Regioselective synthesis and anti-inflammatory activity of novel dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-triones

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Abstract

Novel dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-triones **5a-j** were obtained regioselectively by 1,3-dipolar cycloaddition reaction of 4-arylidene-1-phenylpyrazolidine-3,5-diones **2a-e** as dipolarophiles with non-stabilized azomethine ylides, generated in situ via decarboxylative condensation of isatins **3a,b** and sarcosine **4** in dry ethanol. The prepared compounds were screened for their anti-inflammatory activity "at a dose of 10 mg/kg body weight", especially **5d**, **5f**, **5h**, and **5j** which reveal remarkable activities relative to indomethacin which was used as a reference standard in this study.

Keywords: Azomethine ylides, 1,3-dipolar cycloaddition, spiroheterocycles, anti-inflammatory

Introduction

Cycloadditions are an important group of reactions in organic synthesis. [1] The azomethine ylide represents one of the most reactive and versatile classes of 1,3-dipoles and is trapped readily by a range of dipolarophiles, either inter or intramolecularly, forming substituted pyrrolidines.[2]
Spiropyrrolidinyl-oxoindole represents the main alkaloid skeleton of naturally occurring substances such as spirotryprostatine **A** and spirotryprostatine **B** which were found to be inhibitors of mammalian cell cycle at G2/M phase, from the secondary metabolites of *Aspergillus fumigatus* (Figure 1).[3-5] N-Arylpyrazoles are a very interesting class of heterocyclic compounds that have remarkable pharmacological activities in areas such as antibacterial-antifungal,[6] hypoglycemic,[7] tumor necrosis inhibition,[8] anti-thrombo-embolic disorders, as anti-angiogenic-,[9] and anti-inflammatory effects.[10, 11]

In the present work, it is intended to utilize a natural product isatin scaffold for formation of non-stabilized azomethine ylides following the previously described and successful methods,[12-15] 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 1-phenylpyrazolidine-3,5-dione. The anti-inflammatory properties of the prepared

compounds will be screened. This work is a continuation of our research directed towards the construction of bio-active spiroheterocycles.[16-19]

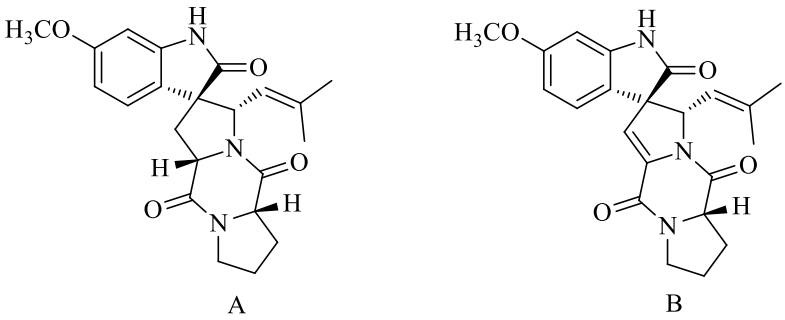
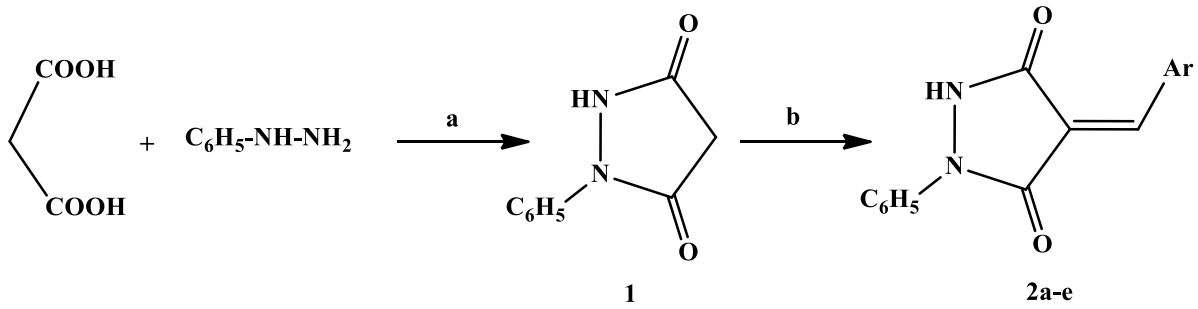


Figure 1. Representative naturally occurring spiropyrrolidinyl-oxoindole alkaloids.

Results and Discussion

Chemistry

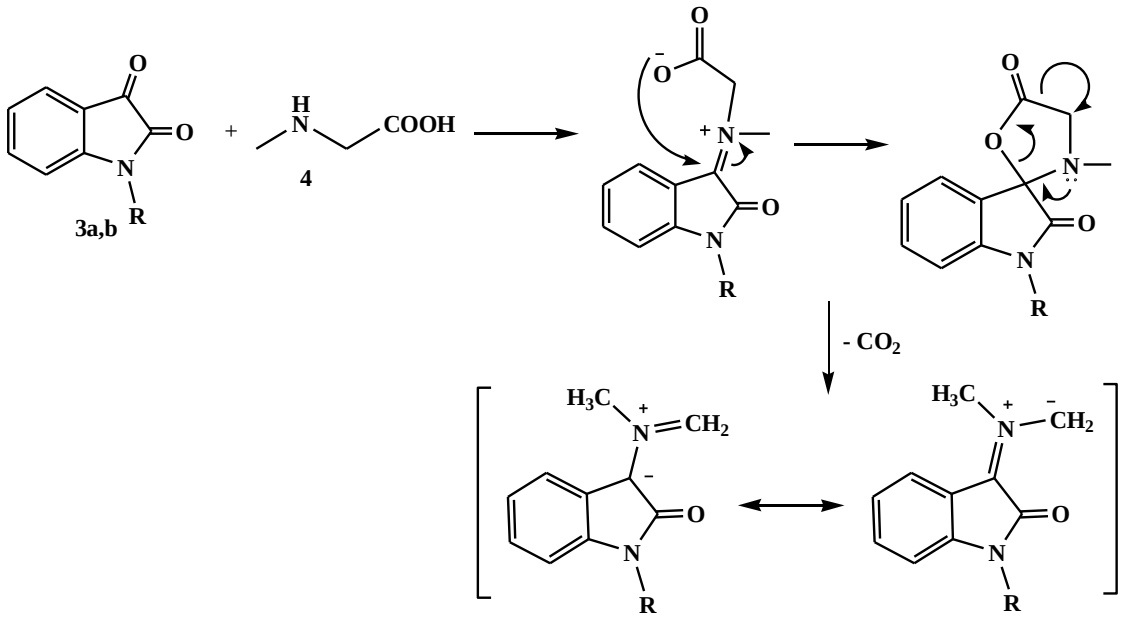
1-Phenylpyrazolidine-3,5-dione **(1)** was prepared from malonic acid and phenyl hydrazine, and the different substituted 4-arylidene-1-phenylpyrazolidine-3,5-dione derivatives **2a-e** were prepared in accordance with the literature procedure (Scheme 1).[20]



Scheme 1. Synthesis of 1-phenylpyrazolidine-3,5-dione and 4-Arylidene-1-phenylpyrazolidine-3,5-dione derivatives: (a) $POCl_3$, $CHCl_3$, reflux 2h., (b) aromatic aldehydes, glacial AcOH, reflux 30 min.

Reaction of the 4-arylidene-1-phenylpyrazolidine-3,5-diones **2a-e** with non-stabilized azomethine ylides, generated *in situ via* decarboxylative condensation of sarcosine **4** and isatins **3a,b** (Scheme 2)[12-15] in refluxing ethanol afforded only one product, as indicated by TLC, in a highly regio- and stereo- selective manner (Scheme 3). This cycloaddition is regioselective with the electron- rich carbon of the dipole adding to the β-carbon of the α,β-unsaturated moiety of **2a-e,** and is stereoselective, affording only one diastereomer exclusively, despite the presence of three stereo-centers in the product as found in many similar cycloaddition studies.[21,22]

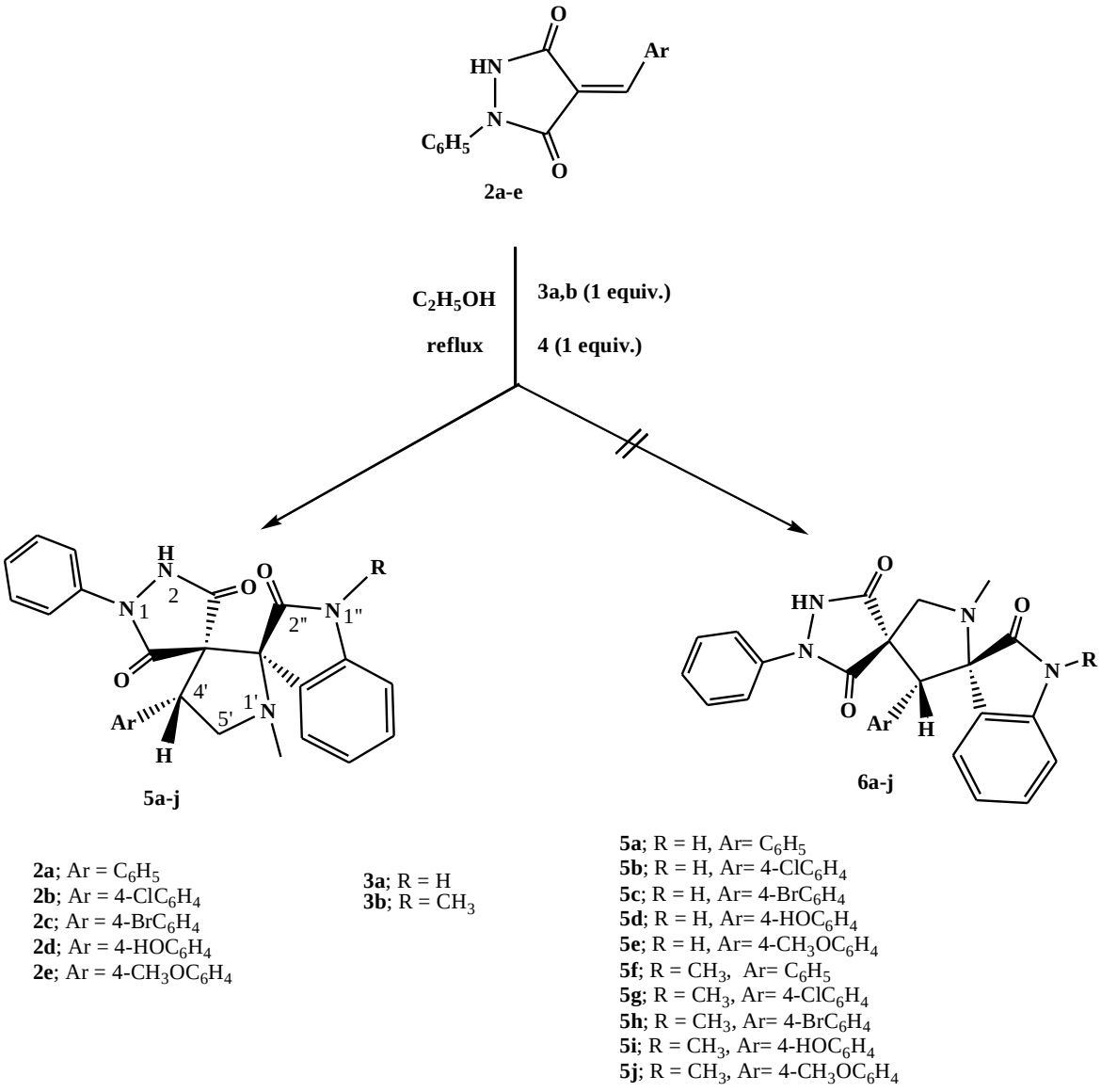
The regioselectivity of the product formation can be explained by considering secondary interaction of the orbitals of the carbonyl group of the dipolarophiles **2a-e** with those of the azomethine ylide, as shown in (Figure 2).[23] Accordingly, the formation of the observed regio-isomers **5a-j** *via* path **A** is more favorable than **6a-j** due to the secondary orbital interaction (SOI) which is not possible in path **B**. Hence, only one set of regio-isomers **5a-j** was formed.



Scheme 2. Mechanism for the generation of azomethine ylide.

The structures of the isolated products were established to be dispiro[pyrazolidine-4,3'-pyrrolidine-2',3''-indoline]-2'',3,5-triones, **5a-j**, rather than the regio-isomeric form dispiro[pyrazolidine-4,4'-pyrrolidine-2',3''-indoline]-2'',3,5-triones, **6a-j**, based on spectroscopic (IR, ^{1}H-, ^{13}C-NMR, MS) and elemental analysis data. ^{1}H-NMR spectra of **5a-j** reveal the presence of three triplet signal sets at δ = 3.54-3.63, 3.95-4.02 ppm (assignable for the magnetically non-equivalent pyrrolidinyl methylene protons *H*$_2$*C*-5', coupled with each other and in turn with the vicinal methine proton *HC*-4') and 4.86-5.01 ppm (corresponding to the pyrrolidinyl methine proton *HC*-4'), excluding the formation of the other regio-isomeric form **6**. If the other isomers **6a-j** were formed, one would expect a singlet instead of a triplet for the pyrrolidinyl methylene and methine protons. The ^{13}C-NMR spectrum of **5b**, as a representative example, exhibits the pyrrolidinyl spiro-carbons *C*-4 (*C*-3'), *C*-2' (*C*-3'') at δ = 64.99, 76.33 ppm beside the pyrrolidinyl methine carbon (*HC*-4') at δ = 45.25, the pyrrolidinyl methylene carbon (*H*$_2$*C*-5') appeared at δ = 55.06 ppm. In addition, the indolyl and pyrazolyl carbonyl carbon atoms appeared at δ = 174.67, 174.85 respectively. The mass spectrum of **5b**, as a representative example, showed the M^+ peak at *m/z* = 372.35 (20 %) and the base peak at *m/z* = 173.92.

Along with the experimental investigation, and for further confirmation of the structures of the synthesized compounds **5a-j**, we also carried out some theoretical studies on selected prepared compounds, with the help of molecular modeling software. For example, the calculated heat of formation (ΔH) of **5b** (Figure 3) showed that the most stable form (with the lowest heat of formation) is 2'*S*, 3'*R*, 4'*R* (ΔH= 68.622 kcal/mol).



Scheme 3. Synthesis of dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-triones.

Anti-inflammatory activity

The anti-inflammatory activity of the target compounds **5a-j** (at a dose of 10 mg/kg body weight) was determined *in vivo* by the acute carrageenan-induced paw oedema standard.[24] The anti-inflammatory properties were recorded at successive time intervals 0.5, 1, 2, 3, and 4h and

compared with that of indomethacin (at a dose of 10 mg/kg body weight) which was used as a reference standard. From the obtained results (Table 1) it was noticed after 1 h that many of the tested compounds exhibit considerable anti-inflammatory properties (especially **5d**, **5f**, **5h**, and **5j**) which reveal remarkable activities, with potency (percentage oedema inhibition of the tested compounds relative to percentage oedema inhibition of indomethacin) of 0.72, 0.64, 0.56, and 0.72 respectively.

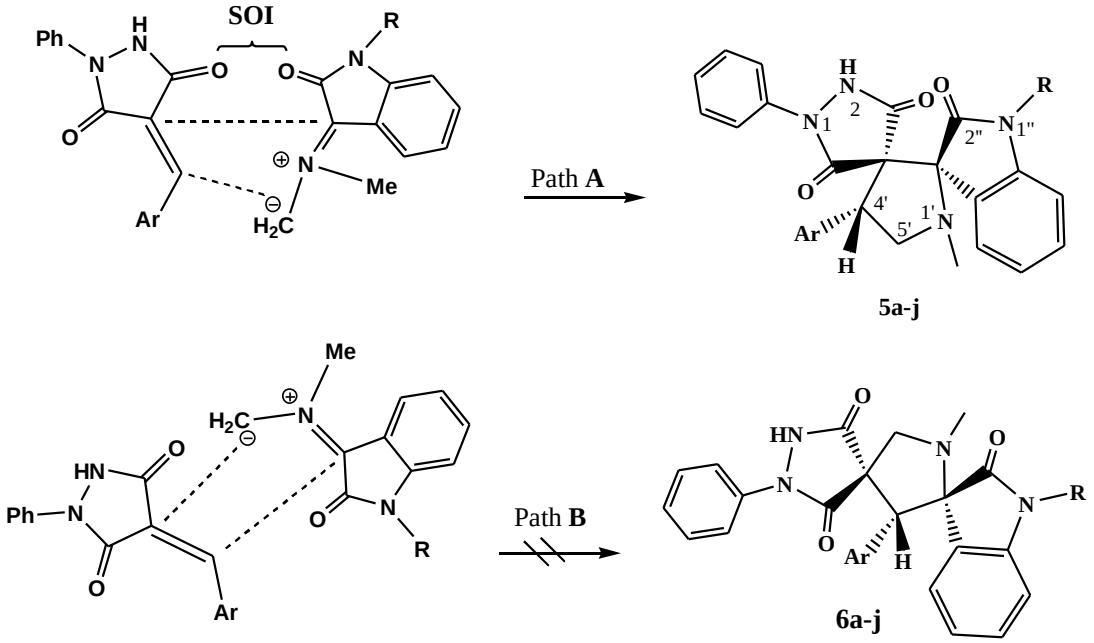
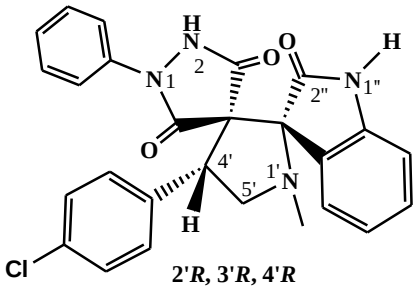


Figure 2. Mode of approach of azomethine ylide.

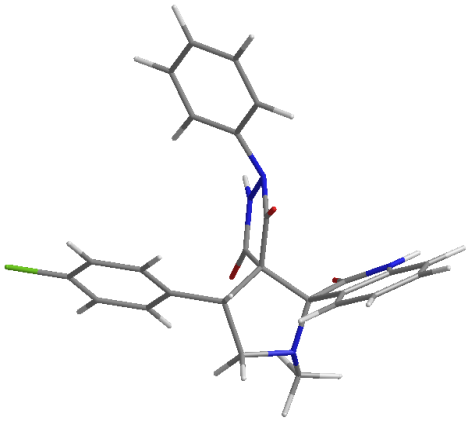
The structure-activity relationships based on the obtained results indicated that the type of substituents attached to *N*-1" and *C*-4' are controlling factors in developing the total pharmacological properties. The best observed anti-inflammatory property is that in which *N*-1" bears a methyl group and *C*-4' is attached to a phenyl group substituted with an electron-donating group (methoxy) in **5j** (potency 0.72). However, with an unsubstituted phenyl group, or substitution with electron-withdrawing groups (chlorine and bromine) the function was relatively decreased. It was also noticed that when *N*-1" is attached to a hydrogen atom, the anti-inflammatory activity is increased only when *C*-4' is attached to a phenyl group substituted with an electron-donating hydroxyl group in **5d** (potency 0.72).

Acute toxicity (*LD*$_{50}$)

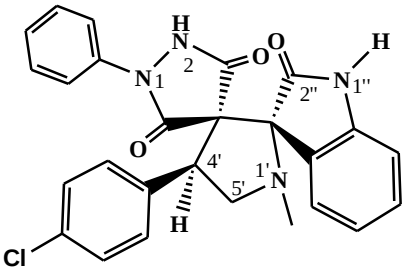
The median lethal dose (LD_{50}) of the most active compounds **5d**, **5f**, **5h**, and **5j** was determined in mice, according to reported procedures.[25] The results showed that the tested compounds **5d**, **5f**, **5h**, and **5j** were non-toxic at doses up to 200 mg / kg.



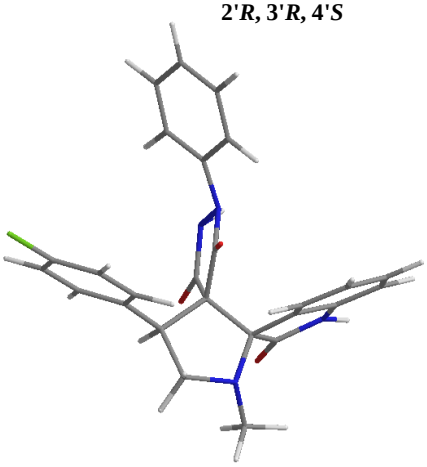
2'*R*, 3'*R*, 4'*R*



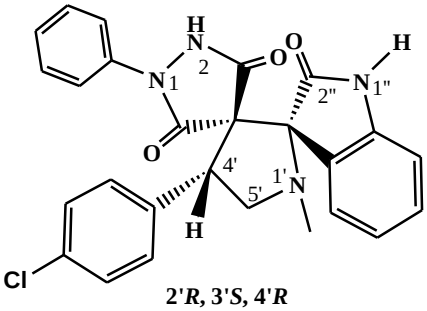
△ *H*= 72.242 kcal/mol



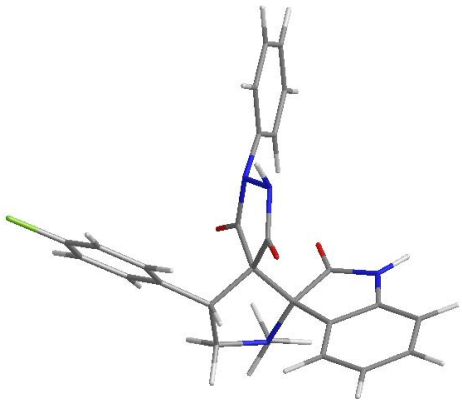
2'*R*, 3'*R*, 4'*S*



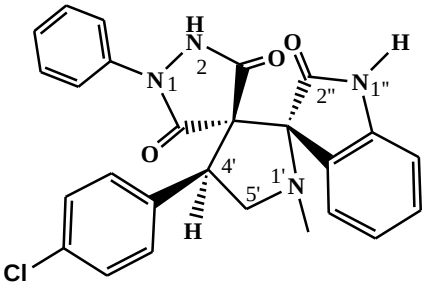
△ *H*= 74.077 kcal/mol



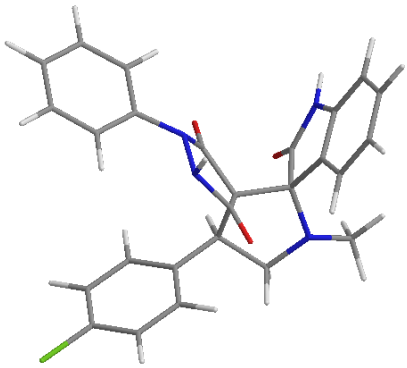
2'*R*, 3'*S*, 4'*R*



△ *H*= 71.663 kcal/mol

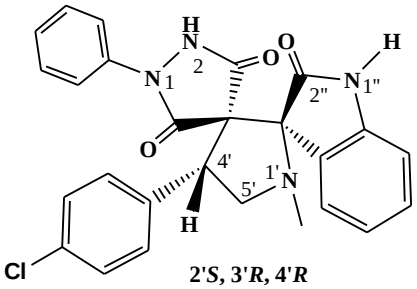


2'*R*, 3'*S*, 4'*S*

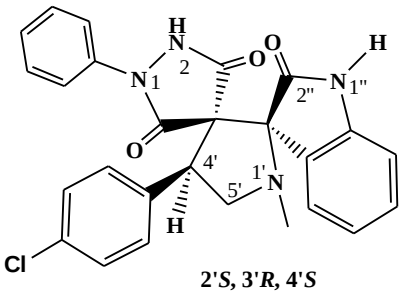


△ *H*= 68.727 kcal/mol

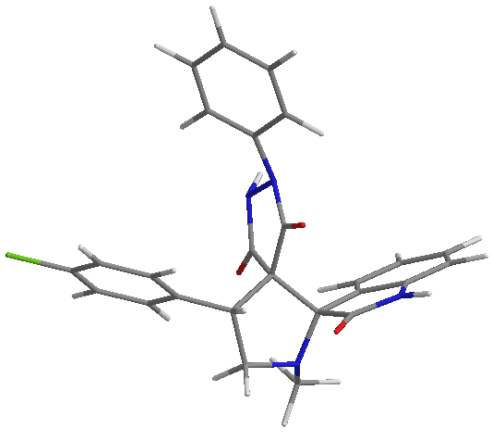
Figure 3. The molecular modeling of **5b**.



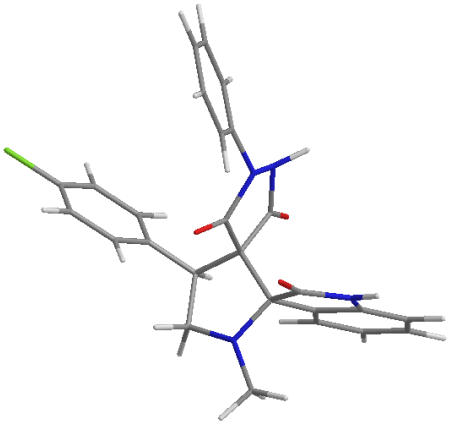
2'*S*, 3'*R*, 4'*R*



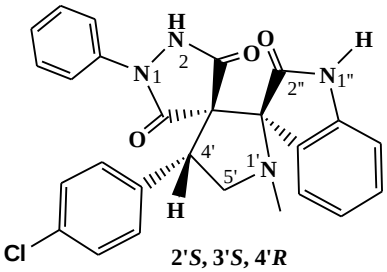
2'*S*, 3'*R*, 4'*S*



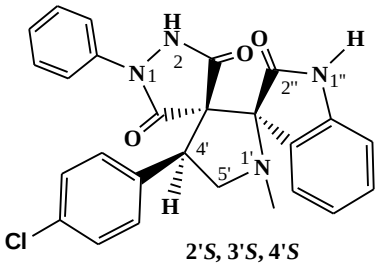
△*H*= 68.622 kcal/mol (most stable)



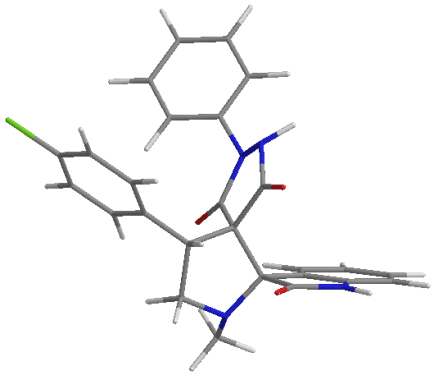
△*H*= 68.622 kcal/mol



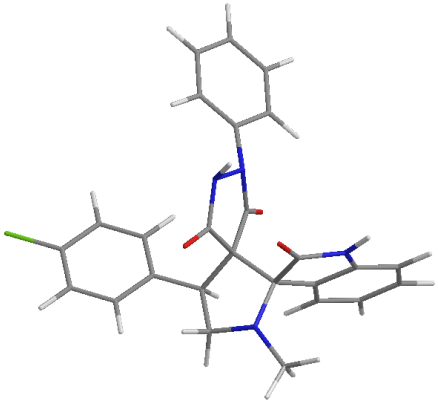
2'*S*, 3'*S*, 4'*R*



2'*S*, 3'*S*, 4'*S*



△*H*= 69.685 kcal/mol



△*H*=74.856 kcal/mol

Figure 3 (*continued*). The molecular modeling of **5b**.

Experimental Section

General. 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-, and ^{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. The molecular modeling software is *MOPAC* (ChemOffice ultra, version 9, 2005). Elemental analyses (C, H, N, and S) were performed on an elemental analysis system GmbH VarioEL $V_{2.3}$; the results were found to be in good agreement with the calculated values.

Synthesis of dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-triones (5a-j). General procedure

A mixture of the appropriate compounds **2a-e** (5 mmol), **3a,b** (5 mmol) and sarcosine **4** (0.445 g, 5 mmol) in dry ethanol (20 mL) was boiled 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 and crystallized from a suitable solvent.

1,4'-Diphenyl-1'-methyl-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5a). Reaction time 11 h. Recrystallized from dioxane; m.p. 281-282 °C, yield 1.99 g (91 %). IR (KBr): ν_{max}/cm^{-1} 3250 (NH), 3100 (NH), 1710 (C=O), 1705 (C=O), 1700 (C=O), 1618, 1460 (C=C); ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.20 (s, 3H, CH_3), 3.48 (t, 1H, upfield H of *CH_2-CH*, *J* = 18.0 Hz), 4.02 (t, 1H, downfield H of *CH_2-CH*, *J* = 18.0 Hz), 5.00 (t, 1H, *CH-CH_2*, *J* = 16.0 Hz), 6.71-7.74 (m, 14H, arom. H), 10.50 (s, 1H, NH), 10.55 (s, 1H, NH). Anal. Calcd. for $C_{26}H_{22}N_4O_3$ (438.17): C, 71.22; H, 5.06; N, 12.78. Found: C, 71.00; H, 4.91; N, 12.57%.

1-Phenyl-1'-methyl-4'-(4-chlorophenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"indoline] -2",3,5- trione (5b). Reaction time 8 h. Recrystallized from dioxane, m.p. 222-224 °C, yield 2.24 g (95 %). IR (KBr): ν_{max}/cm^{-1} 3250 (NH), 3095 (NH), 1725 (C=O), 1695 (C=O), 1616, 1465 (C=C); ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.14 (s, 3H, CH_3), 3.54 (t, 1H, upfield H of *CH_2-CH*, *J* = 14.0 Hz), 3.95 (t, 1H, downfield H of *CH_2-CH*, *J* = 14.0 Hz), 4.95 (t, 1H, *CH-CH_2*, *J* = 16.0 Hz), 6.63-7.77 (m, 13H, arom. H), 10.47 (s, 1H, NH), 10.53 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ (ppm) 34.63 (CH_3), 45.25 (*HC*-4'), 55.06 (*H_2C*-5'), 69.99 [spiro *C*-4 (*C*-3')], 76.39 [spiro *C*-2' (*C*-3")], 109.72, 118.91, 119.16, 121.63, 123.42, 125.59, 128.36, 128.80, 128.87, 129.46, 130.00, 130.18, 132.13 (arom. CH), 135.11, 135.23, 135.59, 143.13, 143.33 (arom. C), 174.67 (*C*-2"), 174.85 (*C*-5), 176.44 (*C*-3); MS: *m/z* (%) 472.35 (M^+, 20), 297.92 (53), 229.85 (82), 173.92 (100). Anal. Calcd. for $C_{26}H_{21}ClN_4O_3$ (472.92): C, 66.03; H, 4.48; Cl, 7.50; N, 11.85. Found: C, 65.84; H, 4.25; Cl, 7.27; N, 11.66%.

Table 1. *In-vivo* anti-inflammatory activity of tested compounds using acute carrageenan-induced paw oedema in rats

Compound	Mean swelling volume ± S.E.M[a] (% inhibition of oedema)					
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**	**Potency[b]**
Control	0.762± 0.047 (00.0)	0.762 ± 0.047 (00.0)	0.775 ± 0.028 (00.0)	0.762 ± 0.047 (00.0)	0.762 ± 0.047 (00.0)	-
Indo-methacin	0.672 ± 0.150 (11.8)	0.606 ± 0.020 (20.4)	0.529 ± 0.010 (32.2)	0.426 ± 0.090 (44.1)	0.360 ± 0.040 (52.7)	1.00
5a	0.762 ± 0.047 (0.0)	0.750 ± 0.057 (1.6)	0.725 ± 0.050 (6.4)	0.750 ± 0.057 (1.6)	0.750 ± 0.057 (1.6)	0.08
5b	0.750 ± 0.057 (1.6)	0.762 ± 0.047 (00.0)	0.775 ± 0.028 (00.0)	0.762 ± 0.047 (00.0)	0.725 ± 0.050 (4.8)	0.00
5c	0.762 ± 0.047 (00.0)	0.750 ± 0.057 (1.6)	0.712 ± 0.062 (8.1)	0.637 ± 0.047 (16.4)	0.612 ± 0.062 (19.7)	0.08
5d	0.733 ± 0.076 (3.8)	0.650 ± 0.070 (14.7)	0.650 ± 0.040 (16.1)	0.587 ± 0.025 (23.0)	0.625 ± 0.050 (18.0)	0.72
5e	0.762 ± 0.047 (0.00)	0.750 ± 0.057 (1.6)	0.750 ± 0.057 (3.2)	0.725 ± 0.050 (4.8)	0.725 ± 0.050 (4.8)	0.08
5f	0.737 ± 0.025 (3.28)	0.662 ± 0.047 (13.1)	0.625 ± 0.028 (19.3)	0.575 ± 0.028 (24.5)	0.537 ± 0.047 (29.5)	0.64
5g	0.750 ± 0.057 (1.6)	0.737 ± 0.047 (3.3)	0.737 ± 0.047 (4.9)	0.725 ± 0.028 (4.8)	0.737 ± 0.025 (3.3)	0.16
5h	0.762 ± 0.047 (00.0)	0.675 ± 0.064 (11.4)	0.625 ± 0.064 (19.3)	0.600 ± 0.040 (21.2)	0.537 ± 0.047 (29.5)	0.56
5i	0.762 ± 0.047 (00.0)	0.750 ± 0.040 (1.6)	0.750 ± 0.040 (3.2)	0.712 ± 0.047 (6.5)	0.700 ± 0.070 (8.1)	0.08

Table 1 (continued)

Compound	Mean swelling volume ± S.E.M[a] (% inhibition of oedema)					
	0.5 h	**1 h**	**2 h**	**3 h**	**4 h**	**Potency[b]**
5j	0.700 ± 0.040 (8.1)	0.650 ± 0.040 (14.7)	0.625 ± 0.028 (19.3)	0.587 ± 0.025 (23.0)	0.537 ± 0.047 (29.5)	0.72

[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 is expressed as percentage oedema inhibition of the tested compounds relative to percentage oedema inhibition of indomethacin "reference standard" at 1 h effect.

1-Phenyl-1'-methyl-4'-(4-bromophenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5c). Reaction time 9 h. Recrystallized from dioxane, m.p. 235-236 °C, yield 2.30 g (89 %). IR (KBr): ν_{max}/cm^{-1} 3220 (NH), 3095 (NH), 1725 (C=O), 1695 (C=O), 1615, 1465 (C=C); ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.10 (s, 3H, CH_3), 3.51 (t, 1H, upfield H of CH_2-CH, J = 18.0 Hz), 3.92 (t, 1H, downfield H of CH_2-CH, J = 18.0 Hz), 4.93 (t, 1H, CH-CH_2, J = 16.0 Hz), 6.62-7.68 (m, 13H, arom. H), 10.43 (s, 1H, NH), 10.50 (s, 1H, NH); ^{13}C-NMR (DMSO-d_6): δ (ppm) 33.27 (CH_3), 45.20 (*HC*-4'), 56.13 (H_2C-5'), 69.89 [spiro *C*-4 (*C*-3')], 76.84 [spiro *C*-2' (*C*-3")], 109.72, 118.91, 119.22, 121.63, 123.42, 125.59, 128.35, 128.80, 128.87, 129.46, 130.09, 130.18, 132.13 (arom. CH), 135.11, 135.20, 135.59, 143.13, 143.36 (arom. C), 174.60 (*C*-2"), 174.84 (*C*-5), 176.52 (*C*-3). *m/z* (%) 518.88 (M^+ + 2, 33), 516.85 (M^+, 45), 354.80 (27), 305.98 (33), 175.02 (100). Anal. Calcd. for $C_{26}H_{21}BrN_4O_3$ (517.37): C, 60.36; H, 4.09; Br, 15.44; N, 10.83. Found: C, 60.11; H, 3.85; Br, 15.21; N, 10.68%.

1-Phenyl-1'-methyl-4'-(4-hydroxyphenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5d). Reaction time 12 h. Recrystallized from ethanol; m.p. 243-244 °C, yield 1.77 g (78 %). IR (KBr): ν_{max}/cm^{-1} 3250 (br., OH), 3115 (NH), 1715 (C=O), 1700 (C=O), 1695 (C=O), 1610, 1461 (C=C); ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.20 (s, 3H, CH_3), 3.59 (t, 1H, upfield H of CH_2-CH, J = 16.0 Hz), 4.00 (t, 1H, downfield H of CH_2-CH, J = 16.0 Hz), 4.99 (t, 1H, CH-CH_2, J = 18.0 Hz), 6.74-7.37 (m, 13H, arom. H), 10.52 (s, 1H, NH), 10.58 (s, 1H, NH), 11.42 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6): δ (ppm) 34.63 (CH_3), 45.24 (*HC*-4'), 55.00 (H_2C-5'), 65.20 [spiro *C*-4 (*C*-3')], 76.38 [spiro *C*-2' (*C*-3")], 110.12, 118.89, 119.16, 121.61, 123.42, 125.59, 128.24, 128.77, 128.87, 129.46, 130.02, 130.18, 132.13 (arom. CH), 135.11, 135.33, 135.69, 143.13, 143.37 (arom. C), 174.67 (*C*-2"), 174.85 (*C*-5), 176.44 (*C*-3). Anal. Calcd. for $C_{26}H_{22}N_4O_4$ (454.48): C, 68.71; H, 4.88; N, 12.33. Found: C, 68.41; H, 4.65; N, 12.25%.

1-Phenyl-1'-methyl-4'-(4-methoxyphenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5e). Reaction time 10 h. Recrystallized from methanol, m.p. 230-231 °C, yield 1.89 g (81 %). IR (KBr): ν_{max}/cm^{-1} 3225 (NH), 3100 (NH), 1715 (C=O), 1700 (C=O), 1610, 1460 (C=C); ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.19 (s, 3H, CH_3), 2.97 (t, 1H, upfield H of

CH$_2$-*CH*, *J* = 14.0 Hz), 3.72 (s, 3H, *CH*$_3$-O), 3.97 (t, 1H, downfield H of *CH*$_2$-*CH*, *J* = 14.0 Hz), 4.94 (t, 1H, *CH*-*CH*$_2$, *J* = 12.0 Hz), 6.66-7.81 (m, 13H, arom. H), 10.48 (s, 1H, NH), 10.53 (s, 1H, NH). Anal. Calcd. for $C_{27}H_{24}N_4O_4$ (468.5): C, 69.22; H, 5.16; N, 11.96. Found: C, 69.05; H, 5.05; N, 11.85%.

1,4'-Diphenyl-1',1"-dimethyl-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5f). Reaction time 18 h. Recrystallized from ethanol, m.p. 181-182 °C, yield 1.74 g (77 %). IR (KBr): ν_{max}/cm^{-1} 3100 (NH), 1705 (C=O), 1700 (C=O), 1696 (C=O), 1605, 1460 (C=C); ^{1}H-NMR (DMSO-*d*$_6$): *δ* (ppm) 2.21 (s, 3H, CH$_3$), 2.89 (t, 1H, upfield H of *CH*$_2$-*CH*, *J* = 16.0 Hz), 3.04 (s, 3H, CH$_3$), 3.67 (t, 1H, downfield H of *CH*$_2$-*CH*, *J* = 16.0 Hz), 4.87 (t, 1H, *CH*-*CH*$_2$, *J* = 14.0 Hz), 6.70-7.72 (m, 14H, arom. H), 10.46 (s, 1H, NH). Anal. Calcd. for $C_{27}H_{24}N_4O_3$ (452.5): C, 71.67; H, 5.35; N, 12.38. Found: C, 71.51; H, 5.22; N, 12.19%.

1-Phenyl-1',1"-dimethyl-4'-(4-chlorophenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5g). Reaction time 16 h. Recrystallized from ethanol; mp 191-192 °C, yield 1.94 g (80 %). IR (KBr): ν_{max}/cm^{-1} 3200 (NH), 1715 (C=O), 1700 (C=O), 1690 (C=O), 1615, 1465 (C=C); ^{1}H-NMR (DMSO-*d*$_6$): δ (ppm) 2.16 (s, 3H, CH$_3$), 3.13 (s, 3H, CH$_3$), 3.65 (t, 1H, upfield H of *CH*$_2$-*CH*, *J* = 18.0 Hz), 4.02 (t, 1H, downfield H of *CH*$_2$-*CH*, *J* = 18.0 Hz), 4.98 (t, 1H, *CH*-*CH*$_2$, *J* = 16.0 Hz), 6.74-7.76 (m, 13H, arom. H), 10.51 (s, 1H, NH). Anal. Calcd. for $C_{27}H_{23}ClN_4O_3$ (486.95): C, 66.60; H, 4.76; Cl, 7.28; N, 11.51. Found: C, 66.49; H, 4.57; Cl, 7.10; N, 11.39%.

1-Phenyl-1',1"-dimethyl-4'-(4-bromophenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5h). Reaction time 20 h. Recrystallized from ethanol; m.p. 198-199 °C, yield 2.10 g (79 %). IR (KBr): ν_{max}/cm^{-1} 3100 (NH), 1710 (C=O), 1700 (C=O), 1690 (C=O), 1610, 1460 (C=C); ^{1}H-NMR (DMSO-*d*$_6$): *δ* (ppm) 2.19 (s, 3H, CH$_3$), 3.16 (s, 3H, CH$_3$), 3.62 (t, 1H, upfield H of *CH*$_2$-*CH*, *J* = 18.0 Hz), 4.00 (t, 1H, downfield H of *CH*$_2$-*CH*, *J* = 18.0 Hz), 4.96 (t, 1H, *CH*-*CH*$_2$, *J* = 16.0 Hz), 6.74-7.77 (m, 13H, arom. H), 10.52 (s, 1H, NH). Anal. Calcd. for $C_{27}H_{23}BrN_4O_3$ (531.4): C, 61.03; H, 4.36; Br, 15.04; N, 10.54. Found: C, 60.89; H, 4.44; Br, 14.90; N, 10.36%.

1-Phenyl-1',1"-dimethyl-4'-(4-hydroxyphenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5i). Reaction time 18 h. Recrystallized from ethanol, m.p. 155-156 °C, yield 1.64 g (70 %). IR (KBr): ν_{max}/cm^{-1} 3250 (br. OH), 1705 (C=O), 1700 (C=O), 1695 (C=O), 1605, 1460 (C=C); ^{1}H-NMR (DMSO-*d*$_6$): *δ* (ppm) 2.20 (s, 3H, CH$_3$), 2.92 (t, 1H, upfield H of *CH*$_2$-*CH*, *J* = 12.0 Hz), 3.14 (s, 3H, CH$_3$), 3.63 (t, 1H, downfield H of *CH*$_2$-*CH*, *J* = 12.0 Hz), 4.79 (t, 1H, *CH*-*CH*$_2$, *J* = 10.0 Hz), 6.68-7.71 (m, 13H, arom. H), 10.51 (s, 1H, NH), 10.82 (s, 1H, OH); ^{13}C-NMR (DMSO-*d*$_6$): *δ* (ppm) 33.24 (CH$_3$), 39.15 (CH$_3$), 45.56 (*HC*-4'), 55.14 (*H*$_2$*C*-5'), 69.91 [spiro *C*-4 (*C*-3')], 76.37 [spiro *C*-2' (*C*-3")], 109.72, 118.79, 119.16, 121.63, 123.42, 125.59, 128.36, 128.80, 128.82, 129.46, 130.00, 130.18, 132.13 (arom. CH), 135.11, 135.31, 135.59, 143.13, 143.33 (arom. C), 174.65 (*C*-2"), 174.77 (*C*-5), 176.43 (*C*-3). Anal. Calcd. for $C_{27}H_{24}N_4O_4$ (468.5): C, 69.22; H, 5.16; N, 11.96. Found: C, 69.00; H, 5.02; N, 11.78%.

1-Phenyl-1',1"-dimethyl-4'-(4-methoxyphenyl)-dispiro[pyrazolidine-4,3'-pyrrolidine-2',3"-indoline]-2",3,5-trione (5j). Reaction time 19 h. Recrystallized from ethanol, m.p. 139-141 °C, yield 1.81 g (75 %). IR (KBr): ν_{max}/cm^{-1} 3200 (NH), 1705 (C=O), 1700 (C=O), 1605, 1460

(C=C); ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.19 (s, 3H, CH_3), 2.92 (t, 1H, upfield H of CH_2-CH, J = 16.0 Hz), 3.14 (s, 3H, CH_3), 3.53 (s, 3H, CH_3-O), 3.69 (t, 1H, downfield H of CH_2-CH, J = 16.0 Hz), 4.91 (t, 1H, CH-CH_2, J = 18.0 Hz), 6.64-7.70 (m, 13H, arom. H), 10.43 (s, 1H, NH); MS: *m/z* (%) 482.03 (M^+, 0.5), 226.08 (100), 211.06 (37), 147.03 (48). Anal. Calcd. for $C_{28}H_{26}N_4O_4$ (482.53): C, 69.70; H, 5.43; N, 11.61. Found: C, 69.51; H, 5.22; N, 11.43%.

Anti-inflammatory activity screening

The anti-inflammatory activity screening for the prepared compounds **5a-j** was determined *in vivo* by the acute carrageenan-induced paw oedema standard method in comparison to indomethacin as reference drug.[24] 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 paw 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 the rat paw was measured by a Vernier 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 nine 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 oedema. The measurement was carried out at 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 oedema volume in treated animals in comparison with the control group (see Table 1).

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

where *Vc* and *Vt* are the volumes of oedema 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:

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

Determination of acute toxicity (LD_{50}). The median lethal dose (LD_{50}) of the most active compound **5d**, **5f**, **5h**, and **5j** was determined in mice. A group of male adult albino mice of five animals (25-30 g) was injected (i.p.) at a certain grade. The percentage of mortality was determined 72 h after injection. The computation of LD_{50} was processed by a graphical method.[25]

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