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3-Methyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester: an exploration of the C-2 position. Part I

Fabrizio Micheli *, Romano Di Fabio *, Roberto Benedetti, Anna Maria Capelli, Palmina Cavallini, Paolo Cavanni, Silvia Davalli, Daniele Donati, Aldo Feriani, Sylvie Gehanne, Mahmoud Hamdan, Micaela Maffeis, Fabio Maria Sabbatini, Maria Elvira Tranquillini, Monica Valeria Angela Viziano [1]

GlaxoSmithKline Medicine Research Centre, via Fleming 4, Verona 37135, Italy

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Abstract

Following the recent disclosure of 3-methyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester, a potent and selective mGluR1 non-competitive antagonist, we report here a detailed exploration of the C-2 position of this scaffold with the preparation of differently substituted amides. Great improvement of the pharmacokinetic properties has been achieved through this exercise.

Keywords: Pyrrole; Metabotropic receptors; Glutamate; Antagonists

1. Introduction

Glutamate is a key neurotransmitter in the central nervous system and it exerts its activity through ionotropic (NMDA, AMPA and kainate) [1–4] and metabotropic receptors (mGluRs) [5–7].

The former are ligand-gated cation channels, while the latter belong to the C family of G-protein-coupled receptors (GPCRs) and are characterized by a large amino-terminal domain where the agonists bind. The ability of these GPCRs to interact with a large number of key effectors within the cell [8–16] provides a variety of targets to the medicinal chemists.

To date, eight mGluRs subtypes and multiple splice variants 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).

Recent molecular modeling studies as well as receptor models based on crystallographic data shed light on the basis of the interactions between agonists and antagonists with these receptors [17–20].

We have recently reported [21–25] a new pyrrole class of selective non-competitive mGluR1 antagonists. Among them, 3,5-dimethyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester (**1,** Fig. 1) and 3-methyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester (**2,** Fig. 1) are potent compounds endowed with nanomolar potency and in vivo activity in different animal models of pain. Not only are these compounds active in chronic/inflammatory situations, but they also show a clear "opiate-like" antinociceptive profile, both in vitro and in vivo.

In order to expand our knowledge of this pyrrole class and to check its pharmacokinetic (PK) properties, we decided to probe the C-2 position by replacing the ester oxygen with a nitrogen, preparing a series of amides, hydroxamates and hydrazides.

This work is reported in the present study.

* Corresponding authors.
E-mail address: fabrizio.e.micheli@gsk.com (F. Micheli).
[1] Present address: Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, CA 94404, USA

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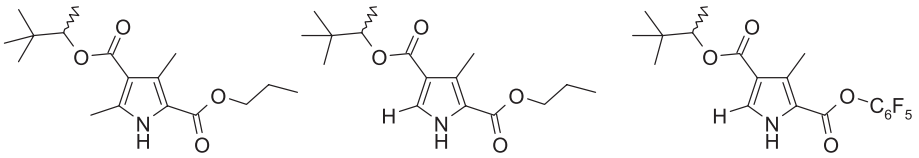


1 2 3

Fig. 1. The recently discovered pyrrole class.

2. Results

As previously reported [23–25], the pyrrole C-2 position of derivatives **1** and **2** was able to accept a number of esters of different steric bulk and pattern of substitution without major loss of potency at the mGluR1. However, basic or acidic groups were poorly tolerated. Moreover, the calculated physico-chemical properties of the pyrrole series seemed to correlate very well with potency values [24]. To further investigate the tolerance of this position, a set of different amines, hydrazines and hydroxylamines was selected on the basis of their chemical diversity. They were reacted with the pentafluorophenyl ester **3** (Fig. 1) [25] in array format as reported in Scheme 1 to give the desired products in good yields.

The results of this array are reported in Table 1 and show that the selected amides were better tolerated than the hydrazides or the hydroxamates derivatives. Among the amides prepared, the piperazine series was poorly tolerated, suggesting that the introduction of a basic group was detrimental for the in vitro potency as previously observed in the C-2 esters series.

The most potent derivatives prepared in this array, namely **4, 9** and **10,** were further characterized along the screening cascade [23–25]. Out of these products, derivative **9** showed an interesting in vivo [26] profile when assessed in the formalin test [24] in mice.

When administered to mice per os at 0.3 mg/kg, **9** showed a 45% reduction of the thermal hyperalgesia in the early phase of the formalin test. At the same dose, a 50% reduction in the late phase of this test was observed, showing a comparable in vivo activity to derivatives **1** and **2** despite a lower in vitro potency (35 vs. 16 and 4 nM, respectively).

A better PK profile was therefore predicted for **9** given the more favorable physico-chemical properties (calculated and measured) with respect to **1.** This hypothesis was confirmed in different animal species as reported in Table 2.

In order to maximize the probability of generating compounds with an in vivo PK profile similar to **9,** a strategy for the selection of monomers to be used in a further array was needed.

All the in vivo data generated on the pyrroles prepared in house were analyzed and modeled with respect to a number of different calculated physico-chemical properties. From this exercise, a strict correlation between the calculated lipophilicity values of the molecules tested and their measured plasma concentrations after oral administration in rats was detected: only molecules endowed with a *C*log*P* lower than 3.5 were able to show C_{max} levels greater than 0.5 μg/ml.

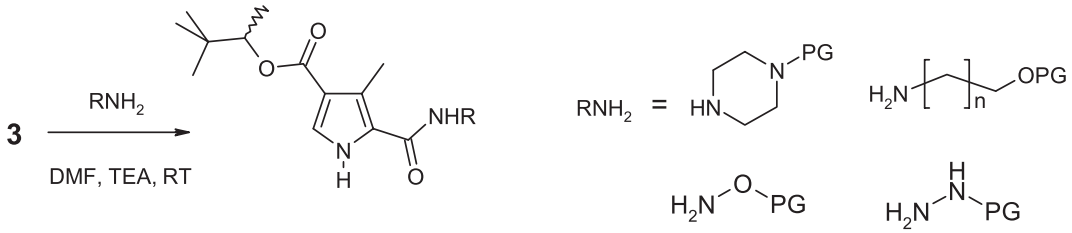
As the *C*log*P* values for the derivatives analyzed correlated sufficiently well with the measured chromatographic hydrophobicity index [27], a high-throughput alternative to classical log *P*/log*D* (see Table 5 in Section 4), it was decided to rely on this property for the monomer selection. Consequently, a virtual library was computationally designed using commercially available amines to achieve the maximum possible chemical diversity [28] and it was subsequently pruned by applying a *C*log*P* cutoff of 3.5 on the final compounds to identify the appropriate monomers.

In addition, a few close analogs of **9** (five amines out of the 50 chosen for this array) were used to bias this selection, and the results are reported in Table 3 (entries from **22** to **40**).

In vivo PK data in rats confirmed the validity of the working hypothesis as acceptable plasma concentrations (>0.5 μg/ml) were obtained; however, only derivative **23** showed an acceptable potency value.

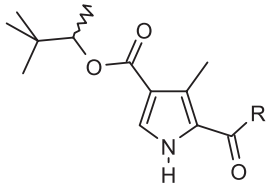
Nonetheless, the unexpected activity exhibited by compound **36** (all the free acids previously tested on the pyrrole derivatives were inactive [21–25]) represented a starting point for further investigation.

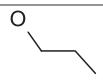
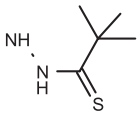
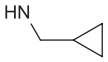
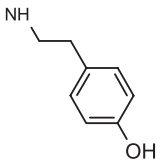
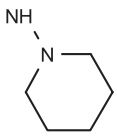
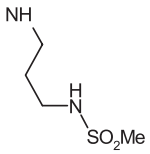
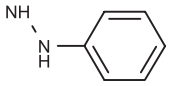
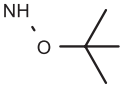
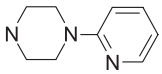
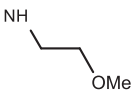
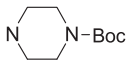
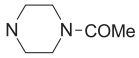
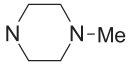
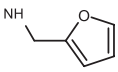
The free carboxylic acid, as expected proved to be unsuitable for achieving good brain penetration, and was replaced with appropriate bio-isosteres. Among the different derivatives prepared, it is worth mentioning, in terms of potency achieved, the triflamide **43.** This derivative was prepared according to Scheme 2, and the protected diaminopropane



Scheme 1. Nucleophilic displacements of the pentafluorophenyl ester **3.**

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



Entry	R	IC_{50} (μM)	Entry	R	IC_{50} (μM)
2		0.004	13		N.A.
4		0.020	14		N.A.
5		0.12	15		0.69
6		0.35	16		12
7		N.A.	17		4.35
8		0.31	18		27
9		0.035	19		N.A.
10		0.020	20		N.A.
11		0.074	21		N.A.
12		0.052			

IC_{50}s were measured from at least six-point inhibition curves and they are the geometric means of at least three independent experiments. NA: not active up to 100 μM.

used for this task was synthesized following literature procedure [29]. However, the high lipophilicity measured for this product negatively influenced its oral absorption, in line with the above-mentioned hypothesis.

As a further attempt to exploit the unexpected in vitro potency of derivative **36,** a mechanism of active transportation [30] was looked for. Accordingly, **36** was activated as pentafluorophenyl ester and coupled with appropriately protected amino acids to give the desired products in high yields.

Table 2
A direct comparison of the physico-chemical and PK properties of derivatives **1** and **9**

	1	**9**
C log*P*	5.96	2.5
Water solubility	0.34 μg/ml	53 μg/ml
C_{max} (rat, p.o. at 10 mg/kg)	<0.01 μg/ml	1.05 μg/ml
C log*P*	NT	61 ml/min/kg
F % (rat)	<1	30
F % (dog)	NT	25
Protein binding	NT	83.7% (rat), 92.8% (man)

NT: not tested.

As expected, none of these products had detectable in vitro activity on mGluR1, but some of them were relatively well absorbed both in rats and in dogs showing good bioavailability. Unfortunately, their stability after in vivo administration was extremely high and the hydrolytic process was too slow for any practical application.

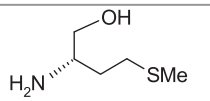
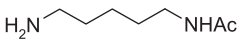
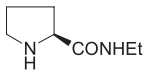
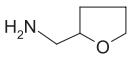
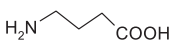
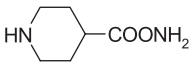
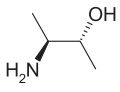
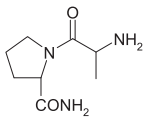
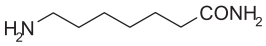
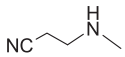
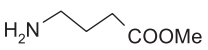
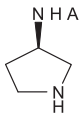
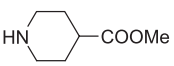
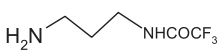
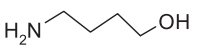
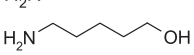
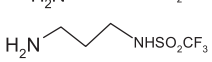
Finally, to complete the C-2 exploration, a more pragmatic approach was followed and close analogs of derivative **9,** carrying the oxygen atom in beta position with respect to the nitrogen, were prepared as reported in Table 4.

It is worth noting that almost none of the prepared compounds matched the potency of **9,** suggesting strict requirements in this region of the receptor. Only derivative **46** showed an acceptable in vitro profile, but its instability in vivo precluded further developments in this area.

3. Conclusions

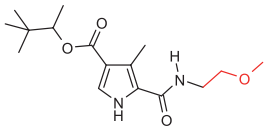
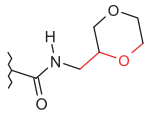
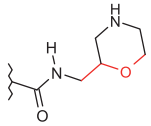
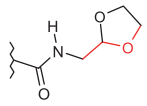
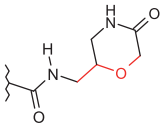
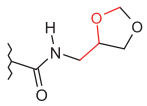
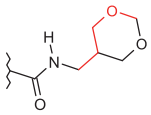
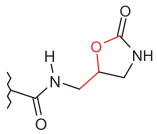
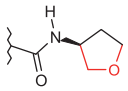
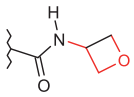
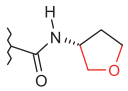
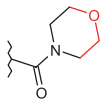
We have reported the C-2 exploration of 3-methyl pyrrole-2,4-dicarboxylic acid 2-propyl ester 4-(1,2,2-trimethyl-propyl) ester **2** using a number of differently substituted

Table 3
Potency values of the pyrrole derivatives on r-mGluR1a–CHO cells using the CDP–DAG accumulation method [23]

Entry	Amine	IC_{50} (μM)	Entry	Amine	IC_{50} (μM)
22	H_2N-$(CH_2)_3$-OiPr	0.39	**33**		N.A.
23	H_2N-$(CH_2)_3$-Oet	0.043	**34**		0.32
24	H_2N-$(CH_2)_3$-OMe	0.17	**35**		N.A.
25		0.26	**36**		0.39
26		0.063	**37**		1.00
27		0.36	**38**		N.A.
28		0.11	**39**		N.A.
29		0.32	**40**		N.A.
30		2.23	**41**		0.63
31		0.16	**42**		N.A.
32		0.10	**43**		0.040

IC_{50}s were measured from at least six-point inhibition curves and they are the geometric means of at least three independent experiments. NA: not active up to 100 μM.

Table 4
Potency values of the pyrrole derivatives on r-mGluR1a–CHO cells using the CDP–DAG accumulation method [23]

Entry	Structure	IC_{50} (μM)	Entry	Structure	IC_{50} (μM)
9		0.035	**49**		0.50
44		N.A.	**50**		0.14
45		N.A	**51**		0.26
46		0.087	**52**		1.15
47		0.12	**53**		0.40
48		0.18	**54**		10

IC_{50}s were measured from at least six-point inhibition curves and they are the geometric means of at least three independent experiments. NA: not active up to 100 μM.

Table 5
Comparison between the calculated log*P* values and the measured CHI ones for derivatives reported in Tables 1, 4 and 5. Data according to references [24] and [27]

Entry	CHI values pH 7.4 [27]	CHI log*D* [27]	*C* log*P* [24]
1	121.95	4.94	5.97
5	80.48	2.76	3.89
6	68.69	2.14	5.03
9	76.15	2.53	2.53
10	86.44	3.07	3.3
17	66.76	2.04	2.26
50	77.45	2.6	2.16
51	78.04	2.63	2.16

nitrogen nucleophiles. Amides seem to be much better tolerated than hydrazides and hydroxamates. Greater stability in vivo was obtained with respect to the corresponding esters in different animal species as well as acceptable PK profiles. In particular derivative **9** showed promising pharmacological activities in pain models together with an acceptable PK profile in different animal species. This compound represents a new tool for a further investigation of the role of the mGluR1 in different physio-pathological conditions in animal models and its further characterization will be reported in due course.

4. Experimental section

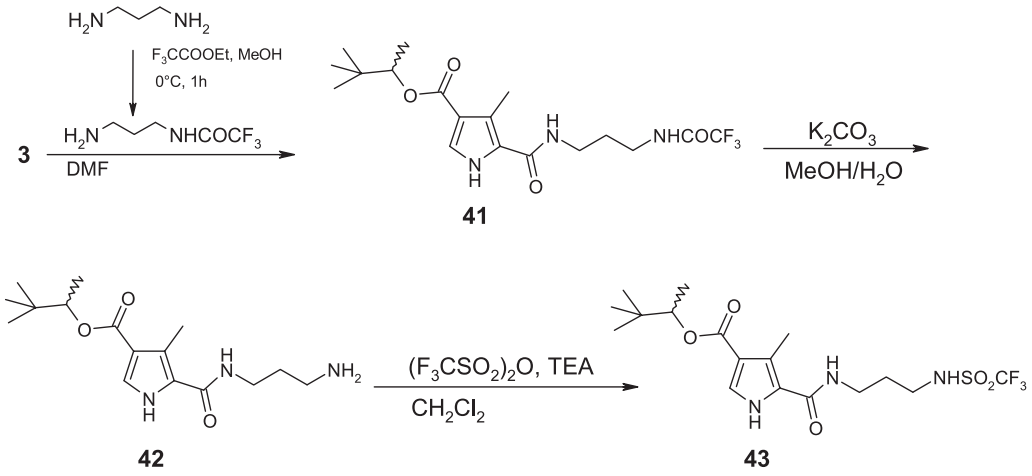
4.1. General procedure for array synthesis of the reported amides, hydrazides and hydroxamates

A solution of **3** [25] (30 mg; 0.072 mmol) and the desired amine/hydrazine/hydroxylamine (0.072 mmol) in dry dimethylformamide (DMF) (1 ml) was stirred at 23 °C for 24 h under a nitrogen atmosphere. The solution was concentrated in vacuo and the residue was dissolved in water (5 ml) and extracted with ethyl acetate (2 × 10 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give the title compound (28.6 mg) as a yellow oil.

4.2. Selected examples

4.2.1. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 2-methoxy-1-ethylamide ***9***

A solution of **3** (120 mg; 0.286 mmol) and 2-methoxymethylamine (29.5 μl; 0.34 mmol) in dry DMF (3 ml) was stirred at 23 °C for 24 h under a nitrogen atmosphere. The solution was diluted with ethyl acetate (15 ml) and washed with a 5% hydrochloric acid solution (2 × 15 ml) and brine (15 ml). The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give a residue that was purified by



Scheme 2. Preparation of the triflamide **43.**

flash chromatography, eluting with ethyl acetate, to give the title compound (80 mg) as a white solid.

Y = 90%.

TLC: ethyl acetate, $R_f = 0.60$.

m.p.: 103–105 °C.

NMR (d_6-DMSO): δ 11.7 (s, 1H), 7.62 (t, 1H), 7.39 (d, 1H), 4.70 (q, 1H), 3.45–3.35 (m, 4H), 3.26 (s, 3H), 2.45 (s, 3H), 1.12 (d, 3H), 0.91 (t, 9H).

IR (nujol): cm^{-1} 3149 (NH), 1693 (C=O).

MS (FAB/NBA): *m/z* 311 $[M + H]^+$.

HPLC: column: inertsil ODS-2 (25 cm × 4.6 mm); phase: acetonitrile/water 7:3; flow = 1 ml/min; $\lambda = 225$ nm; retention time: 9.8 min.

Solubility 0.0533 mg/ml.

4.2.2. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, isopropyl amide ***10***

Y = 95% (white foam).

NMR (d_6-DMSO): δ 11.7 (bs, 1H), 7.7 (bs, 1H), 7.48 (d, 1H), 4.76 (q, 1H), 4.06 (m, 1H), 2.24 (s, 3H), 1.15–1.05 (m, 9H), 0.90 (s, 9H).

MS (FAB/NBA): *m/z* 295 $[M + H]^+$.

4.2.3. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 4-(2-pyridyl)piperazinamide ***18***

NMR ($CDCl_3$): δ 9.8 (bs, 1H), 8.2 (m, 1H), 7.55–7.49 (m, 2H), 6.71–6.65 (m, 2H), 4.87 (q, 1H), 3.75 (m, 4H), 3.61 (m, 4H), 2.38 (s, 3H), 1.22 (d, 3H), 0.97 (s, 9H).

Y = quant.

MS (FAB/NBA): *m/z* 399 $[M + H]^+$.

*4.2.4. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 4-*tert*-butoxycarbonyl-piperazinamide* ***19***

Y = quant. (yellow oil).

NMR ($CDCl_3$): δ 8.92 (bs, 1H), 7.48 (d, 1H), 4.86 (q, 1H), 3.6 (m, 4H), 3.47 (m, 4H), 2.34 (s, 3H), 1.47 (s, 9H), 1.21 (d, 3H), 0.97 (s, 9H).

MS (FAB/NBA): *m/z* 422 $[M + H]^+$.

4.2.5. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 4-acetyl-piperazinamide ***20***

Y = quant. (yellow oil).

NMR ($CDCl_3$): δ 9.1 (bs, 1H), 7.5 (d, 1H), 4.87 (q, 1H), 3.67 (m, 4H), 3.63 (m, 2H), 3.53 (m, 2H), 2.35 (s, 3H), 2.14 (s, 3H), 1.22 (d, 3H), 0.96 (s, 9H).

MS (FAB/NBA): *m/z* 364 $[M + H]^+$.

4.2.6. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 4-methyl-piperazinamide ***21***

Y = 89% (yellow oil).

NMR ($CDCl_3$): δ 9.91 (bs, 1H), 7.45 (s, 1H), 4.85 (q, 1H), 3.74 (m, 4H), 2.61 (m, 4H), 2.41 (s, 3H), 2.34 (s, 3H), 1.2 (d, 3H), 0.95 (s, 9H).

MS (FAB/NBA): *m/z* 336 $[M + H]^+$.

4.2.7. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 3-isopropoxy-1-propylamide ***22***

Y = 83.4% (white waxy solid).

TLC: ethyl acetate, $R_f = 0.72$.

m.p.: 80–81 °C.

NMR (d_6-DMSO): δ 11.67 (bs, 1H), 7.52 (t, 1H), 7.37 (d, 1H), 4.70 (q, 1H), 3.5 (m, 1H), 3.39 (t, 2H), 3.25 (m, 2H), 2.44 (s, 3H), 1.68 (m, 2H), 1.12 (d, 3H), 1.06 (d, 6H), 0.91 (t, 9H).

IR (nujol): cm^{-1} 3381 and 3186 (NH), 1689 and 1630 (C=O).

MS (FAB/NBA): *m/z* 353 $[M + H]^+$.

4.2.8. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 3-ethoxy-1-propylamide ***23***

Y = 91% (white solid).

TLC: ethyl acetate, $R_f = 0.72$.

m.p.: 108–111 °C.

NMR (d_6-DMSO): δ 11.66 (s, 1H), 7.54 (t, 1H), 7.37 (d, 1H), 4.70 (q, 1H), 3.42 (q, 2H), 3.38 (m, 2H), 3.26 (m, 2H), 2.44 (s, 3H), 1.71 (m, 2H), 1.12 (d, 3H), 1.09 (t, 3H), 0.91 (s, 9H).

IR (nujol): cm^{-1} 3204 (NH), 1697 (C=O).

MS (ES/–): *m/z* 339 $[M + H]^+$.

HPLC: column: inertsil ODS-2 (25 cm × 4.6 mm); phase: acetonitrile/water 7:3; flow = 1 ml/min; λ = 225 nm; retention time: 6.16 min.

*4.2.9. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 3-(1,3-oxazine)amide **26***

Y = 56% (colorless oil).

TLC: ethyl acetate, R_f = 0.66.

NMR (d_6-DMSO): δ 11.83 (bs, 1H), 7.38 (d, 1H), 4.88 (s, 2H), 4.69 (q, 1H), 3.81 (t, 2H), 3.66 (t, 2H), 2.19 (s, 3H), 1.62 (m, 2H), 1.12 (d, 3H), 0.9 (s, 9H).

IR (nujol): cm^{-1} 3261 (NH), 1700 (C=O).

MS (ES/+): *m/z* 323 $[M + H]^+$.

*4.2.10. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 2-(3-hydroxy)butylamide **27***

Y = 89.9% (white foam).

TLC: ethyl acetate, R_f = 0.57.

NMR (d_6-DMSO): δ 11.74 (m, 1H), 7.4 (d, 1H), 7.38 (d, 1H), 4.70 (q, 1H), 4.67 (d, 1H), 3.81 (m, 1H), 3.61 (m, 1H), 2.46 (m, 3H), 1.12 (d, 3H), 1.06 (d, 3H), 1.04 (d, 3H), 0.91 (s, 9H).

IR (nujol): cm^{-1} 3384 (NH), 1689 (C=O).

MS (ES/+): *m/z* 325 $[M + H]^+$, 347 $[M + Na]^+$.

*4.2.11. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 1-(6-aminocarbonyl)hexylamide **28***

Y = 65.9% (white solid).

TLC: ethyl acetate, R_f = 0.13.

m.p.: 159–160 °C.

NMR (d_6-DMSO): δ 11.65 (bs, 1H), 7.54 (t, 1H), 7.38 (d, 1H), 7.20 (bs, 1H), 6.66 (bs, 1H), 4.70 (q, 1H), 3.18 (m, 2H), 2.44 (s, 3H), 2.01 (t, 2H), 1.48–1.26 (m, 8H), 1.12 (d, 3H), 0.91 (t, 9H).

IR (nujol): cm^{-1} 3410 (NH), 1695, 1669 and 1645 (C=O).

MS (ES/+): *m/z* 380 $[M + H]^+$, 402 $[M + Na]^+$.

*4.2.12. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 1-(3-methoxycarbonyl)propylamide **29***

A solution of **3** (210 mg; 0.5 mmol), methyl 4-aminobutyrate hydrochloride (92.2 mg; 0.6 mmol) and triethylamine (83.6 µl, 0.6 mmol) in dry DMF (4 ml) was stirred at 23 °C for 18 h under a nitrogen atmosphere. The solution was diluted with ethyl acetate (50 ml) and washed with 5% hydrochloric acid solution (30 ml), 5% sodium hydrogen carbonate solution (30 ml) and brine (30 ml). The organic phase was dried (Na_2SO_4) and concentrated in vacuo to give a residue that was purified by flash chromatography, eluting with cyclohexane/ethyl acetate, 3:7, to give the title compound (0.17 g) as a colorless oil.

Y = 96.4%.

TLC: ethyl acetate, R_f = 0.83.

NMR (d_6-DMSO): δ 11.65 (s, 1H), 7.6 (t, 1H), 7.38 (d, 1H), 4.70 (q, 1H), 3.57 (s, 3H), 3.22 (m, 2H), 2.44 (s, 3H), 2.36 (t, 2H), 1.74 (m, 2H), 1.12 (d, 3H), 0.91 (t, 9H).

IR (film): cm^{-1} 3348 and 3236 (NH), 1730 and 1699 (C=O).

MS (FAB/NBA): *m/z* 353 $[M + H]^+$.

*4.2.13. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 1-(3-carboxy)propylamide **36***

Lithium hydroxide monohydrate (39.4 mg; 0.938 mmol) was added to a solution of **29** (0.165 g; 0.469 mmol) in distilled tetrahydrofuran (20 ml) and water (10 ml). The resulting solution was stirred at 23 °C for 1 h, then concentrated to half volume and washed with ethyl acetate (20 ml). The aqueous layer was acidified to pH 2 with 5% hydrochloric acid and extracted with ethyl acetate (2 × 30 ml). The combined organic extracts were washed with brine (20 ml), dried (Na_2SO_4) and concentrated in vacuo to give the title compound (148 mg) as a colorless oil.

Y = 93%.

NMR (d_6-DMSO): δ 12.04 (bs, 1H), 11.64 (bs, 1H), 7.59 (t, 1H), 7.38 (d, 1H), 4.70 (q, 1H), 3.22 (m, 2H), 2.44 (s, 3H), 2.26 (t, 2H), 1.71 (m, 2H), 1.13 (d, 3H), 0.91 (s, 9H).

IR (film): cm^{-1} 3433 (NH), 1747 and 1696 (C=O).

MS (FAB/NBA): *m/z* 339 $[M + H]^+$.

4.2.14. 3-Methyl-4-(1,2,2-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, N-*(4-carboxy)piperidinamide **37***

Y = 78% (off-white solid).

TLC: ethyl acetate/methanol, R_f = 0.58.

m.p.: 234–236 °C.

NMR (d_6-DMSO): δ 11.71 (m, 1H), 7.35 (d, 1H), 7.28 (s, 1H), 6.79 (s, 1H), 4.69 (q, 1H), 3.97 (m, 2H), 2.92 (m, 2H), 2.34 (m, 1H), 2.18 (s, 3H), 1.71 (m, 2H), 1.43 (m, 2H), 1.12 (d, 3H), 0.91 (s, 9H).

IR (nujol): cm^{-1} 3450 and 3180 (NH), 1707 and 1671 (C=O).

MS (ES/+): *m/z* 364 $[M + H]^+$, 386 $[M + Na]^+$.

*4.2.15. 3-Methyl-4-(1,2,2,-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 1-(3-trifluoromethylcarbonylamino)propylamide **41***

Y = 78% (white solid).

TLC: ethyl acetate, R_f = 0.74.

m.p.: 75–76 °C.

NMR ($CDCl_3$): δ 11.68 (bs, 1H), 9.4 (s, 1H), 7.59 (t, 1H), 7.39 (d, 1H), 4.7 (q, 1H), 3.2 (m, 4H), 2.45 (s, 3H), 1.7 (m, 2H), 1.12 (d, 3H), 0.91 (s, 9H).

IR (nujol): cm^{-1} 3312 (NH), 1700 (C=O).

MS (FAB/NBA): *m/z* 406 $[M + H]^+$.

*4.2.16. 3-Methyl-4-(1,2,2,-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 1-(3-amino)propylamide **42***

A solution of **41** (60 mg; 0.148 mmol) and potassium carbonate (41.2 mg; 0.296 mmol) in methanol (2 ml) and water (2 ml) was heated to 85 °C for 2 h. The solution was allowed to cool to room temperature, concentrated in vacuo to remove the alcohol, diluted with further water (5 ml) and extracted with ethyl acetate (20 ml). The organic layer was

washed with brine (10 ml), dried (Na_2SO_4) and concentrated in vacuo to give the title compound (10 mg) as a whitish solid.

Y = 21% (white solid).

m.p.: 69–71 °C.

NMR (d_6-DMSO): δ 7.73 (bt, 1H), 7.37 (s, 1H), 4.7 (q, 1H), 3.27 (m, 2H), 2.65 (m, 2H), 2.45 (s, 3H), 1.61 (m, 2H), 1.13 (d, 3H), 0.91 (s, 9H).

IR (nujol): cm^{-1} 3352 (NH + NH_2), 1698 (C=O).

MS (ES/+): *m/z* 310 $[M + H]^+$.

*4.2.17. 3-Methyl-4-(1,2,2,-trimethylpropyloxycarbonyl)pyrrole-2-carboxylic acid, 1-(3-trifluoromethylsulphonylamido)propylamide **43***

A solution of trifluoromethanesulfonic anhydride (65 μl; 0.3876 mmol) in dry dichloromethane (3 ml) was added to a solution of **42** (100 mg; 0.323 mmol) and triethylamine (47 μl; 0.339 mmol) in dry dichloromethane (17 ml) previously cooled to –78 °C under a nitrogen atmosphere. The solution was stirred at –78 °C for 1 h, then quenched with water (15 ml). The layers were separated and the organic phase was washed with brine (15 ml), dried (Na_2SO_4) and concentrated in vacuo to give a residue that was purified by flash chromatography, eluting with ethyl acetate, to give the title compound (47 mg) as a white foam.

Y = 33%.

TLC: ethyl acetate, R_f = 0.88.

m.p.: 75–76 °C.

NMR (d_6-DMSO): δ 11.67 (bs, 1H), 9.39 (s, 1H), 7.61 (t, 1H), 7.39 (d, 1H), 4.71 (q, 1H), 3.28–3.18 (m, 4H), 2.44 (s, 3H), 1.73 (m, 2H), 1.12 (d, 3H), 0.91 (s, 9H).

IR (nujol): cm^{-1} 3366 and 3200 (NH), 1683 (C=O).

MS (ES/+): *m/z* 442 $[M + H]^+$.

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