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Antinociceptive Properties of Chloromaleinimides and their Sulphonyl Derivatives

This report describes the synthesis of new cyclic imides obtained from the reaction between aniline and dichloromaleic anhydride with further chlorosulphonation as well as the reaction between different amines and 4-methoxyphenol for the synthesis of imidobenzenesulphonyl derivatives. These compounds were tested as antinociceptive agents using the writhing test on mice. Some compounds, when intraperitoneally injected, proved to be potent and dose-related antinociceptives, being several times more active than many known reference drugs.

Keywords: Chloromaleinimides; Sulphonamides; Antinociception; Writhing test; Mice

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Introduction

Studies carried out in this laboratory previously have shown that both, cyclic imides [1, 2] and sulphonamides have potent analgesic action, exceeding the anti-inflammatory potency observed under the same conditions by either aspirin or paracetamol. Kalgutkar et al. [3] have demonstrated that some N-substituted maleinimides inhibit the prostaglandine endoperoxide synthase (PGHS). The cyclooxygenase activity of PGHS is inhibited by several compounds known as nonsteroidal anti-inflammatory drugs (NSAIDs). In this context, the scope of the present investigation was the synthesis of new imidic and imidobenzenesulphonyl compounds aiming at the development of novel active compounds related to cyclic imides. We also investigated whether the maleinimides in this study act by the same mechanism of those indicated by Kalgutkar et al. [3]. The analgesic potential of the synthesized compounds was tested using a writhing test in mice.

Results and discussion

The synthesis method used is represented in Scheme 2. The compounds (**1-22**) were readily characterized by conventional spectral data.

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The results summarized in Table 1 reveal that the compounds synthesized show a significant antinociceptive effect as compared to nonsteroidal drugs such as aspirin and paracetamol, based on the acetic acid-induced abdominal contortion model. The N-phenylsuccinimide displayed quite a different activity behavior with respect to maleinimides. The former exhibited a very low activity compared with that of maleinimides. It was also observed that the substitution of the double bond hydrogens of the imido ring by chlorine atoms increased the activity of the N-phenylmaleinimide significantly.

Acetic acid releases several inflammatory mediators, including prostaglandines [4, 5], when administered in the peritonaeum of animals. It has been suggested that the analgesic and anti-inflammatory effects of NSAIDs result, at least in part, from suppression of prostaglandin synthesis due to PGHS inhibition [6, 7]. Considering the structural similarity of the maleinimides in this study with those studied by Kalgutkar et al. along with the fact that both, the former (*in vivo*) and the latter (*in vitro*) studies, found succinimides to be almost inactive in comparison to maleinimides, it is reasonable to assume that these maleinimides are acting by the same mechanism: inhibition of PGHS. However, it should be noted that some differences exist between the effects of the compounds used in this study and those of Kalgutkar's which are probably due to the distinct behavior of the compounds involved in *in vivo* and *in vitro* experiments. According to Kalgutkar et al., N-(carboxyheptyl)-maleinimide inhibits enzyme activity within seconds after mixing with a stoi-

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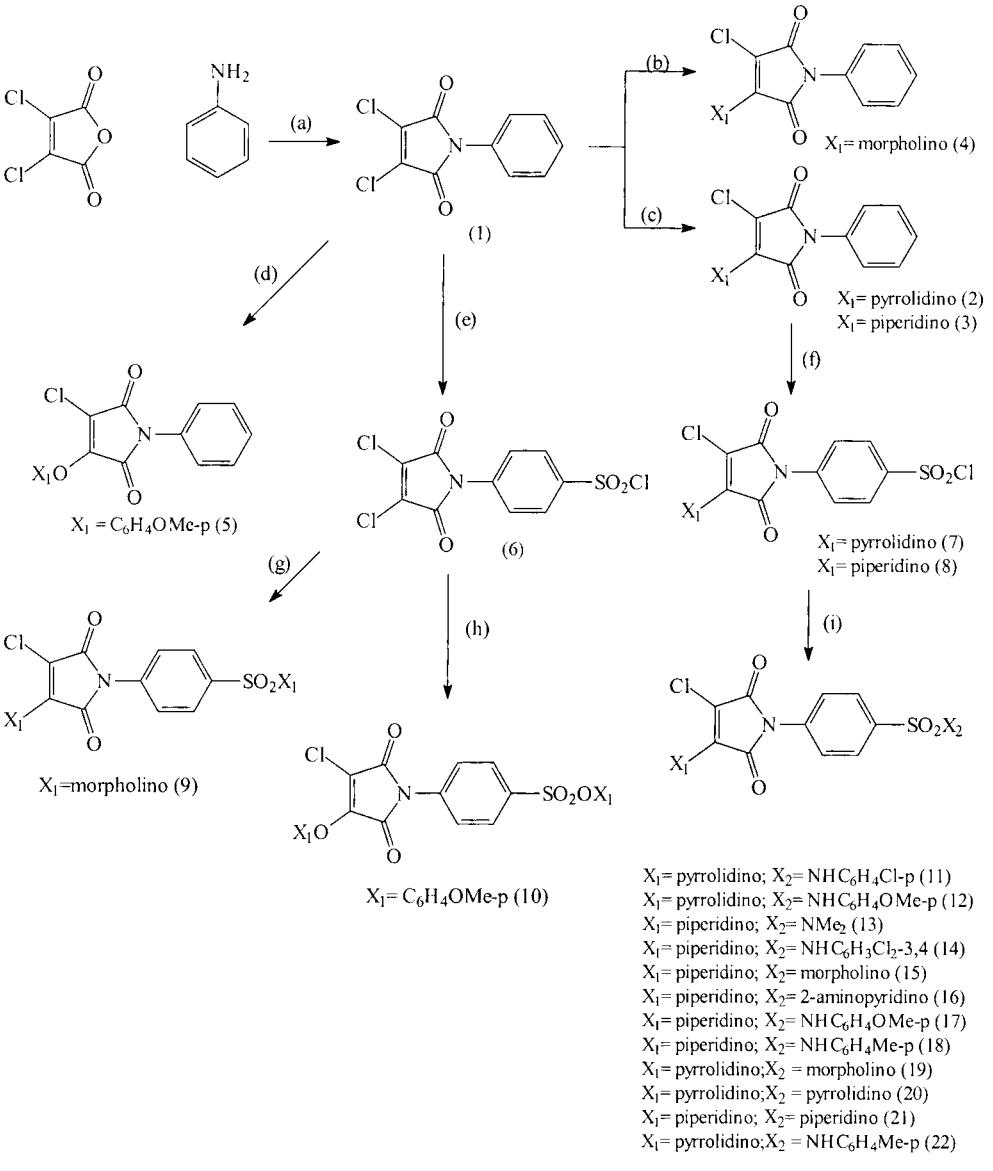
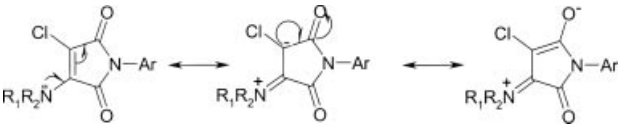


(a) HOAc/reflux; (b) morpholine; (c) pyrrolidine /piperidine; (d) 4-methoxyphenol/NEt_3; (e) HSO_3Cl; (f) HSO_3Cl; (g) morpholine; (h) 4-methoxyphenol/NEt_3;(i) amines.

Scheme 1.

chiometric amount of PGHS protein. The lack of PGHS inhibition of N-(carboxyheptyl)-succinimide suggests that this inhibition results from a covalent modification of the enzyme. Compound **1** shows very rapid reaction with some nucleophilic reagents that replace one of the chlorine atoms. This fact supports the hypothesis of Kalgutkar, who proposed that maleinimides inactivate prostaglandin-endoperoxide synthase (PGHS) due to a covalent modification of the protein [3]. The high activity of **1** compared with compounds **2-4** suggests that a covalent bond modification takes place by displacement of a chlorine atom of the imido ring. However, when a chlorine atom from **1** is replaced by nitrogen bases, as in compounds **2**–**4**, a nucleophilic attack on the double bond along with the substitution of the second chlorine atom is not expected to occur, as can be inferred from the resonance structures in Scheme 2, resulting in a low activity. The reaction of compounds **2**–**4** with different amines at room temperature did not take place.



Scheme 2.

Compounds **2**–**5** were submitted to chlorosulphonation in an attempt to improve their activity. In the process, compounds **4** and 5 produced multiple products that could not be isolated. On the other hand, compounds **2** and **3** were successfully processed and the corresponding benzenesulphonyl chlorides (compounds **7** and **8**) reacted with nitrogen bases producing compounds **11**–**22**. The chlorosulphonation of compound **1** with subsequent synthesis of compounds **9** and **10** was also carried out successfully.

The activity of the sulphonamides **9**, **11**–**22** and the ester **10** improved with respect to their corresponding parent compounds (compounds **2**–**5**). Compound **22** was approximately 13 times more active than aspirin, suggesting additional non-covalent interactions between imidobenzenesulphonyl compounds and the residues of aminoacid present in the active site of PGHS. This implied that non-covalent interactions played an important role in determining the activity of **11**–**22** which lead us to think that the mechanism of

Table 1. Effect of imides, sulphonyl compounds, and reference drugs given intraperitoneally against acetic acid-induced abdominal constriction (writhing test) in mice.

Compounds	Dosage range (mg/kg)	ID_{50} (mg/kg)	ID_{50} (μmol/kg)
1	0.5–10	0.67 (0.48–0.77)†	2.80 (2.00–3.20)†
2	10–60	22.65 (14.36–22.84)	81.88 (51.93–82.54)
3	3–10	11.24 (3.23–15.40)	38.66 (11.14–53.00)
4	3–30	25.41 (16.28–29.36)	86.84 (55.62–100.33)
5	1–10	4.38 (3.58–5.36)	13.31 (10.86–16.26)
9	10–60	28.90 (18.49–45.20)	64.95 (41.86–102.29)
10	1–10	3.45 (2.99–6.89)	7.36 (5.81–13.37)
11	3–30	12.23 (8.97–16–67)	26.24 (19.25–35.76)
12	10–60	29.40 (12.27–36.69)	50.66 (29.44–79.45)
13	3–30	17.88 (13.55–23.59)	44.96 (34.08–59.31)
14	3–30	11.71 (9.96–13.75)	22.75 (19.36–26.72)
15	3–30	8.30 (8.05–8.55)	18.87 (18.30–19.44)
16	3–30	5.36 (3.91–6.10)	10.92 (8.77–13.65)
17	3–30	9.09 (4.27–12.31)	19.10 (8.99–25.88)
18	3–30	11.80 (8.20–22.39)	25.66 (17.83–48.70)
19	3–30	5.66 (4.43–11.57)	12.78 (10.41–27.18)
20	10–60	20.59 (4.18–29.31)	50.25 (10.20–71.53)
21	3–30	6.30 (2.14–14.34)	13.94 (4.75–31.75)
22	3–30	4.61 (2.33–9.99)	10.36 (5.24–22.42)
N–Phenylsuccinimide ([8], unpublished results)			321.1(280.10–480.2)
N–Phenylmaleinimide [9]			19.00 (11.50–31.80)
Aspirin [10]			133.00 (73.–247.00)
Paracetamol [10]			125.00 (104.–250.00)

† 95% confidence limit.

inhibition of the PGHS, involving compounds **11**−**22**, might occur without the formation of a covalent interaction, since the substitution of the nitrogen bases and/or the remaining chlorine atom is not expected to occur as discussed previously.

Acknowledgments

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Experimental

Chemistry

Compound **1** was directly obtained from the reaction of 3,4-dichloromaleic anhydride and aniline with acetic acid under reflux, according to a methodology described elsewhere [11]. Compounds **2**−**4** were obtained from the reaction of compound **1** (1 mol) and appropriate amines (2 mol) in dichloromethane at room temperature. Compound **5** was obtained from the reaction of compound **1** (1 mol) with 4-methoxyphenol (1mol) in the presence of triethylamine (2 mol) at room temperature. Compounds **6**−**8** were obtained from the reaction of compounds **1, 2** and **3** (1 mol) with chlorosulphonic acid (6.0 mol). Compound **10** was obtained from the reaction of compound **6** (1 mol) with 4-methoxy-phenol (1 mol) also in the presence of triethylamine (2 mol) in dichloromethane at room temperature. Compounds **9, 11**−**22** were obtained from the reaction between compounds **6, 7** and **8** (1 mol) and amines (morpholine, piperidine, pyrrolidine, and substituted anilines, 2 mol) in methanol at 0 °C. The mixture was allowed to warm to room temperature for 2 hours and was then poured onto ice-water. The precipitate was filtered off, washed with water and dried under an infrared lamp. All the compounds were characterized by ^{1}H-NMR, IR, and microanalysis. The purity of these compounds was determined by TLC using several solvent systems of different polarity. Infrared spectra were determined with a Perkin Elmer 16PC spectrophotometer (Perkin Elmer, Wellesley, MA, USA). Nuclear magnetic resonance spectra were recorded with a Bruker AC-200F spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) using tetramethylsilane as internal standard. Mass spectra were obtained with a Shimadzu GC-MS-2000A instrument (Shimadzu, Kyoto, Japan). Microanalyses were carried out with a Perkin Elmer 2400 instrument.

Physico-chemical data of synthesized compounds

3,4-Dichloro-1 phenyl-1H-pyrrole-2,5-dione (**1**)

mp (°C): 204−205 (lit. [11] 204−206 °C); Yield: 70 %.

3-Chloro-1-phenyl-4-pyrrolidin-1-yl-1H-pyrrole-2,5-dione (**2**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7,40- 7,34 (m, 5H, ArH); 4,00 (t, 4H, $N(CH_2)_2$); 1,96 (t, 4H, 2 × CH_2); IR (KBr, cm^1) = 1762, 1706, 1634 (C=O), 1590 (Ar C=C); $C_{14}H_{13}ClN_2O_2$ requires C,60.77; H,4.74; N,10.12; found C, 60.50; H, 5.00; N, 9.85 %; mp (°C) 135−136 (dry-flash chromatography, ethyl acetate:hexane, 1:1); Yield: 76.5 %.

3-Chloro-1-phenyl-4-piperidin-1-yl-1H-pyrrole-2,5-dione (**3**)

mp (°C) 126-127 (lit. [12] 127−128 °C); Yield: 87 %.

3-Choro-4-morpholin-4-yl-1-phenyl-1H-pyrrol-2,5-dione (**4**)

mp (°C) 160-161 (lit. [12] 160−161 °C); Yield: 67 %.

3-Chloro-4-(4-metoxyphenoxy)-1-phenyl-1H-pyrrole-2,5-dione (**5**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.46−7.35 (m, 5H, ArH), 7.16−6.89 (m, 4H, ArH-OC_6H_4OMe-p), 3.83 (s, 3H, OCH_3); IR (KBr ,cm^1) = 1782, 1726, 1656 (C=0), 1598 (Ar C=C); $C_{17}H_{12}ClNO_4$ requires C, 61.92; H, 3.67; N, 4.25; found C, 61.58; H, 4.00; N, 4.45 %; mp (°C): 128.6−129.8 (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 67 %.

4-(3,4-Dichloro-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-benzenesulphonyl chloride (**6**)

m.s. 339, 341, 343, 345 (M^+), 304, 306, 308 (M^+-Cl), 240, 242, 244 (M^+-SO_2Cl), 184, 166, 122, 118, 87; mp (°C): 186,5−187,5; Yield: 85 %.

4-(3-Chloro-2,5-dioxo-4-pyrrolidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)benzenesulphonyl chloride (**7**)

m.s. 378, 376, 374 (M^+), 341, 339 (M^+-Cl); $C_{14}H_{12}Cl_2N_2O_4S$ requires C, 44.81; H, 3.22; N, 7.47; found C, 44.38; H, 3.60; N, 7.73 %; mp (°C): 122-123; Yield: 90 %.

4-(3-Chloro-2,5-dioxo-4-piperidin-2,5-dihydro-1H-pyrrol-1-yl)benzenesulphonyl chloride (**8**)

m.s. 392, 390, 388, (M^+), 355, 353, (M^+-Cl), 289, 291(M^+-SO_2Cl), 218, 108, 87; $C_{15}H_{14}Cl_2N_2O_4S$ requires C, 46.28; H, 3.63; N, 7.20; found C, 46.50; H, 3.45; N, 7.05 %. mp (°C): 88 decomp.; Yield: 74 %.

3-Chloro-4-morpholin-4-yl-1-[4-(morpholin-4-ylsulphonyl)-phenyl]-1H-pyrrole-2,5-dione (**9**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.85- 7.60 (m, 4H, ArH); 4.08−3.01 (m, 16H, morpholino); IR (KBr, cm^1) = 3470 (NH), 1772, 1716, 1632 (C=O), 1592 (Ar C=C), 1343,1166 (SO_2); $C_{18}H_{20}ClN_3O_6S$ requires C, 48.92; H, 4.56; N, 9.51; found C, 48.50; H, 4.34; N, 9.72 %; mp (°C): 150-151 (from ethanol); Yield: 58 %.

4-Metoxyphenyl-4-[3-chloro-4-(4-metoxyphenoxy)-2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl]benzenesulphonate (**10**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.93−7.65 (m, 4H, ArH); 7.15−6.73 (m, 8H, ArH, C_6H_4OMe-p); 3.84 (s, 3H, OCH_3); 3.77 (s, 3H, OCH_3); IR (KBr, cm^1) = 3502 (NH), 1754, 1738, 1658 (C=O), 1594 (Ar C=C), 1340, 1146 (SO_2); $C_{24}H_{18}ClNO_8S$requires C, 55.87; H, 3.52; N, 2.71; found C, 55.39; H, 3.70; N, 2.95 %; mp (°C): 140-141 (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 12 %.

4-(3-Chloro-2,5-dioxo-4-pyrrolidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N-(4-chlorophenyl) benzenesulphonamide (**11**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.72-7.55 (m, 5H, ArH, NH); 7.10−6.50 (m, 4H, ArH, C_6H_4Cl-p); 3.90−4.10 [m, 4H, $N(CH_2)_2$]; 1.90−2.10 (m, 4H, 2 × CH_2); IR (KBr, cm^1) = 3458 (NH),1766, 1704, 1640 (C=O),1592 (Ar C=C), 1342,1164 ($S0_2$); $C_{20}H_{17}Cl_2N_3O_4S$ requires C, 51.51; H, 3.67; N, 9.01; found C, 51.30; H, 3.93; N, 8.75 %; mp (°C): 177 decomp (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 56 %.

4-(3-Chloro-2,5-dioxo-4-pyrrolidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N-(4-metoxyphenyl) benzenesulphonamide (**12**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.71-7.54 (m, 4H, ArH); 6.96−6.60 (m, 4H, ArH, C_6H_4OMe-p); 6.30 (s, 1H, NH); 3.90−4.10 [m, 4H, $N(CH_2)_2$]; 3.76 (s, 3H, OCH_3); 1.97−2.10 (m, 4H, 2 × CH_2); $C_{21}H_{20}ClN_3O_5S$ requires C, 54.60; H, 4.36; N, 9.10; found C, 54.20; H, 4.67; N, 9.46%; mp (°C): 148-149 (dry-flash chromatography, ethyl acetate: hexane, 1:1); Yield: 58.5%.

4-(3-Chloro-2,5-dioxo-4-piperidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N,N-dimethylbenzenesulphonamide (**13**)

mp (°C): 217,5−219,5 (lit. [1] 218−219°C); Yield: 47.5%.

4-(3-Chloro-2,5-dioxo-4-piperidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N-(3,4-dichlorophenyl) benzenesulphonamide (**14**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 8.70 (s, 1H, NH),.8.0-6.9 (m, 7H, ArH), 3.80-4.10 [m, 4H, $N(CH_2)_2$], 1.50−2.00 (m, 6H, 3 × CH_2); IR (KBr, cm^1) = 3450 (NH), 1758,1706, 1620 (C=O), 1594 (Ar C=C), 1332, 1164 (SO_2); $C_{21}H_{18}Cl_3N_3O_4S$ requires C, 48.99; H, 3.52; N, 8.16; found C, 49.23; H, 3.15; N, 8.39%; mp (°C) 205-206 (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 44.5%.

3-Chloro-1-[4-(morpholin-4yl-sulphonyl)phenyl]-4-piperidin-1-yl-1H-pyrrole-2,5-dione (**15**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.83- 7.61 (m, 4H, ArH); 4.10-3.90 [m, 4H, $N(CH_2)_2$]; 3.80−3.70 [m, 4H, $O(CH_2)_2$], 2.90-3.10 [m, 4H, $N(CH_2)_2$], 1.65−1.75 (m, 6H, 3 × CH_2); IR (KBr, cm^1) = 1766, 1718, 1642 (C=O), 1586 (Ar C=C), 1340, 1162 (SO_2); $C_{19}H_{22}ClN_3O_5S$ requires C, 51.87; H, 5.04; N, 9.55; found C, 51.46; H, 5.43; N, 9.32%; mp (°C): 207 (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 55%.

4-(3-Chloro-2,5-dioxo-4-piperidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N-pyridin-2-yl-benzenesulphonamide (**16**)

^{1}H-NMR (C_3D_6O/DMSO, ppm) δ: 7.40-7.80 (m, 4H, ArH); 8.10−7.90 and 7.1−6.8 (m, 4H, 2-pyridyl); 4.00-3,90 [m, 4H, $N(CH_2)_2$]; 1.80−1,60 (m, 6H, 3 × CH_2); IR (KBr, cm^1) = 3318 (NH), 1764, 1710, 1634 (C=O), 1670(Ar C=C), 1398,1182 ($S0_2$); $C_{20}H_{19}ClN_4O_4S$ requires C, 53.75; H, 4.29; N, 12.54; found C, 53.48; H, 4.70; N%, 12.42; mp (°C): 194-196 (from ethanol); Yield:17%.

4-(3-Chloro-2,5-dioxo-4-piperidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N-(4-metoxyphenyl) benzenesulphonamide (**17**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.75-7.48 (m, 4H, ArH); 7.00-6.75 (m, 4H, ArH, C_6H_4OMe-p); 6.37 (s, 1H, NH); 4.00−3.90 [m, 4H, $N(CH_2)_2$]; 3.76 (s, 3H, OCH_3); 1.80−1.70 (m, 6H, 3 × CH_2); IR (KBr, cm−1) = 3284 (NH), 1760, 1708, 1622 (C=O), 1340, 1160 (SO_2); $C_{22}H_{22}ClN_3O_5S$ requires C, 55.52; H, 4.66; N, 8.83; found C, 55.21; H, 4.75; N, 8.75%; mp (°C) 172-173 (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 73%.

4-(3-Chloro-2,5-dioxo-4-piperidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N-(4-methylphenyl) benzenesulphonamide (**18**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.75−7.55 (m, 4H, ArH); 7.10−6.90 (m, 4H, ArH, $C_6H_4CH_3$-p); 6.35 (s, 1H,NH); 4.00−3.90 [m, 4H, $N(CH_2)_2$]; 2.26 (s, 3H, CH_3); 1.80−1.70 (m, 6H, 3 × CH_2); IR (KBr, cm−1) = 3450 (NH), 1758, 1706, 1620 (C=O), 1594 (Ar C=C), 1332, 1164 (SO_2); $C_{22}H_{22}ClN_3O_4S$ requires C, 57.45; H, 4.82; N, 9.14; found C, 57.62; H, 4.45; N, 9.50%; mp (°C) :189-190 (dry-flash chromatography, ethyl acetate:hexane, 1:1); Yield: 32%.

3-Chloro-1-[4-(morpholin-4-ylsulphonyl)phenyl]-4-pyrrolidin-1-yl-1H-pyrrole-2,5-dione (**19**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.83-7.63 (m, 4H, ArH); 4.00-4.10 [m, 4H, $N(CH_2)_2$]); 3.70−3,80 [m, 4H, $O(CH_2)_2$], 3.00-3.08 [m, 4H, $N(CH_2)_2$], 1.92−2.02 (m, 4H, 2 × CH_2); IR (KBr, cm^1) = 1766, 1718, 1642 (C=O), 1586 (Ar C=C), 1340, 1162 (SO_2); $C_{18}H_{23}ClN_4O_5S$ requires C, 48.81; H, 5.23; N, 12.65; found C, 48.63; H, 5.30; N, 12.48%; mp (°C): 147.8-149.0 (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 63%.

3-Chloro-4-pyrrolidin-1-yl-1-[4-pyrrolidin-1-yl-sulphonyl)-phenyl]-1H-pyrrole-2,5-dione (**20**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.91−7.58(m, 4H, ArH); 4.01−4.10 [m, 4H, $N(CH_2)_2$]; 3.30−3.20 [m, 4H, $SO_2N(CH_2)_2$]; 2.10−1.97 (m, 4H, 2 × CH_2); 1.80-1.70 (m, 4H, 2 × CH_2); IR (KBr, cm^1) = 3458 (NH), 1766, 1716, 1632 (C=O), 1594 (Ar C=C), 1344,1158 (SO_2); $C_{18}H_{20}ClN_3O_4S$ requires C, 52.74; H, 4.92; N, 10.25; found C, 52.46; H, 4.68; N, 9.95%; mp (°C): 178.7-180.0 (from ethanol); Yield: 70%.

3-Chloro-1-[4-(piperidin-1-yl-sulphonyl)benzyl]-4-piperidin-1-yl-1H-pyrrol-2,5-dione (**21**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.71−7.45(m, 4H, ArH); 4.00−3.88 [m, 4H, $N(CH_2)_2$]; 3.00−2.99 [m, 4H, $SO_2N(CH_2)_2$]; 1.73-1.50 (m, 12H, 6 × CH_2); IR (KBr, cm^1) = 3452 (NH), 1764, 1709, 1628 (C=O), 1344, 1166 ($S0_2$). $C_{21}H_{26}ClN_3O_4S$ requires C, 55.81; H, 5.80; N, 9.30; found C, 56.05; H, 5.46; N, 9.62%; mp (°C): 87-88 (from ethanol); Yield: 60%.

4-(3-Chloro-2,5-dioxo-4-pyrrolidin-1-yl-2,5-dihydro-1H-pyrrol-1-yl)-N-(4-methylphenyl) benzenesulphonamide (**22**)

^{1}H-NMR ($CDCl_3$, ppm) δ: 7.80−7.55 (m, 4H, ArH); 7.10−6.90 (m, 4H, ArH, C_6H_4Me-p); 6.35 (s, 1H, NH); 4,10−4.00 [m, 4H, $N(CH_2)_2$]; 2.28 (s, 3H, CH_3); 2.00−1.95 (m, 4H, 2 × CH_2); IR (KBr, cm^1) = 3452 (NH), 1762, 1712, 1638 (CO), 1594 (Ar C=C), 1336, 1160 (SO_2); $C_{21}H_{20}ClN_3O_4S$ requires C, 56.56; H, 4.52; N, 9.42, found C, 56.15; H, 4.78; N, 9.73%; mp (°C): 219-220 (dry-flash chromatography, ethyl acetate : hexane, 1:1); Yield: 43%.

Pharmacology

Writhing test

Male Swiss mice (25−30 g, N = 6−8 animals for each dose) were used. The abdominal constriction induced by intraperitoneal injection of acetic acid (0.6%) was carried out according to the previously described procedures [13, 14] with minor modifications. Within each of the dose ranges (Table 1) of the studied compounds, three doses were selected and the animals were pre-treated intraperitoneally with them 30 min before the injection of acetic acid. The vehicle used for injecting of the test compounds was 0.9% NaCl solution. When necessary, an aqueous solution of DMSO (2%) was used in order to dissolve some of the compounds. Control animals received a similar volume of vehicle (10 mL kg^{-1}, i.p.). All experiments were carried out at 23 ± 2°C. Pairs of mice were placed in separate boxes and the number of abdominal constrictions of the abdominal muscles together with a stretching, were cumulatively counted over a period of 20 min. Antino-

ciceptive activity was expressed as the reduction of the number of abdominal contractions between control animals and mice pre-treated with the studied compound.

Statistical Analysis

The results are presented as mean ± s.e.m., and the statistical significance between the groups was analyzed by means of variance followed by Dunnett's multiple comparison test. P values less than 0.05 were considered as indicative of significance. The ID_{50} values (concentration of the compound that reduced responses by 50% with respect to control values) were estimated by graphical interpolation from individual experiments. ID_{50}'s are presented as mean values with the 95% confidence interval.

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