy	*t*-BuO	**17**	3
H		*t*-BuO	**18**	0.048
H	*n*-OPr		**19**	0.004
H	*t*-BuO		**20**	0.017
H			**21**	0.16
H			**22**	0.39
Cl	*n*-OPr		**23**	0.16
H			**24**	0.72
H			**25**	0.26
H			**26**	1.45

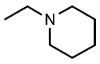
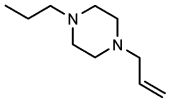
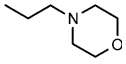
IC_{50}s were measured from at least six-point inhibition curves and they are the geometric means of at least three independent experiments. The standard error of the mean was less than 0.05; N.A., not active up to 100 μM.

acidic and basic group, and allowed the discovery of a more potent pyrrole derivative, 3-methyl PPP **19**.

This increased potency has allowed the preparation of more water-soluble compounds, which will be reported in future communications.

Table 2. Potency values of the pyrrole derivatives on r-mGluR1a-CHO cells using the CDP-DAG accumulation method[23]



X	Entry	IC_{50} (μM)
CH_2CONMe_2	**27**	N.A.
$CH_2COOAllyl$	**28**	12.6
	**29**	N.A.
CH_2COOH	**30**	N.A.
	**31**	N.A.
	**32**	N.A.
Me	**33**	1
BOC	**34**	2.3
$(CH_2)_3OSi$-*t*-$BuPh_2$	**35**	N.A.
$(CH_2)_2OSi$-*t*-$BuPh_2$	**36**	N.A.
$(CH_2)_3OH$	**37**	5.8

IC_{50}s were measured from at least six-point inhibition curves and they are the geometric means of at least three independent experiments. The standard error of the mean was less than 0.05; N.A., not active up to 100 μM.

Acknowledgements

We would like to thank Dr. Carla Marchioro and co-workers and Dr. Mahmoud Hamdan and co-workers for their help and analytical support. We would also like to thanks Dr. Frank King and Dr. Annarosa Ugolini for helpful discussions during the preparation of this manuscript.

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