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Preliminary communication

Pyrrolobenzodiazepines with antinociceptive activity: synthesis and pharmacological activities

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Summary — The synthesis of some *N*-[2-(1*H*-pyrrol-1-yl)benzyl]arylacetamides and new 4-arylmethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*a*]-[1,4]benzodiazepines as their conformationally restricted analogues is reported. The reduction of arylacetamides and *N*-methylation of pyrrolobenzodiazepines led to the corresponding *N*-[2-(1*H*-pyrrol-1-yl)benzyl]arylethylamines and the 4-arylmethyl-5-methyl-5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepines, respectively. The new compounds were subjected to pharmacological tests for evaluation of antinociceptive effects. Neurobehavioural assays were also carried out on selected compounds to acquire data on neurotoxicity. Among the derivatives tested, arylacetamides **7b** and **7e** and 4-(4-methoxybenzyl)-5-methyl-5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine **10d** were the most active derivatives in antinociceptive assays, showing high significance in both hot-plate and acetic-acid-induced writhing tests in mice without sedative or myorelaxant effects.

***N*-[2-(1*H*-pyrrol-1-yl)benzyl]arylacetamide / pyrrolo[1,2-*a*][1,4]benzodiazepine / antinociceptive activity / hot-plate test / acetic-acid-induced writhing test / myorelaxant action**

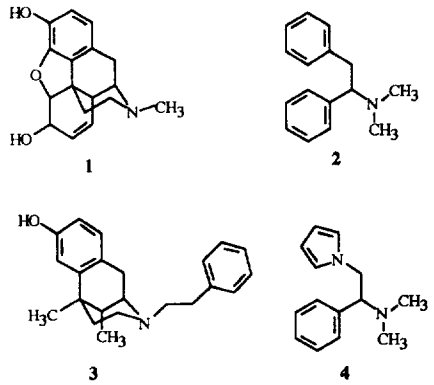
Introduction

The development of new potent analgesic agents with the efficacy of morphine **1** as pain killers but which lack the serious, undesired and use-limiting side effects (such as respiratory depression, dependence-inducing liability, nausea, and inhibition of gastrointestinal motility) has been widely pursued for many decades. A large variety of compounds related to natural opiates or with completely novel chemical structures have been investigated, and interesting hypothetical models for the opiate receptors have been proposed to explain the structure–activity data.

Research on simpler bioisosters of **1** has led to the discovery of many therapeutically useful analgesics, *eg*, lefetamine **2** and phenazocine **3**. The pyrryl analog **4** of lefetamine has also been described [1, 2].

Recently, several new selective κ-agonists, such as the benzodiazepine derivative tifluadom **5** and the phenylacetamide **6** (ICI-199441), have been reported to exert potent and efficacious analgesic activity without displaying the behavioural properties associated with morphine **1** and its congeners [3].

Following our search for new antinociceptive agents containing a pyrrole moiety, we decided to synthesize derivatives **7** and **8**, which share structural similarities with derivatives **2**, **4** and **6**. Furthermore, the Bischler–Napieralsky intramolecular cyclization of **7** to the



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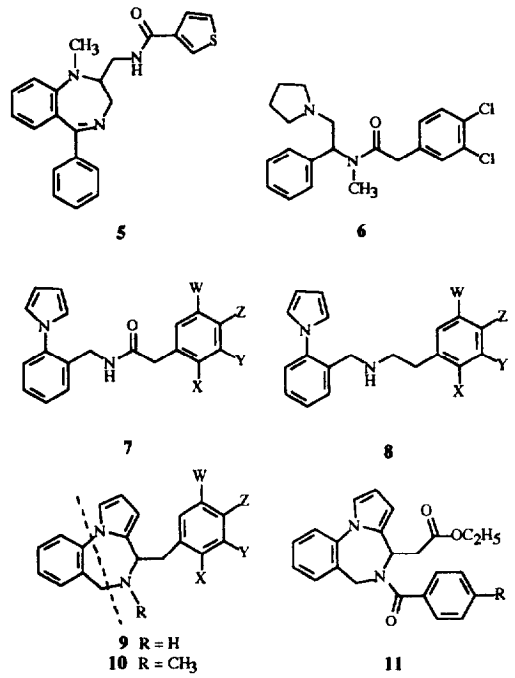


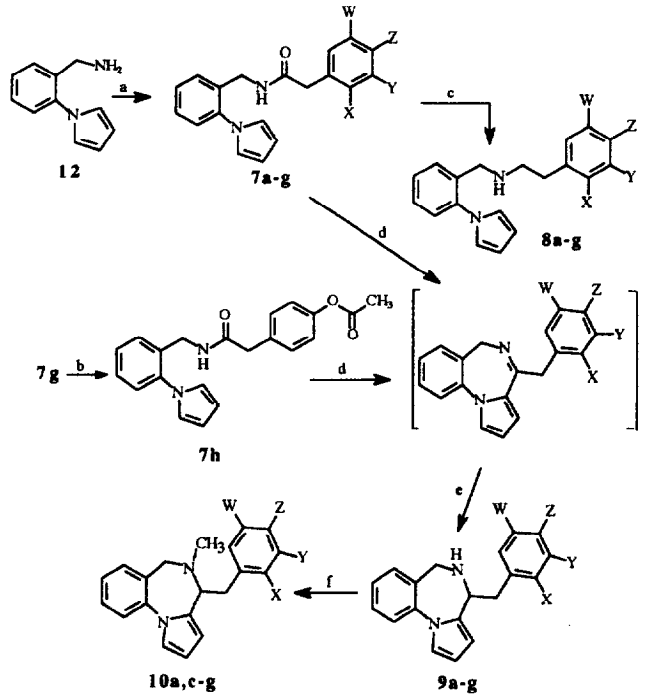
tricyclic pyrrolobenzodiazepine **9** could led to potentially active analgesics, due to the structural affinities existing between **9**, **5** and the pyrrolo[1,2-*a*][1,4]-benzodiazepines **11**, described by us in previous research on new tricyclic systems with a bridge-head nitrogen atom. In that work we highlighted the pyrrolobenzodiazepine nucleus as a useful pharmacophoric supporting model to design novel antinociceptive agents [4–6]. Among the chemical features that would favourably affect the pharmacological activity, we envisaged that compounds **9**, and their *N*-5 methyl derivatives **10**, would incorporate the isomeric structure of **4**, a pyrrole derivative which was found to be as potent as lefetamine [2], into the pyrrolobenzodiazepine skeleton.

It is also noteworthy that all the new derivatives, with the sole exception of amides **7**, contain a basic centre, as required by the classical opiates receptor model.

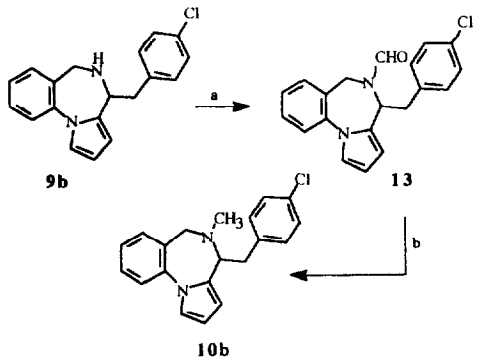
Chemistry

The synthesis of 4-arylmethyl-5,6-dihydro-4*H*-pyrrolo-[1,2-*a*][1,4]benzodiazepines **9** was carried out by the sequence of reactions shown in scheme 1. Preparation of arylacetamides **7a–g** was performed by reacting 1-(2-aminomethylphenyl)-1*H*-pyrrole **12** with substituted arylacetyl chlorides in the presence of triethylamine (*Method A*) or, alternatively, with the proper arylacetic acids and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) as carboxyl activator (*Method B*). 4-Hydroxyphenyl derivative **7g** was then converted by standard procedures into the protected 4-acetoxyderivative **7h**. Reduction of arylacetamides **7a–g** to the corresponding amines **8a–g** was accomplished by sodium borohydride and glacial acetic acid in refluxing dioxane. Bischler–Napieralsky intramolecular cyclization of **7a–g** in phosphoryl chloride afforded crude 4-arylmethyl-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepines, which without further purification were reduced by sodium borohydride to the corresponding 4-arylmethyl-5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]-benzodiazepines **9a–g**. Finally, the reductive formylation of amines **9a,c–g** with formaldehyde in a Parr apparatus yielded the desired compounds **10a,c–g**.

4(4-Chlorobenzyl)-5-methyl-5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine **10b** was achieved by $LiAlH_4/AlCl_3$ reduction of formyl derivative **13** obtained by reacting 4-(4-chlorobenzyl)-5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine **9b** with butyl formate (scheme 2). Characteristic chemical and spectroscopic data of new compounds are listed in tables I



Scheme 1. X, Y, W = H, OCH_3; Z: H, Cl, OCH_3, OH, $OCOCH_3$. a: arylacetylchloride, $(C_2H_5)_3N$ (*Method A*) or arylacetic acid, EDCI (*Method B*); b: acetylchloride, $(C_2H_5)_3N$; c: $NaBH_4$, CH_3COOH; d: $POCl_3$, 40°C; e: $NaBH_4$; f: CH_2O, H_2/Pd.



Scheme 2. a: HCOOBu, Δ; b: $LiAlH_4/AlCl_3$.

and II. NMR spectra of cyclic derivatives **9** and **10** show the presence of three pyrrole protons and four benzene protons in accordance with the proposed tricyclic structure.

Pharmacological results and discussion

Experimental data relative to the preliminary screening of new compounds **7–10** for antinociceptive activity (hot-plate test) are presented in table III. Since compounds with actions other than analgesia, *eg*, myorelaxant benzodiazepines such as diazepam, are also responsive to the hot-plate test, we assayed by the Boissier and Simon 'traction test' (table III) independently of whether our derivatives possessed myorelaxant effects. As a further preliminary screening to assess the analgesic activity of our compounds, we performed the acetic-acid-induced writhing test on mice. This test is not selective for opiates, since various classes of compounds, such as antispasmodics, antihistaminics, sympaticolytics, simpaticomimetics and other drugs, behave positively when subjected to the acetic-acid-induced writhing test. For this reason, in order to ascertain the potential opiate-like antinociceptive effect of our compounds, the acetic-acid-

Table I. Characteristic data of compounds **7–10** and **13**.

Compound	*X*	*Y*	*Z*	*W*	*Formula*	*Method*	*Mp (°C)*	*Yield (%)*	*Solvent*[a]	*Chromatography*[b] *eluent*
7a	H	H	H	H	$C_{19}H_{18}N_2O$	A	117–118	95.5	A	–
7b	H	H	Cl	H	$C_{19}H_{17}ClN_2O$	A	144–146	59.3	B	–
7c	H	OCH_3	H	H	$C_{20}H_{20}N_2O_2$	B	108–109	97.5	B	–
7d	H	H	OCH_3	H	$C_{20}H_{20}N_2O_2$	B	119–120	93.3	B	–
7e	H	OCH_3	OCH_3	H	$C_{21}H_{22}N_2O_3$	B	115	98.2	B	–
7f	OCH_3	H	H	OCH_3	$C_{21}H_{22}N_2O_3$	B	117	100.0	B	–
7g	H	H	OH	H	$C_{19}H_{18}N_2O_2$	B	154–155	75.9	C	–
7h	H	H	$OCOCH_3$	H	$C_{21}H_{20}N_2O_3$	–	108–110	100.0	C	–
8a*	H	H	H	H	$C_{19}H_{21}N_2Cl$	–	183–185*	75.0	C	A
8b*	H	H	Cl	H	$C_{19}H_{20}Cl_2N_2$	–	178–179*	56.2	C	A
8c*	H	OCH_3	H	H	$C_{20}H_{23}ClN_2O$	–	183–186*	59.2	C	B
8d*	H	H	OCH_3	H	$C_{20}H_{23}ClN_2O$	–	188–190*	74.8	C	B
8e*	H	OCH_3	OCH_3	H	$C_{21}H_{25}ClN_2O_2$	–	184*	76.1	C	A
8f*	OCH_3	H	H	OCH_3	$C_{21}H_{25}ClN_2O_2$	–	147–149*	56.5	C	A
8g*	H	H	OH	H	$C_{19}H_{21}ClN_2O$	–	157–159*	34.3	C	B
9a	H	H	H	H	$C_{19}H_{18}N_2$	–	80–82	54.0	D	A
9b	H	H	Cl	H	$C_{19}H_{17}ClN_2$	–	82–83	66.0	A	A
9c*	H	OCH_3	H	H	$C_{20}H_{21}ClN_2O$	–	230–231*	93.6	C	A
9d	H	H	OCH_3	H	$C_{20}H_{20}N_2O$	–	110–111	88.4	E	C
9e	H	OCH_3	OCH_3	H	$C_{21}H_{22}N_2O_2$	–	150–151	89.5	C	A
9f	OCH_3	H	H	OCH_3	$C_{21}H_{22}N_2O_2$	–	133–134	41.4	C	A
9g	H	H	OH	H	$C_{19}H_{18}N_2O$	–	208–209	86.9	C	A
10a	H	H	H	H	$C_{20}H_{20}N_2$	–	98–99	85.7	D	A
10b*	H	H	Cl	H	$C_{20}H_{20}Cl_2N_2$	–	224–225*	90.3	C	A
10c	H	OCH_3	H	H	$C_{21}H_{22}N_2O$	–	216–218	93.0	C	A
10d	H	H	OCH_3	H	$C_{21}H_{22}N_2O$	–	213–216	69.4	C	A
10e	H	OCH_3	OCH_3	H	$C_{22}H_{24}N_2O_2$	–	111–112	87.7	B	A
10f	OCH_3	H	H	OCH_3	$C_{22}H_{24}N_2O_2$	–	123–124	77.0	B	A
10g	H	H	OH	H	$C_{20}H_{20}N_2O$	–	184–188	95.4	C	A
13	–	–	–	–	$C_{20}H_{17}ClN_2O$	–	125–126	57.0	D	A

*As hydrochloride. a: A, cyclohexane; B, benzene; C, ethanol; D, hexane; E, ethyl acetate. b: A, ethyl acetate; B, ethyl acetate/chloroform 1:1; C, ethyl acetate/chloroform 1:2; D, chloroform.

Table II. Spectroscopic data of compounds **7–10**.

Compound	*IR (cm^{-1})*	*Solvent*	*^{1}H-NMR* *δ, ppm*
7a	3260 (NH), 1635 (CO)	$CDCl_3$	3.42–3.48 (m, 2H, $COCH_2$), 4.22–4.33 (d, 2H, NCH_3), 5.55 (bd s, 1H, NH), 6.27–6.30 (m, 2H, β-pyrrole-H), 6.63–6.67 (m, 2H, α-pyrrole-H), 7.13–7.47 (m, 9H, Ar-H)
7b	3260 (NH), 1635 (CO)	$CDCl_3$	3.40 (s, 2H, $COCH_2$), 4.30–4.37 (d, 2H, NCH_2), 5.40 (broad s, 1H, NH), 6.27–6.30 (m, 2H, β-pyrrole-H), 6.67–6.73 (m, 2H, α-pyrrole-H), 7.17–7.37 (m, 8H, Ar-H)
7c	3280 (NH), 1638 (CO)	$CDCl_3$	3.45 (s, 2H, $COCH_2$), 3.77 (s, 3H, OCH_3), 4.23–4.33 (d, 2H, NCH_2), 5.42 (broad s, 1H, NH), 6.23–6.27 (m, 2H, β-pyrrole-H), 6.60–6.67 (m, 2H, α-pyrrole-H), 6.73–7.37 (m, 8H, Ar-H)
7d	3260 (NH), 1638 (CO)	$CDCl_3$	3.40 (s, 2H, $COCH_2$), 3.78 (s, 3H, OCH_3), 4.27–4.30 (d, 2H, NCH_2), 5.85 (broad s, 1H, NH), 6.23–6.30 (m, 2H, β-pyrrole-H), 6.60–6.67 (m, 2H, α-pyrrole-H), 6.80–7.37 (m, 8H, Ar-H)
7e	3270 (NH), 1625 (CO)	$CDCl_3$	3.43 (s, 2H, $COCH_2$), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.25–4.32 (d, 2H, NCH_2), 5.43 (broad s, 1H, NH), 6.27–6.30 (m, 2H, β-pyrrole-H), 6.67–6.68 (m, 2H, α-pyrrole-H), 6.73–7.40 (m, 7H, Ar-H)
7f	3270 (NH), 1645 (CO)	$CDCl_3$	3.47 (s, 2H, $COCH_2$), 3.70 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 4.22–4.28 (d, 2H, NCH_2), 5.87 (broad s, 1H, NH), 6.23–6.27 (m, 2H, β-pyrrole-H), 6.63–6.67 (m, 2H, α-pyrrole-H), 6.80–7.37 (m, 7H, Ar-H)
7g	3350 (OH), 3300 (NH), 1635 (CO)	DMF-d_7	3.47–3.53 (m, 3H, $COCH_2$ and NH), 4.20–4.30 (d, 2H, NCH_2), 6.27–6.30 (m, 2H, β-pyrrole-H), 6.97–7.00 (m, 2H, α-pyrrole-H), 6.73–7.47 (m, 8H, Ar-H), 9.43 (s, 1H, OH)
8a	3100 (NH)	CCl_4	0.87 (s, 1H, NH), 2.67–2.77 (m, 4H, NCH_2CH_2), 3.57 (s, 2H, NCH_2), 6.12–6.15 (m, 2H, β-pyrrole-H), 6.67–6.73 (m, 2H, α-pyrrole-H), 7.13–7.33 (m, 9H, Ar-H)
8b	3100 (NH)	CCl_4	0.90 (s, 1H, NH), 2.61–2.67 (m, 4H, NCH_2CH_2), 3.57 (s, 2H, NCH_2), 6.13–6.17 (m, 2H, β-pyrrole-H), 6.67–6.70 (m, 2H, α-pyrrole-H), 6.98–7.33 (m, 8H, Ar-H)
8c	3100 (NH)	CCl_4	0.90 (s, 1H, NH), 2.60–2.70 (m, 4H, NCH_2CH_2), 3.57 (s, 2H, NCH_2), 3.72 (s, 3H, OCH_3), 6.12–6.15 (m, 2H, β-pyrrole-H), 6.60–6.70 (m, 2H, α-pyrrole-H), 7.00–7.33 (m, 8H, Ar-H)
8d	3100 (NH)	CCl_4	0.92 (s, 1H, NH), 2.58–2.68 (m, 4H, NCH_2CH_2), 3.57 (s, 2H, NCH_2), 3.70 (s, 3H, OCH_3), 6.12–6.15 (m, 2H, β-pyrrole-H), 6.63–6.70 (m, 2H, α-pyrrole-H), 6.70–7.23 (m, 8H, Ar-H)
8e	3100 (NH)	CCl_4	0.92 (s, 1H, NH), 2.63–2.70 (m, 4H, NCH_2CH_2), 3.57 (s, 2H, NCH_2), 3.73 (s, 6H, OCH_3), 6.13–6.17 (m, 2H, β-pyrrole-H), 6.58–7.52 (m, 9H, α-pyrrole-H and Ar-H)
8f	3080 (NH)	CCl_4	0.92 (s, 1H, NH), 2.63–2.70 (m, 4H, NCH_2CH_2), 3.55 (s, 2H, NCH_2), 3.60 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 6.12–6.15 (m, 2H, β-pyrrole-H), 6.67–6.73 (m, 2H, α-pyrrole-H), 6.57–7.20 (m, 7H, Ar-H)
8g	3350 (OH), 3200 (NH)	CCl_4	2.62–2.70 (m, 4H, NCH_2CH_2), 3.58 (s, 2H, NCH_2), 4.18 (broad s, 2H, NH and OH), 6.13–6.17 (m, 2H, β-pyrrole-H), 6.43–7.27 (m, 10H, α-pyrrole-H and Ar-H)
9a	3360 (NH)	CCl_4	1.60 (s, 1H, NH), 3.03–3.08 (m, 2H, $CHCH_2$), 3.57 (s, 2H, NCH_2), 3.73–3.92 (m, 1H, CH), 6.17–6.20 (m, 2H, β-pyrrole-H), 6.83–6.90 (m, 1H, α-pyrrole-H), 7.00–7.33 (m, 9H, Ar-H)
9b	3360 (NH)	CCl_4	1.73 (s, 1H, NH), 3.00–3.10 (m, 2H, $CHCH_2$), 3.59–3.61 (m, 2H, NCH_2), 3.70–3.87 (m, 1H, CH), 6.13–6.20 (m, 2H, β-pyrrole-H), 6.83–6.90 (m, 1H, α-pyrrole-H), 7.07–7.27 (m, 8H, Ar-H)
9c	3380 (NH)	CCl_4	1.83 (s, 1H, NH), 2.93–3.05 (m, 2H, $CHCH_2$), 3.55 (s, 2H, NCH_2), 3.70–3.93 (m, 1H, CH), 3.70 (s, 3H, OCH_3 overlapped signal), 6.17–6.20 (m, 2H, β-pyrrole-H), 6.87–6.90 (m, 1H, α-pyrrole-H overlapped signal), 6.67–7.33 (m, 8H, Ar-H)
9d	3260 (NH)	$CDCl_3$	1.92 (s, 1H, NH), 2.93–3.08 (m, 2H, $CHCH_2$), 3.67 (s, 2H, NCH_2), 3.70 (s, 3H, OCH_3), 3.80–3.97 (m, 1H, CH), 6.30–6.33 (m, 2H, β-pyrrole-H), 6.97–7.00 (m, 1H, α-pyrrole-H overlapped signal), 6.75–7.40 (m, 8H, Ar-H)

Table II. *(Continued.)*

Compound	*IR (cm^{-1})*	*Solvent*	*^{1}H-NMR* *δ, ppm*
9e	3020 (NH)	DMF-d_7	1.08 (s, 1H, NH), 2.87–3.07 (m, 2H, CHCH_2), 3.67 (s, 2H, NCH_2), 3.72 (s, 6H, OCH_3), 3.75–3.97 (m, 1H, CH), 6.23–6.27 (m, 2H, β-pyrrole-H), 7.15–7.20 (m, 1H, α-pyrrole-H overlapped signal), 6.77–7.50 (m, 7H, Ar-H)
9f	3360 (NH)	$CDCl_3$	2.02 (s, 1H, NH), 2.90–3.37 (m, 2H, CHCH_2), 3.65 (s, 2H, NCH_2), 3.67 (s, 6H, OCH_3), 3.75–3.97 (m, 1H, CH), 6.30–6.33 (m, 2H, β-pyrrole-H), 6.97–6.98 (m, 1H, α-pyrrole-H), 6.70–7.37 (m, 7H, Ar-H)
9g	3260 (NH), 2800 (OH)	DMF-d_7	1.10 (s, 1H, NH), 2.93–3.02 (m, 2H, CHCH_2), 3.50–3.70 (m, 2H, NCH_2), 3.73–3.87 (m, 1H, CH), 6.20–6.23 (m, 2H, β-pyrrole-H), 7.07–7.13 (m, 1H, α-pyrrole-H overlapped signal), 6.67–7.47 (m, 8H, Ar-H)
10a	–	CCl_4	2.27 (s, 3H, NCH_3), 2.73–2.82 (d, 2H, CHCH_2), 3.37–3.40 (m, 2H, NCH_2), 3.77–3.93 (m, 1H, CH), 6.07–6.20 (m, 2H, β-pyrrole-H), 6.83–6.90 (m, 1H, α-pyrrole-H), 6.97–7.33 (m, 9H, Ar-H)
10b	–	$CDCl_3$	2.25 (s, 3H, NCH_3), 2.68–2.77 (d, 2H, CHCH_2), 3.33–3.37 (m, 2H, NCH_2), 3.43–3.67 (m, 1H, CH), 6.02–6.17 (m, 2H, β-pyrrole-H), 6.80–6.85 (m, 1H, α-pyrrole-H), 6.88–7.45 (m, 8H, Ar-H)
10c	–	DMF-d_7	2.67 (s, 3H, NCH_3), 2.93–2.98 (m, 2H, CHCH_2), 3.47–3.53 (m, 2H, NCH_2), 3.57–3.65 (m, 1H, CH), 3.72 (s, 3H, OCH_3), 6.17–6.25 (m, 2H, β-pyrrole-H), 6.83–7.80 (m, 9H, α-pyrrole-H and Ar-H)
10d	–	$CDCl_3$	2.57 (s, 3H, NCH_3), 2.95–3.23 (m, 2H, CHCH_2), 3.60–3.68 (m, 2H, NCH_2), 3.70 (s, 3H, OCH_3), 3.73–3.98 (m, 1H, CH), 6.25–6.40 (m, 2H, β-pyrrole-H), 6.68–7.67 (m, 9H, α-pyrrole-H and Ar-H)
10e	–	CCl_4	2.30 (s, 3H, NCH_3), 2.65–2.72 (d, 2H, CHCH_2), 3.40–3.42 (m, 2H, NCH_2), 3.48–3.57 (m, 1H, CH), 3.65 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 6.10–6.15 (m, 2H, β-pyrrole-H), 6.87–6.92 (m, 1H, α-pyrrole-H), 6.43–7.37 (m, 7H, Ar-H)
10f	–	$CDCl_3$	2.43 (s, 3H, NCH_3), 2.85–3.02 (m, 2H, CHCH_2), 3.45–3.53 (m, 2H, NCH_2), 3.55–3.77 (m, 1H, CH), 3.65 (s, 3H, OCH_3 overlapped signal), 3.68 (s, 3H, OCH_3 overlapped signal), 6.17–6.27 (m,2H, β-pyrrole-H), 6.90–7.00 (m, 1H, α-pyrrole-H), 6.53–7.50 (m, 7H, Ar-H)
10g	2800 (OH)	DMF-d_7	2.35 (s, 3H, NCH_3), 2.77–2.90 (m, 2H, CHCH_2), 3.30–3.50 (m, 3H, NCH_2 and CH), 6.13–6.33 (m, 2H, β-pyrrole-H), 6.57–7.60 (m, 9H, α-pyrrole-H and Ar-H), 9.27 (broad s, 1H, OH)

induced writhing test on mice was repeated using naloxone as opiate antagonist (see table IV). Finally, to complete the preliminary screening on analgesia we submitted our derivatives to the tail-flick test (table IV) with the aim of investigating a possible κ-opioid receptor involvement in the biological response [7–9].

The data from the pharmacological experiments clearly show that all the groups of test derivatives, even those lacking a basic moiety, exhibited an opiate-like antinociceptive activity without significant myorelaxant effects. In fact, many arylacetamides **7**, arylethylamines **8** and pyrrolobenzodiazepine derivatives **9** and **10** showed significant activities in both hot-plate and acetic-acid-induced writhing tests. Particularly, amides **7b** and **7e**, amine **8d**, and pyrrolobenzodiazepines **9a**, **9f**, and **10d** showed the highest potency as antinociceptive agents (tables III and IV). Good activity was also exhibited by amides **7a** and **7g**, and benzodiazepines **10b** and **10e**.

When arylacetamides **7** were compared with the related amines **8** (table III), no great differences were observed for analgesic activity, with the exception of **8d**, which was more active than its counterpart **7d**. In the phenylacetamide series **7**, 4-chlorophenyl and 3,4-dimethoxyphenyl derivatives (**7b** and **7e**) displayed higher antinociceptive activity than 4-hydroxy, 2,5-dimethoxyphenyl and the unsubstituted phenyl counterparts (**7g**, **7f**, and **7a**), whereas 3-methoxy- and 4-methoxyphenyl derivatives **7c** and **7d** were inactive. In contrast, in the benzodiazepine series **9**, the best analgesic activity was displayed by unsubstituted benzyl derivative **9a**, the other compounds showing only weak significance (**9c**, **9f** and **9g**) or none (**9b**, **9c** and **9d**). Among derivatives **10a–g**, 4-(4-methoxybenzyl)-5-methyl-5,6-dihydro-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine **10d** showed the highest activity in both the hot-plate test and the acetic-acid-induced writhing test. Unfortunately, experimental data relative to the

other derivatives **10** were not so homogeneous, and some had maximum activity in the hot-plate test (**10c**) and some in the acetic-acid-induced writhing test (**10b**, **10e**).

The lack of significance for any of the tested compounds in the tail-flick test ruled out a hypothetical κ-opiate receptor involvement in the analgesic activity of new derivatives **7–10**.

Some differences were observed when the open-chain derivatives were compared with the cyclized counterparts. Chloro-substituted amide and amine **7b** and **8b** showed highly significant activities in the hot-plate test, whereas the cyclized chloro derivatives were inactive (**9b**) or moderately active (**10b**). The 3,4-dimethoxy derivatives **7e–10e** behaved similarly. The best activity in the *N*-methyl pyrrolobenzodiazepine series was associated with 3-methoxy (**10c**) and 4-methoxy (**10d**) substituents, while the related nor-derivatives **9** and the open-chain amides **7** and amines **8** lacked any activity except for **8d** and **9c**.

The most significant results in both the hot-plate and the acetic-acid-induced writhing tests were observed for derivatives **7b**, **7e**, **8d**, **9a** and **10d**. For these compounds, the ED_{50} values in the hot-plate test and the toxic neurobehavioural effects (160 mg/kg ip) were determined (table V).

Concluding, we can remark that in our preliminary pharmacological studies a large number of new derivatives showed a potent opiate-like antinociceptive action. In the hot-plate assay the most active products were found to be arylacetamides (**7b** and **7e**), arylethylamines (**8a** and **8d**), pyrrolobenzodiazepines (**9a**) and their *N*-methyl derivatives (**10c** and **10d**). Furthermore, a number of derivatives (**7b**, **7e**, **8d**, **9a** and **10d**) also confirmed their high activity in the acetic-acid-induced writhing test.

Although the results of our screening indicate derivatives **7b**, **7e**, **8d**, **9a** and **10d** as potent opiate-like analgesics, no attempts were made in this preliminary assay to ascertain the nature of the analgesic action and further studies are indispensable to better define the possible involvement of opiate receptors other than the κ-receptor. In fact, the absence of a basic center in the structure of arylacetamides **7** may exclude any possibility of interaction of these compounds with the opiate receptor and a different mechanism of action could account for their good analgesic activity.

Experimental protocols

Chemistry

Melting points were taken on a Büchi 530 apparatus and are uncorrected. IR spectra (Nujol mulls) were obtained using a Perkin Elmer 1310 spectrophotometer. ^{1}H-NMR spectra were run on a Varian EM-390 (90 MHz) spectrometer with tetramethylsilane as the internal standard. Column chromatography was performed on silica gel Merck (70-230 Mesh). TLC was carried out on Stratocrom SIF Carlo Erba (silica-gel precoated plates with fluorescent indicator). Elemental analyses were taken by the analytical laboratory of Prof A Pietrogrande, University of Padova (Italy) for C, H, N, and, when desired, Cl, and were within ±0.4% of the theoretical values. Organic extracts were dried over anhydrous Na_2SO_4.

Typical procedure for the synthesis of arylacetamides 7a–g
Method A. To an ice-cooled solution of 1-(2-aminomethylphenyl)-1*H*-pyrrole **12** [10] (1.72 g, 0.010 mol) and triethylamine (1.67 ml, 0.012 mol) in anhydrous THF (100 ml), a solution of arylacetyl chloride (1.32 ml, 0.010 mol) in the same solvent (50 ml) was gradually added. After stirring at room temperature for 2 h, the mixture was filtered and the solution

Table III. Hot-plate test and myorelaxant action of derivatives **7–10**[a].

Compounds	*Hot-plate test (time in s)*	*Myorelaxant action*	
		Wire reflex (time in s)	*Wire time (time in s)*
TC	4.30 ± 0.79	1.0 ± 0	120 ± 0
7a	6.56 ± 2.02*	1.0 ± 0	120 ± 0
7b	7 90 ± 2.78***	1.0 ± 0	93.6 ± 45.6*
7c	7.20 ± 4.70	1.7 ± 0.6	120 ± 0
7d	3.76 ± 0.66	1.0 ± 0	120 ± 0
7e	6.26 ± 0.41***	1.7 ± 0.6	120 ± 0
7g	7.40 ± 2.16**	1.3 ± 0.5	88.3 ± 54.8*
7f	5.13 ± 0.68*	1.0 ± 0	120 ± 0
8a	8.13 ± 2.44***	1.3 ± 0.5	120 ± 0
8b	6.16 ± 1.49**	1.3 ± 0.5	120 ± 0
8c	5.33 ± 1.90	1.3 ± 0.5	120 ± 0
8d	7.30 ± 1.57***	1.3 ± 0.5	120 ± 0
8e	6.96 ± 2.40**	1.3 ± 0.5	108 ± 20.7
8f	7.90 ± 3.29**	1.0 ± 0	88.3 ± 54.8*
8g	5.50 ± 0.60*	1.0 ± 0	120 ± 0
9a	6.93 ± 1.80***	1.0 ± 0	86.0 ± 37.3*
9b	5.33 ± 1.80	1.0 ± 0	99.6 ± 35.2
9c	7.30 ± 2.58**	1.3 ± 0.5	96.6 ± 40.4*
9d	5.53 ± 1.76	1.3 ± 0.5	120 ± 0
9e	5.83 ± 3.17	1.3 ± 0.5	120± 0
9f	8.56 ± 4.03**	1.3 ± 0.5	113.3 ± 11.5
9g	6.70 ± 2.35*	1.0 ± 0	120 ± 0
10a	5.16 ± 0.55*	1.0 ± 0	120 ± 0
10b	5.60 ± 1.73*	2.8 ± 0.3*	120 ± 0
10c	7.60 ± 1.90***	1.3 ± 0.5	120 ± 0
10d	7.20 ± 1.42***	1.0 ± 0	120 ± 0
10e	5.53 ± 0.66*	3.0 ± 0.5*	120 ± 0
10f	3.93 ± 0.40	1.3 ± 0.5	120 ± 0
10g	4.13 ± 0.70	1.0 ± 0	120 ± 0
Morphine	14.9 ± 4.49***	7.0 ± 6.1**	107.3 ± 21.9

[a]All values represent the mean ± standard deviation for each group of animals. Drugs were administered ip (20 mg/kg) to groups of 10 mice (5 male and 5 female); a group of mice was treated only with vehicle (treated controls, TC). Statistical significance: *$0.05 > p > 0.02$; **$0.02 > p > 0.001$;

Table IV. Acetic-acid-induced writhing test and tail-flick test of selected derivatives **7–10**[a].

Compounds	*Tail-flick test (time in s)*	*Acetic-acid-induced writhing test*		
		Number of writhes	*% Inhibition*	*Number of writhes after administration of naloxone (2 mg/kg sc)*
TC	4.34 ± 1.67	28.83 ± 2.78	–	24.83 ± 2.78
7a	ND[b]	9.50 ± 11.70*	61.73	ND
7b	6.08 ± 3.38	1.33 ± 2.33***	94.64	38.60 ± 7.36
7e	4.05 ± 1.03	1.33 ± 2.10***	94.64	23.20 ± 9.00
7g	ND	14.00 ± 7.00*	43.61	ND
8a	7.20 ± 2.50*	13.10 ± 6.60	47.24	ND
8b	ND	14.50 ± 10.30	41.60	ND
8d	4.34 ± 1.67	3.83 ± 6.30***	84.57	18.80 ± 13.50
8f	ND	16.60 ± 4.50	33.14	ND
9a	5.45 ± 1.71	3.33 ± 3.00***	86.58	18.30 ± 5.88
9c	ND	15.66 ± 10.32	36.93	ND
9f	4.65 ± 2.04	4.33 ± 2.73***	82.56	19.60 ± 8.35
10b	4.78 ± 0.23	6.16 ± 7.75**	75.19	18.60 ± 11.79
10c	5.25 ± 1.85	24.30 ± 5.88	2.13	ND
10d	4.71 ± 1.22	0.50 ± 0.50***	97.98	ND
10e	ND	5.66 ± 3.14**	77.20	26.50 ± 9.39
Morphine	15.20 ± 2.04***	0 ± 0***	100	21.16 ± 16.80

[a]All values represent the mean ± standard deviation for each group of animals. Drugs were administered ip (20 mg/kg) to groups of 10 mice (5 male and 5 female); a group of mice was treated only with vehicle (treated controls, TC). Statistical significance: *$0.05 > p > 0.02$; **$0.02 > p > 0.001$; ***$p < 0.001$. [b]Not determined.

was evaporated. The residue was taken up in ethyl acetate (100 ml) and washed with 2 N hydrochloric acid (100 ml), saturated solution of sodium hydrogen carbonate (100 ml), brine (100 ml) and water (100 ml). Evaporation of the dried solution gave **7a** as a solid residue which was purified by crystallization.

Method B. To an ice-cooled mixture of 1-(2-aminomethylphenyl)-1*H*-pyrrole **12** hydrochloride (2.09 g, 0.010 mol) and triethylamine (3.06 g, 0.022 mol) in anhydrous THF (100 ml), 3-methoxyphenylacetic acid (1.66 g, 0.010 mol) and *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (2.30 g, 0.012 mol) were added. After stirring at room temperature for

Table V. ED_{50} in hot-plate test and neurobehavioural toxicity test of selected derivatives **7–10**.

Compounds	*Hot plate*	*Neurobehavioural toxicity test*[a]					
	ED_{50} (confidence limits)	*Spontaneous motor act*[b]	*Wire reflex*[c]	*Wire time*[d]	*Rota-rod test*[e]	*% Animals with ES convulsions*	*% Animals not surviving to ES*
7b	19.6 (10.0–30.2)	163.4 ± 43.1*	1.3 ± 0.5	85.2 ± 50.3*	1.5 ± 1.5	70	30
7e	8.3 (4.3–12.6)	123.7 ± 64.9**	2.4 ± 1.7	120 ± 0	1.1 ± 1.1	60	40
8d	15.2 (9.2–24.7)	105.3 ± 47.5***	2.1 ± 1.5	87.5 ± 39.7*	2.2 ± 1.4	70	30
9a	6.2 (5.2–11.6)	158.8 ± 52.5*	1.2 ± 0.7	82.1 ± 47.5*	2.7 ± 2.2*	70	30
10d	5.8 (2.8–8.7)	110.6 ± 30.8***	4.2 ± 4.4	105.0 ± 30.0	2.2 ± 3.3	70	30
TC	–	219.1 ± 35.0	1.2 ± 0.6	120 ± 0	0.7 ± 0.9	50	50

[a]All values represent the mean ± standard deviation for each group of animals. Drugs were administered ip (160 mg/kg) to groups of 10 mice (5 male and 5 female); a group of mice was treated only with vehicle (treated controls, TC). Statistical significance: *$0.05 > p > 0.02$; **$0.02 > p > 0.001$; ***$p > 0.001$. [b]Total number of movements in 5 min. [c]Time in seconds employed to place hind legs on a horizontal wire. [d]Time in seconds on the horizontal wire (cut-off 120 s). [e]Total number of falls in 100 s.

2 h, the mixture was filtered and the solution was evaporated. The residue was taken up in ethyl acetate (100 ml) and washed with saturated solution of sodium hydrogen carbonate (100 ml), brine (100 ml) and water (100 ml). Evaporation of the dried solution gave **7d** as a solid residue then purified by crystallization.

*Typical procedure for the synthesis of arylethylamines **8a–g***
To a suspension of arylacetamide **7a** (2.90 g, 0.010 mol) and sodium borohydride (3.79 g, 0.100 mol) in anhydrous dioxane (100 ml), 6.00 g (0.100 mol) of glacial acetic acid was carefully added. After heating at 100°C for 14 h while stirring, the mixture was cooled, quenched with aqueous sodium hydrogen carbonate, made basic by adding 2 N aqueous sodium hydroxide and stirred at room temperature for further 2 h. The mixture was extracted with chloroform (3 x 50 ml), the organic layer was washed with water (100 ml) and dried. Removal of solvent afforded the required arylethylamine **8a**, which was purified by column chromatography.

*Typical procedure for the synthesis of 4-arylmethyl-5,6-dihydro-4H-pyrrolo[1,2-a][1,4]benzodiazepines **9a–g***
A mixture of **7c** (3.84 g, 0.012 mol) and phosphoryl chloride (25 ml) was heated at 40°C for 2 h, and then treated with crushed ice and made basic by adding concentrated aqueous potassium hydroxide. The brown oil that separated was extracted with chloroform (2 x 100 ml) and the combined extracts were washed with brine (100 ml), dried and evaporated under reduced pressure. The residue was filtered through a silica-gel column eluting with chloroform giving, after evaporation of the solvent, almost pure 4-(3-methoxybenzyl)-4*H*-pyrrolo[1,2-*a*][1,4]benzodiazepine (3.32 g, 0.01 mol; 83% yield), which was dissolved in 95% ethanol (100 ml) without further purification. To this solution sodium borohydride (1.89 g, 0.050 mol) was added, and the reaction mixture was stirred at room temperature for 48 h. The solvent was evaporated under reduced pressure and the residue was diluted with water (100 ml) and extracted with ethyl acetate (3 x 50 ml). The organic layers were washed with brine (100 ml), dried and evaporated. The residue was purified by column chromatography to yield the desired **9c** as a white solid.

Similar reduction of cyclized product derived from **7h** afforded benzodiazepine **9g** by contemporaneous alkaline cleavage of acetoxy group.

*Typical procedure for the synthesis of 4-arylmethyl-5-methyl-5,6-dihydro-4H-pyrrolo[1,2-a][1,4]benzodiazepines **10a,c–g***
To a solution of **9f** (3.34 g, 0.010 mol) in 95% ethanol (70 ml) were added 40% aqueous formaldehyde (2 ml) and 10% palladium on charcoal (0.3 g). The mixture was hydrogenated in a Parr apparatus for 4 h at room temperature and 3 atm. Removal of catalyst by filtration and evaporation of the solution afforded a residue, which was purified by column chromatography to yield **10f** as a white solid.

*4-(4-Chlorobenzyl)-5-formyl-5,6-dihydro-4H-pyrrolo[1,2-a][1,4]benzodiazepine **13***
A solution of **9b** (3.09 g, 0.010 mol) in butyl formate (50 ml) was refluxed for 24 h. After cooling, the solution was evaporated under reduced pressure and the residue was taken up in water (100 ml) and ethyl acetate (100 ml). The organic layer was separated, washed with brine (2 x 100 ml), dried and evaporated to afford a residue, which was purified by column chromatography. The required compound **13** was obtained as a yellow solid.

*4-(4-Chlorobenzyl)-5-methyl-5,6-dihydro-4H-pyrrolo[1,2-a][1,4]benzodiazepine **10b***
To a stirred suspension of lithium aluminum hydride (0.76 g, 0.020 mol) and aluminum trichloride (2.67 g, 0.020 mol) in anhydrous diethyl ether (40 ml), a solution of **13** (3.37 g, 0.010 mol) and aluminum trichloride (1.34 g, 0.010 mol) in anhydrous THF (100 ml) was added dropwise. The resulting suspension was stirred at room temperature for 2 h, and was then very carefully quenced with ice, concentrated under reduced pressure, diluted with water and extracted with chloroform (3 x 50 ml). The organic layer was washed with brine (100 ml), dried and evaporated to furnish a residue which was purified by column chromatography to yield the desired **10b** as a sticky oil.

Pharmacological test procedures

Male and female Swiss inbred mice (Charles River Italia) weighing 23 ± 2 g were used. Animals were housed in standard environmental conditions: temperature 22 ± 1°C, humidity 60–65%, light period 5.0 am–9.0 pm. Each dose of test compounds, dissolved in 1% Tween 80, was administered ip to each group of 10 mice (5 male and 5 female). Morphine was used as a reference standard. Results of experiments were statistically analysed by the 'Dunnet *t* test' [11] and percentage controls were calculated using the 'Fisher exact probability test'. In all methods statistical significance against controls was estimated as follows: $*0.05 > p > 0.02$; $**0.02 > p > 0.001$; $***p < 0.001$. Comparison was performed according to the two-tailed Student's *t* test.

Antinociceptive activity
Three methods were used to assay this activity: the hot-plate test [12], the acetic-acid-induced writhing test [13] and the tail-flick test [14].

Hot-plate test. Test substances were administered ip to mice (20 mg/kg) and 30 min later the animals were gently placed on the surface of the plate maintained at 55.5 ± 0.5°C. When the animal licked its paw, latency (cut-off 15 s) was measured. The ED_{50} (median effective dose required to produce the effect in 50% of experimental animals) and 19/20 confidence limits for selected compounds were determined using the Litchfield–Wilcoxon procedure [15].

Acetic-acid-induced writhing test. For assessment of anti-writhing activity, test compounds were administered ip to mice (20 mg/kg) and were followed 20 min later by ip administration of 3% acetic acid (0.5 ml). Mice were placed in Plexiglas observation cages for 5 min; writhing was then observed during a subsequent 10 min interval (25–35 min postdrug treatment). A writhing response was defined as arching of the back with extension of the hindlimbs and contraction of abdominal muscles. Antinociceptive activity was expressed as percentage of inhibition of the mean number of writhes in the vehicle-treated control group (TC). Concurrent treatment of mice with naloxone (2 mg/kg sc) antagonized the antinociception (acetic-acid-induced writhing) induced by morphine and test compounds.

Tail-flick-test. Mice were placed in Plexiglas cylindrical holders with tails protruding through the base. Tails were immersed to within 5 mm of the base of the tail in a beaker of water maintained at 55°C. The time for the mice to either withdraw their tail from the water or attempt to otherwise escape was recorded to the nearest 0.1 s. After base-line reaction times were determined, mice were treated ip with test substances (20 mg/kg) and 15 min later the time taken for mice to move

their tail in order to escape from the radiant heat source (reaction time) was counted.

Myorelaxant action
Test substances were administered ip to mice (20 mg/kg). The myorelaxant action on mice was analysed either by the Boissier and Simon's 'traction test' (time employed by animals to place their hind legs on a horizontal wire; wire reflex) or by determining how long the animals stayed on wire (cut-off 120 s) [16, 17].

Neurobehavioural toxicity test
Tests were performed on each animal according to the following time schedule [18]: 0 min = administration of substance (160 mg/kg ip); 60 min = spontaneous activity in an open field; 70 min = myorelaxant action; 80 min = motor coordination in Rota-rod test; 90 min = maximal electroshock seizure.

Spontaneous motor activity. Mice were placed individually in standard polypropylene mouse cage (19 x 25 cm). Each cage was placed on the top of a small animal activities monitor platform (Coulbourn Instruments, Lehigh Valley). The sensitivity setting on each platform was adjusted to a level (10.00) that produced a significant number of counts in the absence of any toxin or drug, increased counts after low doses of *d*-amphetamine and decreased counts after high doses of *d*-amphetamine. The activity of each mouse was continuously monitored for 5 min.

Myorelaxant action. The myorelaxant action on mice was analysed as described above (wire reflex and wire time [16, 17]).

Action on motor coordination. This action was examined using the Rota-rod test [19]. The number of falls of the animals during an observation period of 100 s were determined.

Anticonvulsant activity. This was tested by determining the protection from seizures and from death caused by the electroshock intensity produced by the U Basile ECT-Unit 7800 apparatus adjusted as follows: 200 frequency pulses/s; 60 mA current intensity; 0.4 s shock duration; 0.6 ms pulse width. The animals were considered 'protected' when they did not show seizures.

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