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Synthesis and cytotoxic and analgesic activities of some 1, 5-diaryl-3-ethoxycarbonylpyrrole derivatives

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Abstract

Some 1,5-diaryl-3-ethoxycarbonyl-2-methylpyrrole derivatives were obtained by reacting 1-aryl-3-ethoxycarbonylpent-1,4-diones and a suitable aniline derivative or sulfanilamide under Paal-Knorr pyrrole synthesis conditions. The cytotoxicity of the compounds was tested and all compounds, except for compound **2 h**, showed a time-dependent increase in cytotoxic activity. Analgesic activities of the compounds were determined by using the tail-flick and tail-immersion methods; some of the compounds showed potent analgesic activity.

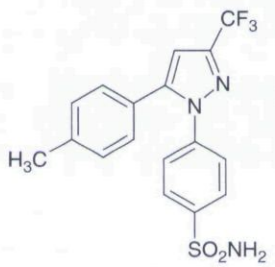
Keywords: *Diarylpyrrole, cytotoxicity, analgesic activity*

Introduction

It was demonstrated that indomethacin inhibited the production of prostaglandins (PGs) by Vane and co-workers in 1971[1]; it is now known that this is the result of inhibition of the enzyme cyclooxygenase (COX)[2,3]. The diarylheterocyclic class of anti-inflammatory agents, considered as structural analogues of indomethacin, were initiated and extensively evaluated in the 1970s. Since then, a large number of compounds have been prepared and evaluated as analgesic and anti-inflammatory agents[2,3] and two of them (i.e. celecoxib **I** and rofecoxib **II**) have recently been marketed. It is also well known that the inhibition of the enzyme COX-2 provides an anticancer activity, because an overproduction of COX-2 to form prostaglandins facilitates proliferation of neoplastic cells.

The heterocyclic residue of these compounds may be five or six membered, such as furan, pyrrole, thiophene, thiazole, oxazole, imidazole, pyrazole, isoxazole, pyrimidine etc. The substituents on the aryl residue have been optimized as fluoro, methyl, methoxy, methylsulphonyl or aminosulphonyl and these are generally in the *para* position[4–14].

In this study, in the light of the above findings, some 1,5-diaryl-3-ethoxycarbonylpyrrole derivatives, which can be considered as analogues of the compounds inhibiting COX-2, were synthesised and their analgesic and cytotoxic activities examined.



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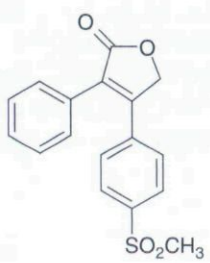

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Materials and methods

Chemistry

Melting points were determined using an Electrothermal 9100 digital melting point apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments, FTIR: Schimadzu 8400S Spectrophotometer, ^{1}H-NMR: Bruker DPX 400 NMR Spectrometer, ES-MS: Agilent 1100 MSD mass spectrometer using the electron spray method. Analyses for C, H, N were within 0.4% of the theoretical values.

1-Aryl-3-ethoxycarbonylpent-1,4-diones **1** were prepared according to literature methods[15]. Some characteristics of the compounds have been given in Table I.

General method for the preparation of 1,5-diaryl-3-ethoxycarbonyl-2-methylpyrrole derivatives **2**. A mixture of **1** (5 mmol) an appropriate aniline derivative or sulfanilamide (5 mmol) in acetic acid was refluxed for 2 h. The cooled mixture was poured into ice water and the formed precipitate was filtered. The crude product was crystallised from ethanol.

2a IR(KBr)ν_{max}(cm^{-1}): 3278, 3124(N-H), 1685 (C = O), 1593–1498(C = C), 1340, 1168(S = O), 1232,1076(C-O). ^{1}H-NMR(400 MHz)(DMSO-d$_6$) ((ppm): 1.29(3H, t, J: 7.1 Hz, OCH_2CH_3), 2.33(3H, s, pyrrole-5-CH_3), 4.24(2H, q, J: 7.1 Hz, OCH_2CH_3), 6.71(1H, s, PyrroleC$_4$-H), 7.05–7.08(2H, m, Ar-H), 7.18–7.24(3H, m, Ar-H), 7.48(2H, d, J: 8.58 Hz, Ar-H), 7.53(2H, s, SO_2NH_2), 7.88(2H, d, J: 8.58 Hz, Ar-H). ES-MS (m/z): 385 (M + 1)(100%).

2b IR(KBr)ν_{max}(cm^{-1}): 3278, 3124(N-H), 1685 (C = O), 1593–1498(C = C), 1340, 1168(S = O), 1232,1076(C-O). ^{1}H-NMR(400 MHz)(DMSO-d$_6$) δ (ppm): 1.29(3H, t, J: 7.1 Hz, OCH_2CH_3), 2.33(3H, s, pyrrole-5-CH_3), 4.24(2H, q, J: 7.1 Hz, OCH_2CH_3), 6.70(1H, s, PyrroleC$_4$-H), 7.11(2H, d, J: 8.10 Hz, Ar-H), 7.25(2H, d, J: 8.12 Hz, Ar-H), 7.49(2H, d, J: 8.58 Hz, Ar-H), 7.55(2H, s, SO_2NH_2), 7.90(2H, d, J: 8.58 Hz, Ar-H).

2d IR(KBr)ν_{max}(cm^{-1}): 1691(C = O), 1600–1494 (C = C), 1218,1074(C-O). ^{1}H-NMR(400 MHz) (DMSO-d$_6$) δ (ppm): 1.29(3H, t, J: 7.05 Hz, OCH_2CH_3), 2.31(3H, s, pyrrole-5-CH_3), 4.22(2H, q, J: 7.05 Hz, OCH_2CH_3), 6.68(H, s, Pyrrole C$_4$-H), 6.99-7.09(4H, m, Ar-H) 7.25–7.27(2H, m, Ar-H), 7.45–7.52(3H, m, Ar-H). ES-MS (m/z): 324 (M + 1)(100%).

2e IR(KBr)ν_{max}(cm^{-1}): 1691(C = O), 1600–1494(C = C), 1218,1074(C-O). ^{1}H-NMR(400 MHz)(DMSO-d$_6$) δ (ppm): 1.28(3H, t, J: 7.08 Hz, OCH_2CH_3), 2.28(3H, s, pyrrole-5-CH_3), 3.78(3H, s, OCH_3), 4.22(2H, q, J: 7.09 Hz, OCH_2CH_3), 6.66(1H, s, PyrroleC$_4$-H), 7.00(2H, d, J: 8.84 Hz, Ar-H), 7.01–7.11–7.09(4H, m, Ar-H), 7.17(2H, d, J: 8.80 Hz, Ar-H).

2f IR(KBr)ν_{max}(cm^{-1}): 3290, 3124(N-H), 1687 (C = O), 1575–1494(C = C), 1330, 1164(S = O), 1234,1091(C-O). ^{1}H-NMR(400 MHz)(DMSO-d$_6$) δ (ppm): 1.29(3H, t, J: 7.09 Hz OCH_2CH_3), 2.33(3H, s, pyrrole-5-CH_3), 4.24(2H, q, J: 7.09 Hz, OCH_2CH_3), 6.69(1H, s, PyrroleC$_4$-H), 6.88–7.12(4H, m, Ar-H), 7.48(2H, d, J: 8.44 Hz, Ar-H), 7.53(2H, s, NH_2), 7.88(2H, d, J: 8.42 Hz, Ar-H). ES-MS (m/z): 403 (M + 1)(95%).

2g IR(KBr)ν_{max}(cm^{-1}): 3290, 3124(N-H), 1687 (C = O), 1575–1494(C = C), 1330, 1164(S = O), 1234,1091(C-O). ^{1}H-NMR(400 MHz)(DMSO-d$_6$) δ (ppm): 1.28(3H, t, J: 7.06 Hz, OCH_2CH_3), 2.33(3H, s, pyrrole-5-CH_3), 4.24(2H, q, J: 7.08 Hz, OCH_2CH_3), 6.75(1H, s, PyrroleC$_4$-H), 7.07(2H, d, J: 8.45 Hz, Ar-H), 7.28(2H, d, J: 8.43 Hz, Ar-H), 7.49(2H, d, J: 8.37 Hz, Ar-H), 7.53(2H, s, NH_2), 7.90(2H, d, J: 8.32 Hz, Ar-H).

Pharmacology

Cytotoxicity of the Compounds

Cell Culture. Rat embryo fibroblast F2408 cells were grown in Dulbecco Modified Eagle Medium (DMEM) (Sigma, Deisenhofen, Germany) and 10% (v/v) of foetal calf serum (FCS) (Gibco, U.K.). The cell culture media was supplemented with penicillin/streptomycin at 100 units/mL and 2 mM L-glutamine and cells were incubated at 37°C under 5% CO_2 / 95% air in a humidified atmosphere.

In vitro cytotoxicity assay. The cytotoxic response of F2408 cell line was determined by using standard

Table I. Some characteristics of the synthesised compounds

Comp.	R	R'	m.p. (°C)	Yield (%)	Molecular formula
2a	H	SO_2NH_2	199–200	82	$C_{20}H_{20}N_2O_4S$
2b	CH_3	SO_2NH_2	182–184	78	$C_{21}H_{22}N_2O_4S$
2c	OCH_3	SO_2NH_2	163–164	71	$C_{21}H_{22}N_2O_5S$
2d	F	H	217–218	82	$C_{20}H_{18}FNO_2$
2e	F	OCH_3	215–218	88	$C_{21}H_{20}FNO_3$
2f	F	SO_2NH_2	94–97	77	$C_{20}H_{19}FN_2O_4S$
2g	Cl	SO_2NH_2	113–115	71	$C_{20}H_{19}ClN_2O_4S$
2h	NO_2	SO_2NH_2	212–215	78	$C_{20}H_{19}N_3O_6$

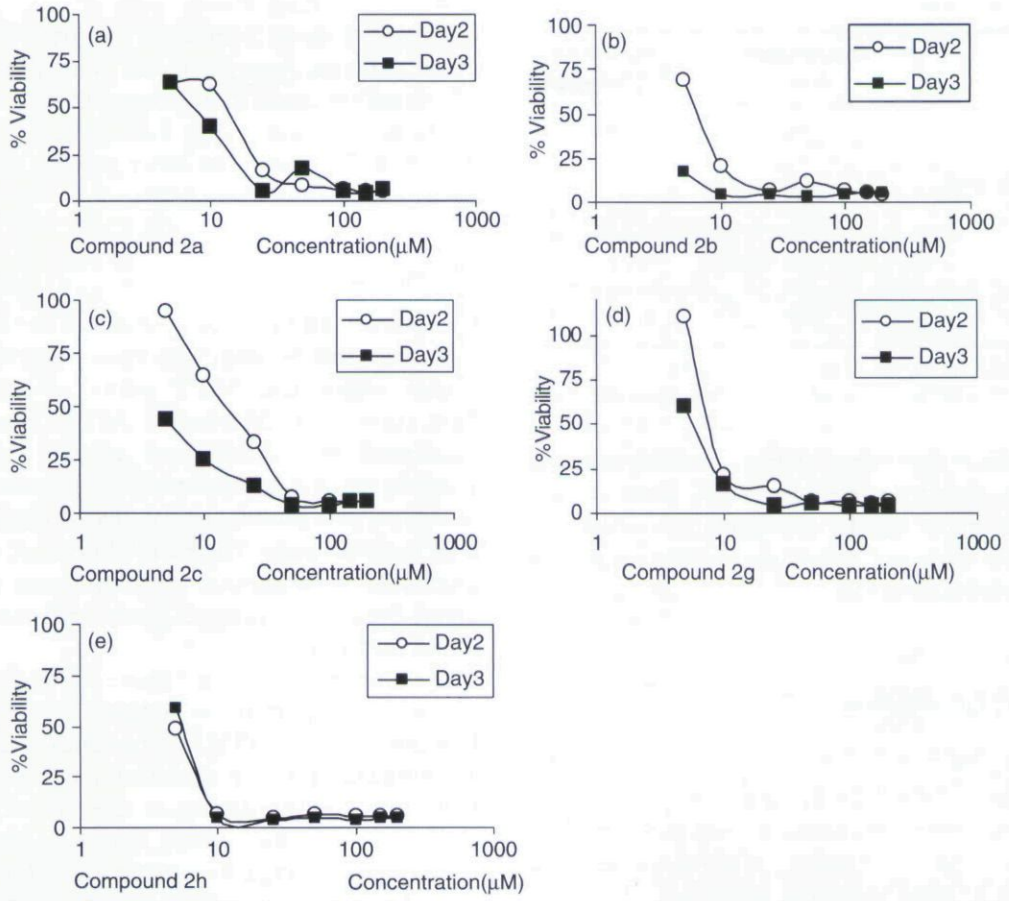


Figure 1. Cytotoxicity of the compounds determined by MTT assay for F2408 fibroblast cell line. Results are the mean of quadruplicate wells. (Standard deviation less than 10%).

tetrazolium MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma, Deisenhofen, Germany) assay[16,17]. Briefly, cells were inoculated into 96-well microtiter plates in 200 μL of complete medium at density 1×10^3 cells/well. Following the addition of drugs (concentrations, 5–10–25–50–100–150–200 μM), the plates were incubated for 48 and 72 h, and 200 μL of MTT solution (5 mg/mL) was added to each well. The cells were returned for 2 h incubation. After removal of supernatant, 200 μL of dimethyl sulfoxide (DMSO) was added to each well. The optical density was determined by using a Bio-Tek (ELx808-IU) ELISA reader at a wavelength of 540 nm. The mean percentage of treated cells calculated relative to the controls as shown[17]:

$$\% \text{ Viable cells} = (A_t - A_b)/(A_c - A_b) \times 100$$

Where A_c is the absorbance of the mean value of control, A_t the absorbance of the mean value of treated cells, and A_b the absorbance of the mean value of blank. The results are shown in Figure 1 and the IC_{50} value of each compound is summarised in Table II.

Analgesic activity

Swiss albino mice of either sex were used for in the vivo tail-clip and tail immersion (52.5°C hot water) analgesic tests[18,19]. Mice were assigned to groups of five animals each. All compounds were dissolved in DMSO and were given to the animals intraperitoneally (i.p.) at 100 mg/kg doses. The control animals received 0.1 ml DMSO i.p. Morphine sulphate (10 mg/kg) and acetylsalicylic acid (100 mg/kg) was used as the reference analgesic agents. Test latencies (in seconds) were assessed 30 min. after the administration of compounds. To avoid irreversible damage in the tail structures of the mice, a maximum latency of 15 s was imposed, if no response was observed within that time. % Analgesia was calculated by the following formula:

Table II. IC_{50} values of the compounds

Compounds	IC_{50}
2a	14 ± 1.5 μM
2b	6.8 ± 2.1 μM
2c	16 ± 1.5 μM
2g	8 ± 0.6 μM
2h	5.5 ± 0.5 μM

Table III. Effects of the compounds on tail-clip response in mice

Compounds	Dose(mg/kg) (i.p.)	%Analgesia (Tail-clip)
Control	–	41.58 ± 6.47
Morphine	10	95.38 ± 0.62*
Acetylsalicylic acid	100	95.15 ± 0.66*
2b	100	3.16 ± 7.03
2c	100	3.02 ± 8.84
2f	100	43.1 ± 23.6
2g	100	90.86 ± 9.14*
2h	100	28.15 ± 9.27

%Analgesia values are expressed as mean ± S.E.M., $n = 5$.
*$p < 0.001$ significant as compared with control, student's t-test.

% Analgesia = {(postdrug latency)-(predrug latency)/(cutoff time)-(predrug latency)} × 100 Results were expressed as mean SEM., and Student's t-test was used to assess statistical significances. Test results are given in Tables III and IV.

Results and Conclusion

Chemistry

The syntheses of the title 1,5-diaryl-3-ethoxycarbonyl-2-methylpyrrole derivatives **2** were accomplished in accordance with the sequence of reactions depicted in Scheme 1. The starting materials, 1-aryl-3-ethoxycarbonylpent-1,4-diones **1** were prepared by reacting ethyl acetoacetate and ω-bromoacetophenones in the presence of metallic sodium in toluene. To obtain the final products **2a–h**, the 1-aryl-3-ethoxycarbonylpent-1,4-diones **1** were reacted with a suitable aniline derivative or sulfanilamide under Paal-Knorr pyrrole synthesis conditions. The structures of the obtained compounds were elucidated using spectral data. In the IR spectra, the characteristic sulfonamide N-H and S = O stretching bands were observed at 3290–3120 and 1340–1160 cm^{-1} respectively. Another common group is the ester and C = O stretching bands due to this group were obtained at about 1690 cm^{-1}. In the NMR spectra, ethyl protons, pyrrole-C_4-H and pyrrole-5-CH_3, which are common in all compounds, were observed at about δ 1.3(CH_3) and 4.2(CH_2), 6.7 (C_4-H) and 2.3 (Ar-CH_3) ppm. The other protons were obtained in the expected positions.

Table IV. Effects of the compounds on the tail-immersion response in mice

Compounds	Dose(mg/kg) (i.p.)	%Analgesia (Tail-immersion)
Control	–	16.35 ± 6.27
Morphine	10	50.7 ± 1.84*
Acetylsalicylic acid	100	21.9 ± 1.41
2a	100	10.05 ± 6.93
2b	100	13.79 ± 7.38
2c	100	45.2 ± 16.0*
2e	100	8.03 ± 16.1
2f	100	47.0 ± 13.4*
2g	100	26.01 ± 6.21
2h	100	−14.7 ± 10.4

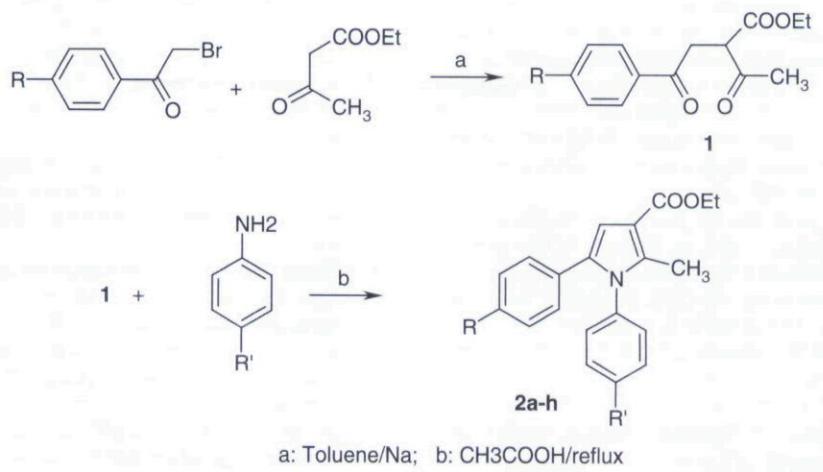
%Analgesia values are expressed as mean ± S.E.M., $n = 5$.
*$p < 0.001$ significant as compared with control, student's t-test

Pharmacology

Cytotoxicity. The cytotoxic effects of the 1,5-diaryl-3-ethoxycarbonyl-2-methylpyrrole derivatives were tested using the MTT assay as described in Materials and Methods. MTT is commonly employed as an indicator of cell number and viability, since it is converted to a coloured formazan derivative via mitochondrial dehydrogenase activity only by viable cells. The F2408 fibroblast cell line was incubated with various concentrations of the 1,5-diaryl-3-ethoxycarbonyl-2-methylpyrrole derivatives for 48 and 72 h.

The results are shown in Figure 1 and the IC_{50} value of each compound is summarised in Table II. However although all the compounds were evaluated, an adequate amount of data for compounds **2d–f**, sufficient for preparing a graph showing their cytotoxic activity, could not be obtained. All compounds, except for compound **2h**, showed a time-dependent increase in cytotoxic activity. F2408 cells were exposed to 5 μM of compound **2c** and **2g** (derivatives bearing either methoxy-or chloro groups on the 1,5-diaryl-3-ethoxycarbonyl-2-methylpyrrole nucleus, respectively) for 48 h and these two compounds showed no cytotoxic effects at all (Figure 1c, d). However, increasing the concentration of **2c** and **2g** to 10 μM resulted in 30–35% and 75% cell death, respectively. The IC_{50} values were 16 ± 1.5 μM and 8 ± 0.6 μM respectively for compounds **2c** and **2g**. Compound **2a**, unsubstituted on the aryl residue and compound **2b**, bearing a methyl group on 1,5-diaryl-3-ethoxycarbonyl-2-methylpyrrole nucleus, demonstrated weak cytotoxicity at 5μM concentration (Figure 1a, b). However, both compounds showed significant cytotoxic activity at high concentrations (IC_{50} value 14 ± 1.5 μM and 6.8 ± 2.1 μM, respectively). Compound **2h**, bearing a nitro group on the arylpyrrole nucleus, demonstrated high cytotoxic activity against the F2408 normal cell line (almost 100% cell death after either 48 h or 72 h incubation time at 10 μM) with an IC_{50} value of 5.5 ± 0.5 μM (Figure 1e).

Analgesic Activity. Central analgesic activities of the compounds were tested by using the "tail-clip" and "tail-immersion" methods. The analgesic activity in each group is shown in Tables III and IV. Morphine sulphate and acetylsalicylic acid were used as positive



Scheme 1. Synthesis compounds **1** and **2**; a: Toluene/Na; b: CH_3COOH/reflux

control analgesic compounds. The results are compared with a control group.

The results, show that compound **2 g** exhibited analgesic activity in the tail clip test equivalent to that of morphine sulphate and acetylsalicylic acid. Compounds **2b** and **2c** did not show any significant analgesic activity in this test. When compound **2f** was compared with the control, it did not exhibit significant analgesic activity. Mice injected with compounds **2a, 2d** and **2e** exhibited sedation and relaxation in skeletal muscles so masking the pain perceived (data not shown). Therefore, the analgesic activity of these compounds in the tail-clip test could not be evaluated.

In the tail immersion test, compounds **2a, 2b, 2e** and **2 h** did not show any significant analgesic activity but compounds **2c** and **2f** resulted gave a significant level of activity when compared with the control group and acetylsalicylic acid. Furthermore, these compounds were two fold more potent as analgesics than acetylsalicylic acid in the test at the same dose level.

In summary, compounds **2c** and **2f** have been found active in the tail immersion test when compared with the control group and acetylsalicylic acid. Compound **2 g** was found active in the tail-clip test when compared with the control group and both standard compounds. These results might lead to the conclusion that these three compounds are central acting analgesic agents. However it was observed that for these compounds **2c**, **2f** and **2 g**, both compounds **2c** and **2 g** also exhibited cytotoxic activity.

Acknowledgements

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