In vivo Evaluation of Substituted -1H-Pyrrole-2,5-Diones as Anxiolytic-Agents

Eric Lattmann1*, Simon Dunn1, Carl H. Schwalbe1, Wanchai Airarat2, Andrew J. Shortt2 and Jintana Sattayasai2

1The School of Pharmacy, Aston University, England
2Department of Pharmacology, Khon Kaen University, Thailand

Abstract

The 3-brominated pyrroldione 1α served as starting material for the preparation of a series of substituted N-methyl 3-amino-pyrrol-2,5-diones, using a Cu catalysed substitution reaction. Initially the newly synthesised compounds were tested at a high dose for CNS depressant activity. When the activity was found significantly different from the control in the rota rod test and the wire mesh grasping test at a high dose the active compounds were evaluated further at a low dose in anxiolytic assays. The pyrroldione 2p showed a good anxiolytic activity at low doses of 1/0.1 mg/kg in the light/dark box and the elevated plus-maze assay. The n-hexyl-amino pyrrol 2e served as lead structure for further structure optimisation. Based on 5-alkoxy 3,4-dichloro-2(5H) furanones 3a and 3b, a second series of bis-substituted pyrroldiones was prepared under Cu catalysis and the pyrroldione 4f occurred the most potent anxiolytic activity.

INTRODUCTION

Cyclic imides, such as succinimides and maleimides, have been found to be pharmaceutically useful [1] as antibacterial [2], anticonvulsant [3] and antitumor agents [4].

Chlorinated 1-arylamino-1H-pyrrole-2,5-diones occurred anti-fungicidal [5] activity and N-substituted imides showed both anti-microbial [6], antidepressant [7], anxiolytic [8] and analgesic activity [9].

Bis-substituted succinimides (Figure 1) such as Ethosuximide represent useful agents in the treatment of epilepsy. A dichlorinated malimide showed analgesic properties against acetic acid-induced writhing in mice [10].

2-alkoxy-4-phenyl-pyrro-2,5-diones [11] and structurally related 3-anilino-4-aryl-maleimides [12] have been prepared with the intent of treating and preventing clinical conditions such as inflammatory diseases and Alzheimer’s disease.

The dimethyl oxazolidindione and phenytoin (Figure 1) showed all sedating, CNS depressant [13] and anxiolytic properties and are mainly in use for the treatment of epilepsy.

These molecules all contain the substituted pyrroldione as privileged structure and served as rationale for the discovery of novel CNS drugs from this template.

The starting point behind this discovery programme was

the desire to use an intermediate, which showed antibiotic properties [14], but was found too toxic in mice due to its high chemical reactivity. These halogenated reactive intermediates served here as ideal chemical starting point for the preparation of novel pyrrolidiones by reacting them with simple commercially available amines.

New chemical entities based on a particular molecular target, such as the 5HT₇- or CCK₂-antagonism did not reach the market, not even later clinical trials.

However, benzodiazepine anxiolytics were discovered by the classical approach using reliable animal models, and that justified evaluating these novel drug-like molecules in mice accordingly.

RESULTS AND DISCUSSION

Chemistry

Maleimides, also known as pyrrole-2,5-diones, can be synthesised by a number of chemical approaches. The majority of these routes are based on reactions of the corresponding maleic anhydride with an amine [15]. Another alternative one-step method involves the action of ammonium acetate on the maleic anhydride in boiling acid [16].

Here, the N-substituents, such as the N-methyl group were introduced with the parent formamide, such as methylformamide. In terms of drug design, the substitution of the halogen attached to the double bond, enabled us to remove a chiral centre from the template, compared with succinimides, which are chiral. The expected desired reaction was according to an IPSO substitution, but proved to deliver the desired molecules in low yields. The use of Cu catalysts finally gave the target molecules in good yields (Scheme 1).

The halogen of the brominated compound 1a and chlorinated entry 1b was readily displaced by a range of diverse primary and secondary amines. The brominated pyrrole 1a is the most preferred building block. A wide range of cyclic, acyclic and aromatic amines were reacted under mild conditions into the desired amino-pyrroldiones 2a-2s.

The metal catalysed coupling reaction provided the non-chiral unsaturated 1H-pyrrolidione template in presence of triethyl amine and Cu (I) catalysis in high yields.

The building block was obtained from mucochloric acid [17], which is commercially available from furfural. Furfural itself is obtained by heating biomass with sulphuric acid. Any further chemical application of furfural represents another important example of using this renewable resource from biomass.

The unmethylated pyrrol template (not shown) also reacted into mono-substituted pyrrole-dione, according to the copper catalysed reaction, but the building block was obtained in a much lower yield and is less lipophilic without the N-methyl group.

In the presence of water the yield is largely reduced. Acetonitril as a solvent could not be replaced by other solvents and a Ni catalyst (NiOAc) also increased the yield of the uncatalysed reaction from 30% range to a 50% range. Without Cu catalysis the aromatic amines 2f and 2g were not obtained at all. For the anilinino-pyrrols 2h and 2i the yield was approximately doubled by using a catalyst.

An overview of the synthesised structures using various chemically diverse amines is outlined in (Figure 2).

The crystal structure of the pyrrolidione 2r is outlined in Fig 3. Interestingly, the hydrogen of C10 formed a hydrogen bond with O7 and this resulted in a pseudo tricyclic structure for the piperazine 2r. Pyrrolidone 2r, as well as the hydrazone 2q showed promising biological activity in further in vivo assays, but this could be due to the biologically active versatile piperazine template and not due the effects of the pyrrol scaffold, on which was focussed in this study (Figure 3).
Having realised the importance of a lipophilic substituent in general [18], in the n-hexyl derivative 2e and in particular for the N,N-substituted pyrrol 2p, further attempts were made to provide other lipophilic bis-substituted pyrrols. The 5-methyl- and the 5-isopropoxy-3,4-dichloro-2(5H)-furanones 3a and 3b, which were described earlier as cytotoxic agents [19], were converted with primary amines into the desired bis-substituted pyrrols 4a-4f. The building blocks 3a and 3b were obtained in high yields from mucochloric acid (Scheme 2).

The addition of triethylamine (TEA), facilitated the ring-opening of the 2(5H)-furanone system and the chlorinated intermediate (Scheme 2) formed then, under Cu catalysis, with primary alkyl and cyclo-alkylamines the desired the 1,3-diaminopyrrol-2,5diones 4a to 4f in good yields. The 4-substituted amino-5-alkoxy-2(5H)-furanones [20], which were formed under these conditions only as a byproduct could be easily separated from the desired bis-substituted pyrroles by chromatography or extraction with petrol ether (PE). The 3-bromo-pyrrole template (not shown) was found of a similar reactivity and formed the same amino pyrroles in similar yields in ether at elevated RT. An overview of synthesised bis-pyrrolidiones 4a-4f using primary amines is outlined in (Figure 4). The cyclohexyl-derivative 4e was recrystallised from ether and the crystal structure is outlined in (Figure 5).

In the crystal the substituents formed a nearly linear chain with a heterocyclic template in the centre. In terms of receptor interaction, the hexyl side chains are flexible and able to interact with a potential receptor via hydrophobic binding (van de Waals interactions), and the anxiolytic effects at low concentrations are likely to be a result of hydrogen binding of the pyrrol-dione (CO) and hydrophobic interactions.

**SAR in vivo pharmacology**

The first step of the pharmacological evaluation was to test for an impairment of co-ordination at a dose that is 100/1000 times higher, than the desired therapeutic dose, using the standard rota-rot and wire mesh grasping test.

The compounds 2a, 2b, 2d, 2j-2o, 2q and 2r were not found significantly different from the control at a 5% level.

If the compound was found initially inactive in these assays, NFI was concluded (no further interest). This is based on the assumption, that no CNS drug in general and no anxiolytic drug in particular is likely to exist, if at a 100/1000 times dose of the potentially active dose is not resulting in an impairment of co-ordination. If the compound was found active, it was tested...
further at two low doses (0.1 and 1 mg/kg) in 2 reliable anxiolytic assays, the light dark box and the elevated plus maze (Table 1).

The butyl derivative 2c showed some initial activity in the light/dark box test and this was enhanced by increasing the size of the side chain, as observed for hexyl derivative pyrrol 2e.

The bis-heterocyclic compounds 2f and 2g showed some interesting analgesic effects, but only at the 1 mg/kg dose. The substituted anilines 2h and 2i displayed an anxiolytic effect in both assays, but were inactive at the 0.1 mg/kg dose. The cyclohexyl-ethyl derivative 2p was found active in both anxiolytic assays for both low doses of 1 and 0.1 mg/kg. Therefore, it was selected for a full dose evaluation.

The second compound of interest is the hexyl derivative 2e, which served as lead structure for further optimisation.

In order to assess the anxiolytic activity, the time spent in the open arms, as well as the number of entries, were observed in the elevated plus maze and these data are displayed in Figure 6 for the cyclohexyl-pyrrol derivative 2p (Figure 6). Diazepam served as positive control at 2 mg/kg. The low dose of the pyrrol 2p of 10 µg/kg was found not different from the negative control (5% DMSO in water). A significant anxiolytic effect of 2p was observed from 100 µg/kg for the time spent in the open arm and the effect of 1 mg/kg 2p was increased dose dependently, similar to the 2 mg/kg dose of diazepam standard. Interestingly the number of entries did not increase as seen for diazepam. Usually, for anxiolytics the time spent in the open arm is increased and the number of entries is increased dose dependently. Here, a mild muscle relaxant effect could be the cause for less spontaneous activity.

A series of disubstituted pyrroldiones 4a-4f was subsequently prepared, evaluated by the same concept and the results are outlined in Table 2. Out of 6 compounds 4 showed a significant impairment of coordination and these 4 compounds were tested at the low doses for desired activity. 4a, 4b and 4c dropped out at the low 0.1 mg/kg dose and 4f is clearly an optimised, more lipophilic version of 4a.

For the bis-n-hexyl derivative 4f, the anxiolytic effects were determined for 0.01, 0.1, 1 mg/kg body weight in mice and the effects are displayed in (Figure 7). The pyrrole 4f was tested at a dose range of 0.01, 0.1 and 1 mg/kg compared with diazepam at a dose of 2 mg/kg as standard. The time, spend in the open arms and the number of entries increased from 0.01 mg/kg, via 0.10 mg/kg to 1 mg/kg in the elevated plus maze and the effects are similar to diazepam in mice. Compared to the previously reported 5-alkoxy 4-amino-furanones, these novel pyrroldiones-ones can be obtained as pure compounds by recrystallization, contain no chlorine in the 3-position, which could be considered a potential toxicophore and are achiral.

The versatile bioactivity of pyrroldiones justified to start our

<table>
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<tr>
<th>Cp</th>
<th>Rota-rod test 100 mg/kg</th>
<th>Wire mesh grasping test 100 mg/kg</th>
<th>Light / dark Box 1 mg/kg / 0.1 mg/kg</th>
<th>Elevated plus maze 1 mg/kg / 0.1 mg/kg</th>
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Selection stage 1: (2 assays); high dose, 100 mg/kg, impairment of coordination

Table 1: In vivo evaluation of selected pyrroldiones.

Yes: Significant, P<0.05 active, No = NS: Not significant in assay at given dose.
**EXPERIMENTAL SECTION**

**Chemistry:** The chemicals were purchased from Aldrich (Gillingham, UK) and Lancaster Synthesis (Lancaster, UK). Mass spectra were obtained by Atmospheric Pressure Chemical Ionisation (APCI), using a Hewlett-Packard 5989b quadrupole instrument (Vienna, Austria). Both proton and carbon NMR spectra were obtained on a Brucker AC 250 instrument (Follanden, Switzerland), calibrated with the solvent reference peak. Infra-red spectra were plotted from KBr discs on a Mattson 300 FTIR spectrophotometer (Coventry, UK). Melting points were recorded from a Stuart Scientific melting point apparatus (Essex, UK). Analytical Thin Layer Chromatography was obtained using aluminum sheets, silica gel , F254, 250 µm and were visualized using ultraviolet light.

**General methods for the preparation of substituted 1-methyl-pyrrole-2,5-diones**

**Method 1:** 3-Bromo-1-methyl-pyrrole-2,5-dione (1a, 3.5x10^{-3} mol) was added to 3 ml acetonitril. 1.5 Equivalents of triethylamine and 1.5 equivalents of the appropriate amine (0.05 mol) with a catalytic amount of CuI (5%) were added and left to stir at 30-35 °C over night. The mixture was worked up using water and the desired compounds were extracted into diethyl ether. The resultant oily crystals were carefully re-crystallised from petrol ether.

**Method 2:** The same method as above was used, with the exception that (1b) 3-chloro-1-methyl-pyrrol-2,5-dione was used as reagent.

### Table 2: In vivo evaluation of selected pyrroldiones.

<table>
<thead>
<tr>
<th>Cp</th>
<th>Rota-rod test 100 mg/kg</th>
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<tbody>
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<td>Yes</td>
<td>Yes</td>
<td>Yes / No</td>
<td>Yes / No</td>
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<tr>
<td>4b</td>
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<td>Yes</td>
<td>Yes / No</td>
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<tr>
<td>4f</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes / Yes</td>
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</tr>
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</table>

Yes: Significant, P<0.05 active, No = NS: Not significant in assay at given dose.

**Table 2: In vivo evaluation of selected pyrroldiones.**

### Figure 7

**A** Dose-effect relationship of 4f on time in the open arms (A) and number of entry (B) in the elevated plus maze test.

The discovery programme and the use of the metal catalyst, such as Cu I, finally enabled us to create these novel pyrroldiones in good yields, which otherwise would only be obtained as by products in low yields. 2 selected molecules of the 2 series were evaluated in vivo in mice using the elevated plus maze and in conclusions the novel chemical entities displayed promising anxiolytic activity.

**Figure 7** Dose-effect relationship of 4f on time in the open arms (A) and number of entry (B) in the elevated plus maze test.

**Table 2:** In vivo evaluation of selected pyrroldiones.
(2d): 3-Isobutylamino-1-methyl-pyrrole-2,5-dione

Yield = 71%; M.P: 150-152°C; Rf (60% ether / 40% petrol ether) = 0.22

Molecular Weight: 247.2; Molecular Formula: C13H16N2O4; MS (APCI(+)): 248 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 7.84 (m, Ar-H, 3H), 7.51-7.60 (m, Ar-H, 3H), 4.99 (s, CH), 4.08 (bs, NH-CH), 2.74 (s, CH3) p.p.m.; IR (KBr-disc) ν max: 3587, 3335, 3098, 2936, 2370, 1710, 1648, 1332, 1161, 1074, 1023 cm⁻¹

(2f): 1-Methyl-3-[1,2,4]triazol-1-yl-pyrrole-2,5-dione

Yield = 80%, M.P: 166-168°C; Rf (60% ether / 40% petrol ether) = 0.34; Molecular Weight: 166.2; Molecular Formula: C11H16N2O2; MS (APCI(+)):167 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 7.56-7.58 (m, Ar-H, 3H), 6.06 (s, CH3), 3.84-4.09 (m, N-CH2), 3.12 (s, N-CH3), 2.05-2.35 (m, overlapping CH-C H2, CH-CH 2-CH2, CH-CH 2-CH2-CH2, 10H) p.p.m.

(2j): 3-Cyclopropylamino-1-methyl-pyrrole-2,5-dione

Yield = 60%; M.P: N/A – Oily Solid; Rf (60% ether / 40% petrol ether) = 0.32

Molecular Weight: 194.2; Molecular Formula: C10H14N2O2; MS (APCI(+)):209 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 7.39-7.42 (m, Ar-H, 3H), 5.42-5.47 (bs, NH), 3.09 (s, N-CH3), 2.55-2.66 (m, overlapping CH2-CH2, 6H) p.p.m.; 'C NMR (CDCl3) 250 MHz: δ = 169.9 (N-CO), 161.6 (CH-CO), 142.4 (CH-N-C), 137.3 (Ar-C), 135.0 (Ar-C) 124.2, (Ar-C) 120.3 (Ar-C), 117.6 (Ar-C), 87.8 (N-CH-CH), 23.0 (N-CH3), 18.6 (Ar-CH2-CH2-CH2) p.p.m. IR (KBr-disc) u max: 3450, 3313, 3099, 2931, 2364, 2331, 1700, 1732, 1623, 1504, 1367 cm⁻¹

(2k): 3-Cyclopentylamino-1-methyl-pyrrole-2,5-dione

Yield = 71%; M.P: 116-118°C; Rf (60% ether / 40% petrol ether) = 0.02

Molecular Weight: 208.3; Molecular Formula: C13H18N3O2; MS (APCI(+)): 231 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 6.79-7.42 (m, Ar-H, 3H), 5.39 (s, CH), 4.52-4.71 (bs, NH), 3.09 (s, N-CH3), 2.55-2.68 (m, overlapping CH2-CH2, 6H) p.p.m.; 'C NMR (CDCl3) 250 MHz: δ = 169.9 (N-CO), 161.6 (CH-CO), 142.4 (CH-N-C), 137.3, 135.0, 132.0 (Ar-C), 124.2 (Ar-C) 120.3 (Ar-C), 117.6 (Ar-C), 87.8 (N-CH-CH), 23.0 (N-CH3), 18.6 (Ar-CH2-CH2-CH2) p.p.m. IR (KBr-disc) u max: 3440, 3299, 2924, 2856, 2368, 2336, 1704, 1640, 1450, 1387, 1263, 1124, 595 cm⁻¹

(2l): 1-Methyl-3-[3-nitro-phenylamino]-pyrrole-2,5-dione

Yield = 71%; M.P: 100-102°C; Rf (60% ether / 40% petrol ether) = 0.37

Molecular Weight: 210.3; Molecular Formula: C9H14N2O2; MS (APCI(+)): 231 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 6.79-7.42 (m, Ar-H, 3H), 5.39 (s, CH), 4.52-4.71 (bs, NH), 3.09 (s, N-CH3), 2.55-2.68 (m, overlapping CH2-CH2, 6H) p.p.m.; 'C NMR (CDCl3) 250 MHz: δ = 169.9 (N-CO), 161.6 (CH-CO), 142.4 (CH-N-C), 137.3 (Ar-C), 135.0 (Ar-C) 124.2, (Ar-C) 120.3 (Ar-C), 117.6 (Ar-C), 87.8 (N-CH-CH), 23.0 (N-CH3), 18.6 (Ar-CH2-CH2-CH2) p.p.m. IR (KBr-disc) u max: 3440, 3299, 2924, 2856, 2368, 2336, 1704, 1640, 1450, 1387, 1263, 1124, 595 cm⁻¹
(2m): 1'-Methyl-2,3,4,5-tetrahydro-[1,3]bipyrrrol-2',5'-dione.

Yield = 86%; M.P: N/A – Oily Solid; Rf (60% ether / 40% petrol ether) = 0.45

Molecular Weight: 180.2; Molecular Formula: C12H20N2O2; MS (APCI(+)): 181 (M+1) m/z; IR (KBr-disc) υ max: 3442, 3339, 3112, 3009, 2924, 2361, 2329, 1718, 1699, 1629, 1441, 1392, 1115, 1044, 1026, 994, 767 cm⁻¹

(2n): 3-(2,6-Dimethyl-morpholin-4-yl)-1-methyl-pyrrole-2,5-dione.

Yield = 89%; M.P: 143-145 °C; Rf (60% ether / 40% petrol ether) = 0.32; Molecular Weight: 250 MHz: δ = 7.25-7.65 (m, Ar-H, 5H), 5.58-5.89 (bs, NH), 4.79 (s, CH), 4.05 (m, ArC-N-CH2, 4H) 3.28-3.41 (m, C-N-CH2, 4H), 2.98 (s, CH3) p.p.m. IR (KBr-disc) υ max: 3432, 2975, 2848, 2361, 2325, 1709, 1607, 1444, 1379, 1133, 1082, 767 cm⁻¹

(2o): 3-Benzylamino-1-methyl-pyrrole-2,5-dione.

Yield = 84%; M.P: 179-181 °C; Rf (60% ether / 40% petrol ether) = 0.25; Molecular Weight: 217.2; Molecular Formula: C11H12N2O2; MS (APCI(+)): 218 (M+1) m/z; IR (KBr-disc) υ max: 3444, 3281, 3209, 2919, 2361, 2352, 1771, 1698, 1599, 1432, 1006, 762 cm⁻¹

(2q): 1-Methyl-3-(N'-phenyl-hydrazino)-pyrrole-2,5-dione.

Yield = 72%; M.P: 179-181 °C; Rf (60% ether / 40% petrol ether) = 0.25; Molecular Weight: 271.3; Molecular Formula: C15H17N3O2; MS (APCI(+)): 272 (M+1) m/z; IR (KBr-disc) υ max: 3444, 2926, 2848, 2361, 2325, 1694, 1614, 1443, 1369, 1340, 1253, 1176, 738 cm⁻¹

Crystal Data - (sample recrystallised from methanol):

C15H17N3O2
Mw = 271.32
T = 293(2) K
Needle
Yellow, transparent
Mo Kα radiation: λ = 0.71073 Å
Monoclinic
C2/c

a = 20.6250(17) Å
b = 6.1567(9) Å
\( \beta = 91.424(8)^\circ \)
\( V = 2727.4(5) Å^3 \)
Dm not measured
R[F2 > 2σ(F2)] = 0.0516
wr(F2) = 0.1639
2699 reflections
182 parameters

Selected geometric parameters (Å, °)

C16H20N2O2
Mw = 271.32
T = 293(2) K
Needle
Yellow, transparent
Mo Kα radiation: λ = 0.71073 Å
Monoclinic
C2/c

a = 20.6250(17) Å
b = 6.1567(9) Å
\( \beta = 91.424(8)^\circ \)
\( V = 2727.4(5) Å^3 \)
Dm not measured
R[F2 > 2σ(F2)] = 0.0516
wr(F2) = 0.1639
2699 reflections
182 parameters

Selected geometric parameters (Å, °)
General method for the preparation of bis-substituted amino-pyrrrole-2,5-diones

0.5 g of the appropriate 5-alkoxy-furan-2-one (method 1/2) was dissolved in DMF (2 ml) and placed into 30 ml glass vials. Suitable amines (3 eq.) and 2 equivalents TEA with a catalytic amount of CuI (5%) were added carefully drop wise to each vial. The vials were placed in a heating block and heated to 45 °C for 48 hours. The compound was extracted with ether (10 ml) and washed twice with water (10 ml). The organic layer was dried using anhydrous magnesium sulphate and removed in vacuum to give a dark brown oil, which was purified using column chromatography (solvent system: 50/50 ether/petrol ether) to give the desired product.

Method 1: 3,4-Dichloro-5-methoxy-5H-furan-2-one 3a

Molecular Weight: 248.3; Molecular Formula: C14H20N2O2; MS (APCI(+)): 249 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 5.19-5.40 (bs, NH), 4.66 (s, CH-O), 3.74-3.99 (m, N-CH2, 8H), 0.82-1.03 (m, overlapping CH3, 6H) p.p.m. 13C NMR (CDCl3) 250MHz: δ = 170.4 (CN-CO), 165.1 (CH-CO), 84.9 (CH-C=O), 53.1 (CH2-CH-NH), 29.5 (CH2-CH-N), 20.1 (NH-CH2-CH2-NH), 19.7 (N-CH2-CH2-NH), 13.7 (NH-CH2-CH2-CH2), 13.6 (N-CH2-CH2-CH2) p.p.m. IR (KBr-disc) v max: 3431, 2966, 2925, 2867, 2361, 2338, 1701, 1638, 1458, 1399 1232, 1105, 919, 893 cm⁻¹.

Method 2: 3,4-Dichloro-5-isopropoxy-5H-furan-2-one 3a

Yield = 73% (method 2); M.P: 130-133 °C; If (50% ether, 50% petrol ether) = 0.77

Molecular Weight: 224.3; Molecular Formula: C12H19N2O2; MS (APCI(+)): 216 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 5.22-5.42 (bs, NH), 4.82 (s, CH-O), 3.10-3.36 (d, N-CH3), 2.97-3.08 (t, NH-CH2-j = 6.0 Hz), 1.89-2.11 (m, overlapping CH2-CH2-N & CH2-CH2-CH2-NH, 2H), 0.87-1.09 (m, overlapping CH3, 12H) p.p.m. 13C NMR (CDCl3) 250MHz: δ = 170.4 (CN-CO), 167.8 (CH-CO), 149.5 (CO-CN) 83.7 (CH-C=O), 52.0 (NH-CH2-N), 45.0 (N-CH2-N), 28.0 (CH2-CH2-NH), 27.9 (CH2-CH2-N), 20.2 (NH-CH2-CH2-CH2), 20.0 (N-CH2-CH2-CH2) p.p.m. IR (KBr-disc) v max: 3399, 2961, 2941, 2882, 2365, 1705, 1635, 1519, 1449, 1416, 1297, 1131, 1035, 786 cm⁻¹.

(4c): 1-sec-Butyl-3-sec-butylamino-pyrrole-2,5-dione

Yield = 69% (method 1); M.P: N/A – Oily Solid; If (50% ether, 50% petrol ether) = 0.80

Molecular Weight: 224.3; Molecular Formula: C12H19N2O2; MS (APCI(+)): 169 (M+), 225 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 5.79 (s, CO-CH), 5.04-5.25 (bs, NH), 3.82-4.16 (m, overlapping N-C&H-NH & CH2-NH-CH2-N), 1.46-1.73 (m, overlapping CH2-CH-N & CH2-CH2-NH, 2H), 1.19-1.31 (m, overlapping CH-CH3-NH, 6H), 0.72-0.97 (m, overlapping CH2-CH2-CH2-NH, 6H) p.p.m. 13C NMR (CDCl3) 250MHz: δ = 173.0 (CN-C=O), 168.3 (CH-C=O), 148.0 (CO-CN), 83.4 (CH-C=O), 51.9 (N-H), 48.3 (N-C), 29.0 (NH-CH2-CH2-NH), 27.1 (CH2-CH2-NH), 19.4 (N-H), 18.4 (N-CH2-CH2), 11.3 (N-H-CH2-CH2), 10.3 (N-CH2-CH2-CH2) p.p.m. IR (KBr-disc) v max: 3428, 2977, 2932, 2867, 2370, 2338, 1703, 1642, 1461, 1393 1229, 1106, 1019, 900 cm⁻¹.

Yield = 45% (method 1); M.P: 142-144 °C; If (50% ether, 50% petrol ether) = 0.78

Molecular Weight: 248.3; Molecular Formula: C14H20N2O2; MS (APCI(+)): 249 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 5.22-5.42 (bs, NH), 4.31-4.49 (m, N-CH2-N), 3.82-3.98 (m, NH, N-C), 1.51-2.13 (m, overlapping CH3, 16H) p.p.m. 13C NMR (CDCl3) 250MHz: δ = 168.6 (CN-C=O), 161.0 (CH-C=O), 148.3 (CH-C=O), 84.3 (CH-C=O), 55.6 (NH-C), 50.5 (N-C), 32.1 (NH-CH2-CH2), 29.5 (NH-CH2-CH2), 24.7 (N-CH2-CH2), 23.8 (N-CH2-CH2) p.p.m. IR (KBr-disc) v max: 3441, 3252, 2924, 2855, 2376, 2338, 1698, 1651, 1623, 1399, 1125, 1075, 989, 892 cm⁻¹.

Yield = 57% (method 2); M.P: 146-148 °C; If (50% ether, 50% petrol ether) = 0.80

Molecular Weight: 276.4; Molecular Formula: C16H24N2O2; MS (APCI(+)): 215 (M+), 277 (M+1) m/z; 'H NMR (CDCl3) 250 MHz: δ = 4.9 Hz), 1.18-1.67 (m, overlapping CH2, 16H) p.p.m. 13C NMR (CDCl3) 250MHz: δ = 169.2 (CN-C=O), 162.0 (CH-C=O), 147.3 (CH-C=O), 83.4 (CH-C=O), 53.1 (NH-CH), 50.0 (N-C), 32.0 (NH-CH2-CH2), 30.1 (N-H), 26.0 (NH-CH2-CH2), 24.7 (N-CH2-CH2), 25.3 (N-CH2-CH2), 24.7 (N-CH2-CH2) p.p.m. IR (KBr-disc) v max: 3431, 2966, 2925, 2867, 2361, 2338, 1701, 1638, 1521, 1458, 1399, 1232, 1105, 1019, 893 cm⁻¹.

Yield = 47% (method 2); M.P: 69-84 °C; If (50% ether, 50% petrol ether) = 0.84
p.p.m. IR (KBr-disc) $\nu$ max: 3447, 2931, 2853, 2370, 2350, 1716 cm$^{-1}$.

**PHARMACOLOGY**

**Animal studies**

Experiments were conducted in male ICR mice obtained from the Animal House, Faculty of Medicine, Khon Kaen University. Each experimental group consisted of 6 animals and the treatment procedures were approved by the ethical committee, Faculty of Medicine, Khon Kaen University (HO 2434-76) accord with current UK legislation. Mice had free access to fresh water and food pellets. They were exposed to automated 12 h light cycles.

Mice were intraperitoneal injected with either test compound dissolved in 5% DMSO at the volume not more than 0.2 ml/animal. At 30 min after treatment, animals were tested as described in the following sections.

**Motor activity tests**

**The rota-rodd test:** Mouse was placed on the rotating drum with the acceleration speed (Accel. Rota-rodd, Jones & Roberts, for mice 7650, Ugo Basile, Italy). The time animal spent on the rod was recorded.

**The wire mesh grasping test:** Mouse was placed on a wire mesh (20x30 cm). After a few seconds, the mesh was turned 180° and the time the animal hold on the mesh was recorded.

**Anxiolytic activity tests**

**The light/dark box:** Mice were placed in the light part of the light/dark box. The box was a plexi glass cage, 25x50x20 cm, having one-third as a dark and two-third as a light compartment. The animals could walk freely between dark and light parts through the opening. The time animals spent in light part during the 5 min interval was recorded. The mouse was considered to be in the light part when its 4 legs were in the light part.

**The elevated plus-maze:** The wooden elevated plus-maze consisted of two open arms (30x10 cm) without any walls, two enclosed arms of the same size with 5-cm high side walls and end wall, and the central arena (10x10 cm) interconnecting all the arms. The maze was elevated approximately 30 cm height from the floor. At the beginning of the experiment the mouse was placed in the central arena facing one of the enclosed arms. During a 5 min interval, the time animals spent in the open arms of plus-maze was recorded. The mouse was considered to be in the open part, when it had clearly crossed the line between the central arena and the open arm with its 4 legs.

**Statistical methods**

The data were expressed as mean ± SD and one-way analysis of variance (ANOVA) and supplementary Tukey test for pair wise comparison were tested to determine for any significant difference at $p<0.05$.

**REFERENCES**


