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Full Length Research Paper

Regioselective synthesis of dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones and evaluation of their anti-inflammatory activities

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Regioselective synthesis of some new dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4a-j) has been generated by 1,3-dipolar cycloaddition reaction of 2-arylidene-indan-1,3-diones (1a-j) as dipolarophiles with non-stabilized azomethine ylides, generated in situ via decarboxylative condensation of isatin (2) and sarcosine (3). Anti-inflammatory activity of the prepared compounds (4a-j) was determined *in vivo* by the acute carrageenan-induced paw edema in rats. Many of the prepared compounds exhibit considerable anti-inflammatory properties "at a dose of 10 mg/kg body weight", especially (4b) and (4c) which reveal promising activities relative to indomethacin which was used as a reference standard in this study.

Keywords Azomethine ylide; Indane-2,3-dione; Isatin; Dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones; Anti-inflammatory

INTRODUCTION

Multi-component 1,3-dipolar cycloaddition reactions are considered to be one of the most useful processes for construction of five membered heterocyclic ring systems (Tsuge et al., 1989; Padua, 1984; Grigg et al., 1993; Suresh Babu and Raghunathan, 2007). Due to high regioselectivity of these reactions many important five membered heterocycles could be obtained, one of these important ring systems are spiropyrrolidinyl-oxoindoles which represent the main alkaloid skeleton of naturally occurring substances such as *elacomine* (James and Williams, 1972) that was isolated from Eleagnus commutate and *horsfiline* (Jossang et al., 1991; Jones and Wilkinson, 1992; Bascop et al., 1994; Pellegrini et al., 1994; Palmisano et al., 1996; Cannell, 2003) was isolated from Horsefieldia superba, a small Malaysian tree, extracts of which have found use in indigenous medicine (Figure 1). Several oxoindole derivatives are well known as anti-tumor and anti-inflammatory (Lane et al., 2001; Jianguo et al., 2003; Marzola et al., 2004; Abadi et al., 2006; Girgis, 2009). In present work, it is intended to utilize an isatin scaffold for formation of non-stabilized azomethine ylides following the previously described and successful methods (Grigg et al., 1984; Ardil et al., 1986; Suresh Badu et al., 2006) through decarboxylative condensation with α-amino acids and trapping the generated reactive intermediate via 1,3-dipolar cycloaddition reaction with the exocyclic olefinic linkage derived from indan-1,3-dione. The anti-inflammatory properties of the prepared compounds will be screened. This work is considered a continuation of our research activity directed towards construction of bio-active spiroheterocyclic compounds (El-Zohry et al., 2008a-c; El-Zohry et al., 2009; Hussein and Abdel-Monem, 2011).

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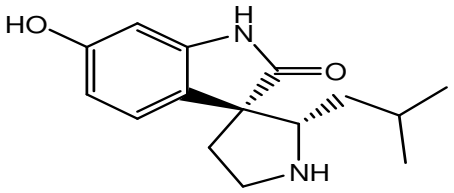
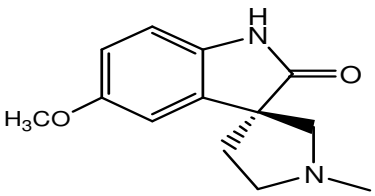
EXPERIMENTAL SECTION

Chemistry

The time required for completion of each reaction was monitored by TLC. All melting points are uncorrected and were measured on a Gallenkamp apparatus. The IR spectra were recorded on a Shimadzu 470 IR spectrometer (KBr) ν_{max}/cm^{-1}. The ^{1}H, ^{13}C-NMR spectra were measured on Varian EM-200 (^{1}H: 200 MHz, ^{13}C: 50 MHz) spectrometer with TMS as internal standard and *DMSO-d_6* as solvent. Mass spectra were determined on a JEOL JMS-600 spectrometer (EI, eV). Elemental analyses (C, H, N, and S) were performed on



(+)-elacomine



(-)-horsfiline

Figure 1. Representative naturally occurring spiropyrrolidinyl-oxoindole alkaloids

an elemental analysis system GmbH VarioEL V2.3; the results were found to be in good agreement with the calculated values. Compounds 2 and 3 are commercially available.

Synthesis of dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4a-j)

General Procedure

A mixture of (1a-j) (5 mmol), isatin (2) (0.735 g, 5 mmol) and sarcosine (3) (0.445 g, 5 mmol) in dry MeOH (20 mL) was heated under reflux for the appropriate time. Upon completion, monitored by TLC, the reaction mixture was allowed to cool to room temperature. The solid product was filtered off, dried, and crystallized from a suitable solvent.

4'-Phenyl-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4a)

Reaction time 5 h, recrystallized from EtOH as pale yellow crystals, mp 273-275 °C, yield 1.81 g (89%). IR: ν_{max}/cm^{-1} 3195 (NH), 1720 (C=O), 1705 (C=O), 1615, 1465 (C=C); ^{1}H-NMR: δ (ppm) 2.22 (s, 3H, CH_3), 3.65 (t, 1H, upfield H of *CH_2*-CH, *J* = 12.0 Hz), 4.11 (t, 1H, downfield H of *CH_2*-CH, *J* = 12.0 Hz), 5.12 (t, 1H, *CH*-CH_2, *J* = 10.0 Hz), 6.63-8.04 (m, 13H, arom. H), 10.49 (s, 1H, NH, D_2O- exchangeable); Anal. Calcd for $C_{26}H_{20}N_2O_3$ (408.45): C, 76.45; H, 4.94; N, 6.86. Found: C, 76.13; H, 4.86; N, 6.69.

4'-(4-Chlorophenyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4b)

Reaction time 4 h, recrystallized from dioxane as pale yellow crystals, mp 256-258 °C, yield 2.01 g (91%). IR: ν_{max}/cm^{-1} 3305 (NH), 1720 (C=O), 1710 (C=O), 1615, 1460 (C=C); ^{1}H-NMR: δ (ppm) 2.20 (s, 3H, CH_3), 3.66 (t, 1H, upfield H of *CH_2*-CH, *J* = 12.0 Hz), 4.07 (t, 1H, downfield H of *CH_2*-CH, *J* = 12.0 Hz), 5.09 (t, 1H, *CH*-CH_2, *J* = 10.0 Hz), 6.63-7.77 (m, 12H, arom. H), 10.49 (s, 1H, NH, D_2O- exchangeable); ^{13}C-NMR: δ (ppm) 34.6 (CH_3), 44.7 (*HC*-4'), 55.6 (*H_2C*-5'), 69.4 [spiro *C*-2' (*C*-3'')], 77.4 [spiro *C*-2 (*C*-3')], 109.8, 121.3, 122.7, 123.3, 125.1, 127.8, 128.3, 130.0, 130.3, 131.8, 132.7, 135.4 (arom. CH), 135.9, 136.3, 136.8, 140.8, 142.1, 142.6 (arom. C), 176.2 (*C*-2''), 196.5, 197.3 (*C*-1, *C*-3); MS: *m/z* (rel. int. %) 442.33 (M^+, 28), 397.26 (21), 369.31 (27), 276.18 (95), 268.13 (28), 267.14 (24), 174.16 (100); Anal. Calcd for $C_{26}H_{19}ClN_2O_3$ (442.89): C, 70.51; H, 4.32; Cl, 8.00; N, 6.33. Found: C, 70.34; H, 4.29; Cl, 7.87; N, 6.17.

4'-(4-Bromophenyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4c)

Reaction time 6 h, recrystallized from dioxane as pale yellow crystals, mp 261-263 °C, yield 2.19 g (90%). IR: ν_{max}/cm^{-1} 3305 (NH), 1715 (C=O), 1700 (C=O), 1618, 1460 (C=C); ^{1}H-NMR: δ (ppm) 2.18 (s, 3H, CH_3), 3.60 (t, 1H, upfield H of *CH_2*-CH, *J* = 12.0 Hz), 4.02 (t, 1H, downfield H of *CH_2*-CH, *J* = 12.0 Hz), 5.08 (t, 1H, *CH*-CH_2, *J* = 10.0 Hz), 6.63-7.81 (m, 12H, arom. H), 10.48 (s, 1H, NH, D_2O-exchangeable); Anal. Calcd for $C_{26}H_{19}BrN_2O_3$ (487.34): C, 64.08; H, 3.93; Br, 16.40; N, 5.75. Found: C, 63.98; H, 3.79; Br, 16.33; N, 5.55.

4'-(4-Hydroxyphenyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4d)

Reaction time 4 h, recrystallized from dioxane as pale yellow crystals, mp 277-278 °C, yield 1.72 g (81%). IR: ν_{max}/cm^{-1} 3500 (OH), 3400 (NH), 1715 (C=O), 1705 (C=O), 1610, 1460 (C=C); ^{1}H-NMR: δ (ppm) 2.25 (s, 3H, CH_3), 3.45 (t, 1H, upfield H of *CH_2*-CH, *J* = 8.0 Hz), 4.00 (t, 1H, downfield H of *CH_2*-CH, *J* = 8.0 Hz), 4.93 (t, 1H, *CH*-CH_2, *J* = 6.0 Hz), 6.61-8.07 (m, 12H, arom. H), 9.53 (s, 1H, OH, D_2O-exchangeable), 10.52 (s, 1H, NH, D_2O-exchangeable); Anal. Calcd for $C_{26}H_{20}N_2O_4$ (424.45): C, 73.57; H, 4.75; N, 6.60. Found: C, 73.49; H, 4.55; N, 6.51.

4'-(4-Methoxyphenyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4e)

Reaction time 4 h, recrystallized from EtOH as yellow crystals, mp 202-203 °C, yield 1.93 g (88%). IR:

v_{max}/cm^{-1} 3350 (NH), 1730 (C=O), 1705 (C=O), 1615, 1420 (C=C). ^{1}H-NMR: δ (ppm) 2.14 (s, 3H, *CH$_3$*-N), 3.20 (s, 3H, *CH$_3$*-O), 3.65 (t, 1H, upfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 4.06 (t, 1H, downfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 5.07 (t, 1H, *CH*-CH$_2$, *J* = 8.0 Hz), 6.67-8.01 (m, 12H, arom. H), 10.43 (s, 1H, NH, D$_2$O-exchangeable); ^{13}C-NMR: δ (ppm) 35.8 (CH$_3$), 44.8 (*HC*-4'), 54.6 (*H$_2$C*-5'), 57.2 (CH$_3$), 69.4 [spiro *C*-2' (*C*-3'')], 77.4 [spiro *C*-2 (*C*-3')], 109.8, 121.3, 122.7, 123.3, 125.3, 127.5, 128.3, 130.0, 130.3, 131.8, 132.7, 135.8 (arom. CH), 135.9, 136.3, 136.8, 140.8, 142.1, 142.6 (arom. C), 176.5 (*C*-2''), 196.7, 197.5 (*C*-1, *C*-3); MS: *m/z* (rel. int. %) 437.97 (M$^+$, 1), 263.71 (100), 248.71 (14), 232.73 (24), 174.80 (4), 164.79 (26); Anal. Calcd for $C_{27}H_{22}N_2O_4$ (438.47): C, 73.96; H, 5.06; N, 6.39. Found: 73.84; H, 4.95; N, 6.30.

4'-(4-Dimethylaminophenyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4f)

Reaction time 5 h, recrystallized from dioxane as pale brown crystals, m.p 230-232 °C, yield 2.00 g (89%). IR: v_{max}/cm^{-1} 3195 (NH), 1730 (C=O), 1705 (C=O), 1610, 1460 (C=C); ^{1}H-NMR: δ (ppm) 2.21 (s, 3H, pyrrolidinyl *CH$_3$*-N), 2.79 (s, 6H, (*CH$_3$*)$_2$N), 3.57 (t, 1H, upfield H of *CH$_2$*-CH, *J* = 8.0 Hz), 4.02 (t, 1H, downfield H of *CH$_2$*-CH, *J* = 8.0 Hz), 5.01 (t, 1H, *CH*-CH$_2$, *J* = 10.0 Hz), 6.49-8.63 (m, 12H, arom. H), 10.39 (s, 1H, NH, D$_2$O-exchangeable). ^{13}C-NMR: δ (ppm) 34.66 (pyrrolidinyl *N-CH$_3$*), 40.8 [(*CH$_3$*)$_2$*N*], 45.2 (*HC*-4'), 56.2 (*H$_2$C*-5'), 69.6 [spiro *C*-2' (*C*-3'')], 77.3 [spiro *C*-2 (*C*-3')], 109.7, 111.9, 121.2, 122.5, 123.7, 125.1, 129.1, 129.8, 130.9, 134.9, 136.0, 136.5 (arom. CH), 137.8, 141.0, 142.2, 142.6, 146.5, 149.2 (arom. C), 175.6 (*C*-2''), 197.0, 197.9 (*C*-1, *C*-3); MS: *m/z* (rel. int. %) 451.34 (M$^+$, 2), 276.58 (100), 173.72 (15); Anal. Calcd for $C_{28}H_{25}N_3O_3$ (451.52): C, 74.48; H, 5.58; N, 9.31. Found: C, 74.28; H, 5.33; N, 9.25.

4'-(3,4-Dihydroxyphenyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4g)

Reaction time 6 h, recrystallized from MeOH as pale yellow crystals, mp 289-291 °C, yield 1.65 g (75%). IR: v_{max}/cm^{-1} 3350 (OH), 3200 (NH), 1715 (C=O), 1700 (C=O), 1610, 1420 (C=C). ^{1}H-NMR: δ (ppm) 2.26 (s, 3H, CH$_3$), 3.33 (t, 1H, upfield H of *CH$_2$*-CH, *J* = 6.0 Hz), 3.97 (t, 1H, downfield H of *CH$_2$*-CH, *J* = 6.0 Hz), 4.95 (t, 1H, *CH*-CH$_2$, *J* = 8.0 Hz), 6.33-8.01 (m, 11H, arom. H), 9.83 (s, 2H, 2OH, D$_2$O-exchangeable), 10.67 (s, 1H, NH, D$_2$O-exchangeable); Anal. Calcd for $C_{26}H_{20}N_2O_5$ (440.45): C, 70.90; H, 4.58; N, 6.36. Found: C, 70.73; H, 4.39; N, 6.15.

4'-(3-Methoxy-4-hydroxyphenyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4h)

Reaction time 7 h, recrystallized from EtOH as pale yellow crystals, mp 281-282 °C, yield 1.49 g (66%). IR: v_{max}/cm^{-1} 3500 (OH), 3300 (NH), 1715 (C=O), 1710 (C=O), 1610, 1460 (C=C). ^{1}H-NMR: δ (ppm) 2.27 (s, 3H, CH$_3$), 3,06 (s, 3H, CH$_3$-O), 4.09 (t, 1H, upfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 4.96 (t, 1H, downfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 5.05 (t, 1H, *CH*-CH$_2$, *J* = 8.0 Hz), 6.31-8.00 (m, 11H, arom. H), 9.86 (s, 1H, OH, D$_2$O-exchangeable), 10.72 (s, 1H, NH, D$_2$O-exchangeable); Anal. Calcd for $C_{27}H_{22}N_2O_5$ (454.47): C, 71.35; H, 4.88; N, 6.16. Found: C, 71.23; H, 4.68; N, 6.01.

4'-(3-Pyridyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4i)

Reaction time 6 h, recrystallized from EtOH as pale yellow crystals, mp 241-243 °C, yield 1.75 g (86%). IR: v_{max}/cm^{-1} 3295 (NH), 1725 (C=O), 1715 (C=O), 1615, 1460 (C=C). ^{1}H-NMR: δ (ppm) 2.19 (s, 3H, CH$_3$), 3.70 (t, 1H, upfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 4.11 (t, 1H, downfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 5.11 (t, 1H, *CH*-CH$_2$, *J* = 8.0 Hz), 6.64-8.33 (m, 12H, arom. H), 10.52 (s, 1H, NH, D$_2$O- exchangeable); ^{13}C-NMR: δ (ppm) 34.6 (CH$_3$), 44.7 (*HC*-4'), 55.6 (*H$_2$C*-5'), 69.4 [spiro *C*-2' (*C*-3'')], 77.4 [spiro *C*-2 (*C*-3')], 121.3, 122.7, 123.3, 125.1, 127.8, 128.3, 130.0, 130.3, 131.8, 132.7, 135.4 (arom. CH), 135.9, 136.3, 136.8, 140.8, 142.1, 142.6 (arom. C), 176.2 (*C*-2''), 196.5, 197.3 (*C*-1, *C*-3); MS: *m/z* (rel. int. %) 408.50 (M$^+$, 10), 334.55 (36), 276.60 (100), 173.77 (51); Anal. Calcd. for $C_{25}H_{19}N_3O_3$ (409.14): C, 73.34; H, 4.68; N, 10.26. Found: C, 73.14; H, 4.44; N, 10.15.

4'-(2-Thienyl)-1'-methyl-dispiro[indane-2,3'-pyrrolidine-2',3''-indoline]-1,2'',3-triones (4j)

Reaction time 5 h, recrystallized from dioxane as yellow crystals, mp 250-251 °C, yield 1.84 g (89%). IR: v_{max}/cm^{-1} 3300 (NH), 1730 (C=O), 1715 (C=O), 1610, 1460 (C=C). ^{1}H-NMR: δ (ppm) 2.19 (s, 3H, CH$_3$), 3.70 (t, 1H, upfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 4.03 (t, 1H, downfield H of *CH$_2$*-CH, *J* = 10.0 Hz), 5.30 (t, 1H, *CH*-CH$_2$, *J* = 8.0 Hz), 6.67-8.00 (m, 11H, arom. H), 10.51 (s, 1H, NH, D$_2$O-exchangeable); MS: *m/z* (%) 413.58 (M$^+$, 29), 340.72 (26), 334.65 (22), 257.69 (97), 238.68 (56), 239.65 (59), 173.80 (100); Anal. Calcd for $C_{24}H_{18}N_2O_3S$ (414.10): C, 69.55; H, 4.38; N, 6.76; S, 7.74. Found: C, 69.24; H, 4.19; N, 6.55; S, 7.70.

Table 1. Anti-inflammatory activity of tested compounds using acute carrageenan-induced paw edema in rats

Compound	Mean swelling volume ± S.E.M[a] (% inhibition of edema)					Potency[b]
	0.5 h	1 h	2 h	3 h	4 h	
Control	0.800 ± 0.040 (00.0)	0.800 ± 0.040 (00.0)	0.812 ± 0.048 (0.00)	0.800 ± 0.040 (00.0)	0.788 ± 0.025 (0.00)	-
Indomethacin	0.706 ± 0.150 (11.7)	0.635 ± 0.020 (20.6)	0.550 ± 0.010 (32.2)	0.447 ± 0.090 (44.1)	0.372 ± 0.040 (52.7)	1.00
4a	0.712 ± 0.048 (11.0)	0.662 ± 0.063 (17.2)	0.662 ± 0.025 (18.4)	0.700 ± 0.041 (12.5)	0.712 ± 0.0478 (9.6)	0.18
4b	0.687 ± 0.075 (14.1)	0.600 ± 0.041 (25.0)	0.575 ± 0.064 (29.2)	0.512 ± 0.025 (36.0)	0.487 ± 0.025 (38.2)	0.73
4c	0.687 ± 0.025 (14.1)	0.650 ± 0.040 (18.7)	0.637 ± 0.048 (21.5)	0.625 ± 0.029 (21.9)	0.562 ± 0.025 (28.7)	0.55
4d	0.725 ± 0.029 (9.4)	0.712 ± 0.025 (11.0)	0.687 ± 0.025 (15.4)	0.700 ± 0.041 (12.5)	0.712 ± 0.025 (9.6)	0.18
4e	0.712 ± 0.085 (11.0)	0.737 ± 0.063 (7.9)	0.712 ± 0.075 (12.3)	0.712 ± 0.048 (11.0)	0.712 ± 0.048 (9.6)	0.18
4f	0.712 ± 0.025 (11.0)	0.700 ± 0.041 (12.5)	0.687 ± 0.025 (15.4)	0.700 ± 0.057 (12.5)	0.750 ± 0.057 (4.8)	0.09
4g	0.687 ± 0.025 (14.1)	0.687 ± 0.025 (14.1)	0.687 ± 0.025 (15.4)	0.700 ± 0.082 (12.5)	0.712 ± 0.025 (9.6)	0.18
4h	0.725 ± 0.050 (9.4)	0.712 ± 0.025 (11.0)	0.700 ± 0.041 (13.8)	0.700 ± 0.071 (12.5)	0.700 ± 0.041 (11.2)	0.21
4i	0.712 ± 0.075 (11.0)	0.687 ± 0.048 (14.1)	0.712 ± 0.048 (12.3)	0.687 ± 0.063 (14.1)	0.700 ± 0.041 (11.2)	0.21
4j	0.687 ± 0.025 (14.1)	0.700 ± 0.091 (12.5)	0.700 ± 0.057 (13.8)	0.687 ± 0.025 (14.1)	0.712 ± 0.025 (9.6)	0.18

[a] S.E.M. = Standard error mean and all showed at least significant difference at $p < 0.05$ in comparison with control group,
[b] Potency was expressed as percentage edema inhibition of the tested compounds relative to percentage edema inhibition of indomethacin " reference standard" at 4 h effect.

Anti-inflammatory Activity Screening

The anti-inflammatory activity of the synthesized compounds (4a-j) was evaluated *in vivo* according to paw induced edema standard method (Winter et al., 1962) in comparison to indomethacin as reference drug. The test is based on the pedal inflammation in rat paws induced by sub-plantar injection of 0.2 ml carrageenan (0.2%) suspension (5% NaCMC) into the right hind of the rats (the tested compounds were dissolved in distilled water with sonication). Male adult albino rats (120-150 g) were divided into groups, each of four animals. The thickness of rat paw was measured by a Veriner caliper (SMIEC, China) before and after 1 h of carrageenan injection to detect the inflammation induced by carrageenan. Test compounds at doses of 10 mg/kg (body weight) were injected *i.p.* to ten groups of rats. Control group received the vehicle (5% NaCMC), while reference group received Indomethacin at 10 mg/kg (body weight). The difference between the thicknesses of the two paws was taken as a measure of edema. The measurement was carried out at 0.5, 1, 2, 3, and 4 h, after injection of the tested compounds, the reference drug, and the vehicle. The anti-inflammatory activity was expressed as percentage inhibition of edema volume in treated animals in comparison with the control group (Table 1).

$$\text{Percentage inhibition of edema} = \frac{Vc - Vt}{Vc} \times 100$$

Where V_c and V_t are the volumes of edema for the control and drug-treated animal groups, respectively.
Potency of the tested compounds was calculated relative to indomethacin "reference standard" treated group according to the following equation:

$$\text{Potency} = \frac{\text{Percentage edema inhibition of tested compound treated group}}{\text{Percentage edema inhibition of indomethacin treated group}}$$

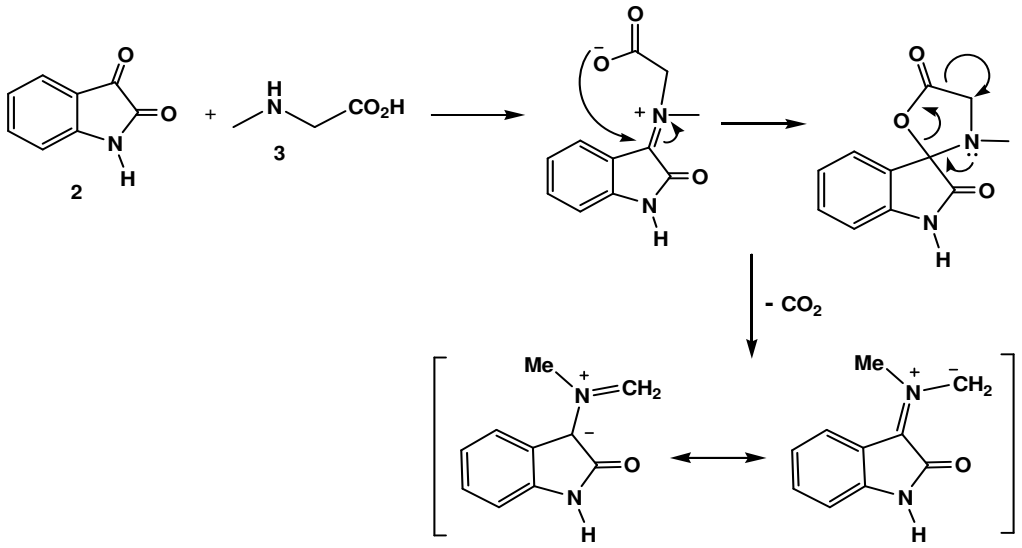


Figure 2. Mechanism for the generation of azomethine ylide

Determination of Acute Toxicity (LD_{50})

The median lethal dose (LD_{50}) of the most active compound (4b) and (4c) was determined in mice. A group of male adult albino mice of five animals (25-30 g) was injected at a certain grade. The percentage of mortality was determined 72 h after injection. Computation of LD_{50} was processed by a reported method (Sztaricskai et al., 1999).

RESULTS AND DISCUSSION

Chemistry

Reaction of 2-arylidene-indan-1,3-diones (1a-j) (Vogel, 1989; Prelicz and Arct, 1968) with non-stabilized azomethine ylide (Rajesh and Raghunathan, 2010), generated in situ via decarboxylative condensation of sarcosine (3) and isatin (2) in refluxing methanol afforded only one product as indicated by TLC in a highly regiooselective manner (Figure 2 and 3).

The structure of the isolated product was established to be dispiro[indane-2,3'-pyrrolidine-2',3"-indoline]-1,2",3-triones (4a-j) rather than the regioisomeric form dispiro[indane-2,4'-pyrrolidine-2',3"-indoline]-1,2",3-triones (5a-j) based on spectroscopic (IR, ^{1}H, ^{13}C-NMR, MS) and elemental analyses data.

^{1}H-NMR spectra of (4a-j) reveal the presence of three signal sets at δ = 3.33-4.09, 3.97-4.96 (assignable for the magnetically diasterotropic pyrrolidinyl methylene protons) and 4.93-5.30 (corresponding to the pyrrolidinyl methine proton *HC*-4'), excluding the formation of the other regioisomeric form 5. If the other isomers 5 were formed, one would expect a singlet instead of a triplet for the pyrrolidinyl methylene and methine protons.

^{13}C-NMR spectrum of (4b) exhibits the pyrrolidinyl spiro-carbons *C*-2 (*C*-3'), *C*-2' (*C*-3") at δ= 77.4, 69.4 respectively, besides the pyrrolidinyl methine carbon (*HC*-4') at δ= 44.7 the pyrrolidinyl methylene carbon (H_2C-5') appeared at δ= 55.6. In addition, the indanyl and indolyl carbonyl carbons appeared at δ= 197.3, 196.5, 176.2 respectively. Mass spectrum of (4b) showed the parent ion peak at *m/z* = 442.33 (28 %), base peak at *m/z* = 174.16.

Anti-inflammatory Activity

Anti-inflammatory activity of the synthesized compounds (4a-j) (at a dose of 10 mg/kg body weight) was determined *in vivo* by the acute carrageenan-induced paw edema standard method (Winter et al., 1962). The anti-inflammatory properties were recorded at successive time intervals 0.5, 1, 2, 3, and 4 h and compared with that of indomethacin (at a dose of 10 mg/kg body weight) that was used as a reference standard (Table 1). After 0.5 h, all the tested compounds seem highly active anti-inflammatory agents (9.4-14.1%) redacting the rat's edema relative to the observations due to used standard reference indomethacin (11.7%).

Structure-Activity relationships based on the obtained results indicated that the type of substituents attached to *C*-4' is controlling factor in developing the total pharmacological properties. The best observed anti-inflammatory property is that in which *C*-4' is attached to a phenyl group substituted with an electron-withdrawing function (chlorine and bromine) as exhibited in compounds (4b) and (4c). When *C*-4' is attached to unsubstituted phenyl ring the anti-inflammatory activity is moderate (4a). However, substituting the phenyl ring

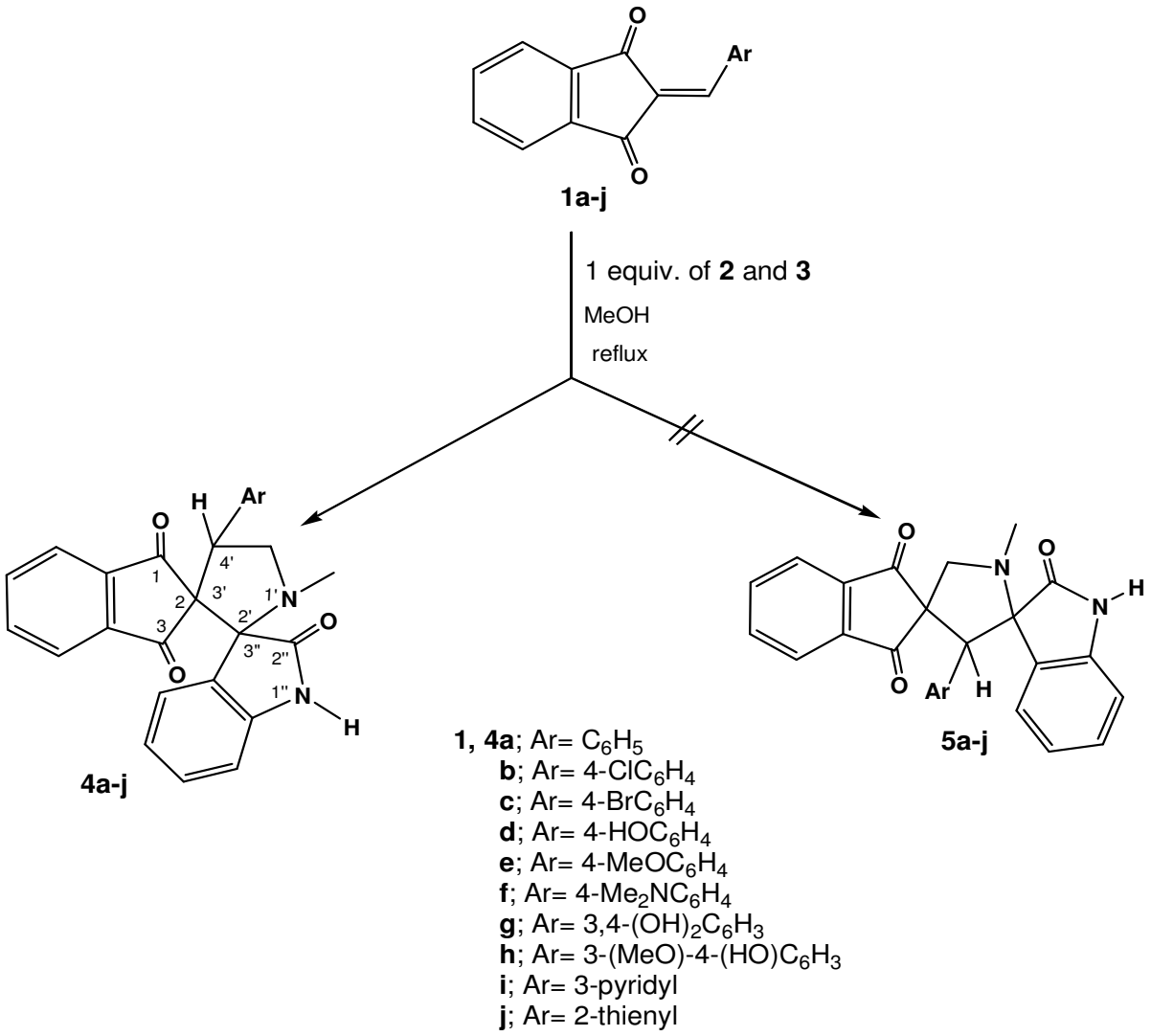


Figure 3. Synthesis of dispiro[indane-2,3'-pyrrolidine-2',3"-indoline]-1,2",3-triones (4a-j)

with an electron-donating function the anti-inflammatory behavior is decreased. It has also, been noticed that attachment of *C*-4' with either pyridine or thiophene moiety as exhibited in (4i) and (4j) decrease in the observed pharmacological properties was noticed compared with the case of using phenyl ring.

Acute Toxicity (LD_{50})

The median lethal dose (LD_{50}) of the most active compound (4b) and (4c) was determined in mice according to reported procedures (Sztaricskai et al., 1999). The animals got injection of a certain grade. The results showed that the (LD_{50}) of tested compound (4b) and (4c) was non-toxic at doses up to 200 mg/kg.

In conclusion, ten new compounds were prepared regioselectively in good yields via 1,3-dipolar cycloaddition of azomethine ylide generated in situ from isatin and sarcosine to 2-arylidene-1,3-indanediones, characterized with analytical data and screened for ant-inflammatory activity. The compounds (4b) and (4c) displayed good "*in vivo*" anti-inflammatory activity relative to indomethacin that was used as a reference standard.

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