

## Pyrazolone Derivatives: Synthesis, Anti-inflammatory, Analgesic, Quantitative Structure–Activity Relationship and *in Vitro* Studies

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Received April 23, 2013; accepted June 2, 2013

Some 1-(4-chlorophenyl or benzenesulfonamide)-2,3- and/or 4-substituted-1*H*-pyrazol-5(4*H*)-one derivatives were synthesized and screened for their anti-inflammatory and analgesic activities, in addition to their ulcerogenic liability. They were found to be active as anti-inflammatory and analgesic agents. Compound **6b** was found to be the most active as anti-inflammatory agent and compound **9b** was found to be the most active one as anti-inflammatory and analgesic agent. On the other hand, cyclooxygenase-1/2 (COX-1)/COX-2 isozyme selectivity was also done and the tested compounds showed equal inhibition to both isoforms. Moreover, 2D-quantitative structure–activity relationship (QSAR) studies revealed well predictive and statistically significant and cross validated QSAR model that helps to explore some expectedly potent compounds.

**Key words** pyrazolone; acetic acid; quantitative structure–activity relationship; cyclooxygenase inhibition; anti-inflammatory

Non steroidal anti-inflammatory drugs (NSAIDs) were known as important class of therapeutic agents for the alleviation of pain and inflammation. The pharmacological activity of NSAIDs is due to the inhibition of prostaglandins biosynthesis from arachidonic acid through inhibiting cyclooxygenases (COXs).<sup>1,2</sup> However, long term use of NSAIDs was associated with several side effects such as gastrointestinal mucosal damage, bleeding, intolerance, and renal toxicity.<sup>3–6</sup> The gastrointestinal (GI) damage from NSAIDs generally attributes to two factors, *i.e.*, local irritation by the carboxylic acid moiety, common to most NSAIDs (topical effect) and decreased tissue prostaglandins production which maintains GI health and homeostasis. Therefore, synthetic approaches based upon chemical modification of NSAIDs were taken with the aim of improving NSAID safety profile.<sup>7–10</sup>

The core pyrazolone structure generally attracted widespread attention because of the diversity of biological activity as they showed anti-inflammatory<sup>11–13</sup> analgesic,<sup>14,15</sup> antitumor,<sup>16</sup> antimicrobial,<sup>17,18</sup> hypoglycemic,<sup>19</sup> and antitubercular<sup>20</sup> activities. One of the first synthetic organic compounds that used as an important drug and having a pyrazolone nucleus was antipyrine **I**.<sup>21</sup> In addition, many pyrazolones like aminophenazone **II**, propyphenazone **III**, and famorofazone **IV** (Fig. 1) are widely used as anti-inflammatory, analgesic, and antipyretic drugs.<sup>7,22,23</sup> Besides, different pyrazolones found to possess significant anti-inflammatory activity as compounds **V**,<sup>24</sup> **VI**,<sup>11</sup> **VII**,<sup>11</sup> and **VIII**<sup>13</sup> (Fig. 2). Thus, our main objective is to design novel pyrazolone derivatives to be further explored as powerful and novel non-ulcerogenic anti-inflammatory lead candidates. Some of these derivatives contain acidic centers with further esterification aiming to decrease the local side effects that might be attained by its acidic moieties.

The synthesized compounds were evaluated for their anti-inflammatory and analgesic activities. Ulcerogenic liability was also performed; in addition, evaluation for their *in vitro* COX-1/COX-2 inhibition was done. Two dimensional-quantitative structure–activity relationship (2D-QSAR) studies were

also performed to correlate between the structures of the synthesized compounds and their pharmacological activities.

### Results and Discussion

**Chemistry** Synthesis of the pyrazolone derivatives was illustrated in the Charts 1–3. The starting intermediates 1-(4-substitutedphenyl)-3-methyl-1*H*-pyrazol-5(4*H*)-ones **2a** and **2c** were synthesized by reacting the commercially available 4-chlorophenylhydrazine hydrochloride **1a** or the synthesized 4-hydrazinylbenzenesulfonamide hydrochloride **1b**<sup>25</sup> with ethyl acetoacetate in acetic acid in the presence of sodium acetate to obtain **2a**<sup>26–28</sup> or in refluxing methanol to obtain **2c**.<sup>29,30</sup> Methylation of **2a** and **2c** with dimethylsulfate (DMS) in alkaline medium afforded the *N*-methyl derivatives **3a**<sup>31</sup> and **3d**.<sup>32</sup> Similarly, 1-(4-substitutedphenyl)-3-phenyl-1*H*-pyrazol-5(4*H*)-ones **2b**<sup>33–35</sup> and **2d** were obtained by reacting **1a** or **1b** with ethyl benzoylacetate. Alkylation of **2b** with DMS gave a mixture of the *O*-methyl and *N*-methyl derivatives **3b** and **3c** in a ratio of 3 : 1, respectively. The two isomers

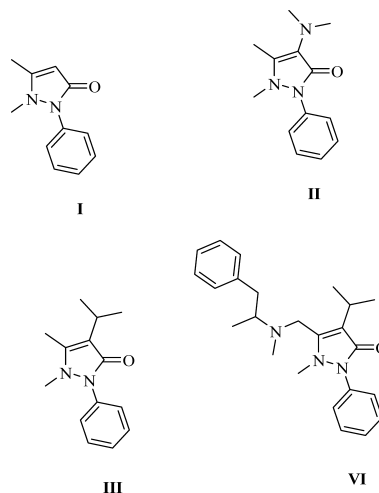


Fig. 1. Anti-inflammatory, Analgesic and Antipyretic Pyrazolone Derivatives

The authors declare no conflict of interest.

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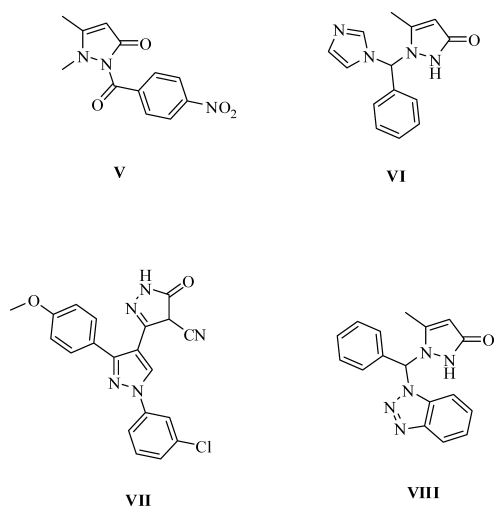


Fig. 2. Pyrazolone Derivatives Exhibited Significant Anti-inflammatory Activity

were separated by preparative TLC using chloroform–methanol (9.5:0.5) as a developing solvent. IR spectrum of **3c** revealed the appearance of C=O absorbance band at  $1762\text{ cm}^{-1}$ , while in case of **3b** this band disappeared.  $^1\text{H-NMR}$  spectrum of **3b** showed a singlet peak at  $\delta$  4.01 ppm corresponding to

(–O–CH<sub>3</sub>), while  $^1\text{H-NMR}$  spectrum of **3c** showed a singlet peak at  $\delta$  3.13 ppm corresponding to (–N–CH<sub>3</sub>). Also, the melting points of the two isomers were different. On the contrary, alkylation of **2d** under the same conditions gave exclusively the *O*-methyl derivative **3e** (Chart 1).

Introduction of dimethylaminomethyl group at 4-position of the pyrazolone derivatives **2a–d** was achieved by Mannich reaction using dimethylamine hydrochloride and formaldehyde to obtain the Mannich bases **4a–d**. The acetic acid derivatives **6a–d** were synthesized through multistep reactions involving quaternarization of Mannich bases with iodomethane, cyanation and finally hydrolysis of the cyanomethyl derivatives **5a–d** with sulfuric acid. Reaction of **6a–d** with thionyl chloride yielded the unstable acid chlorides derivatives which reacted directly without further purification with methanol in sodium methoxide to yield the corresponding ester derivatives **7a–d** (Chart 2).

On the other hand, reaction of **2a–d** with *N,N*-dimethylformamide/diethylacetal (DMF/DEA) in isopropyl alcohol at room temperature led to the incorporation of the dimethylaminomethylene moiety into the position-4 of the pyrazolone derivatives **2a–d** together with protection the sulfamoyl group in case of **2c** and **2d** with *N,N*-dimethylformimidoamide moiety to yield **8a–d**. It is worthy to mention that the literature survey revealed that **8a** was previously synthesized by reac-

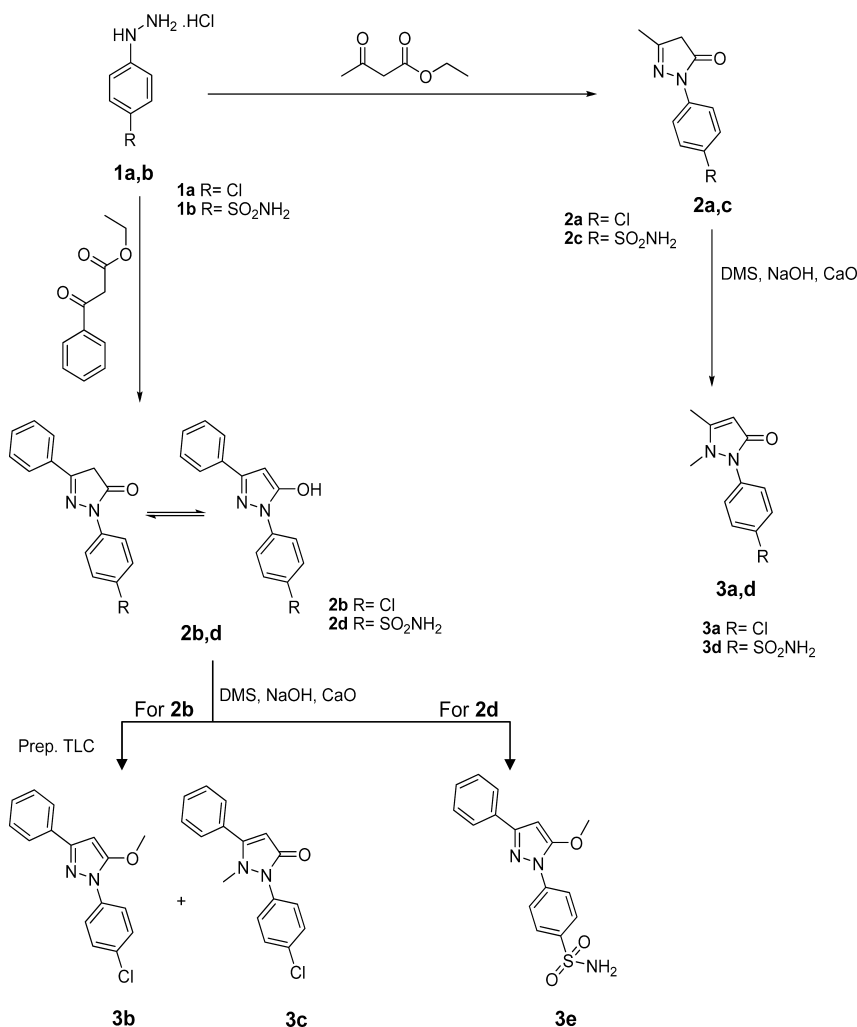


Chart 1. Synthesis of Target Compounds 1–3

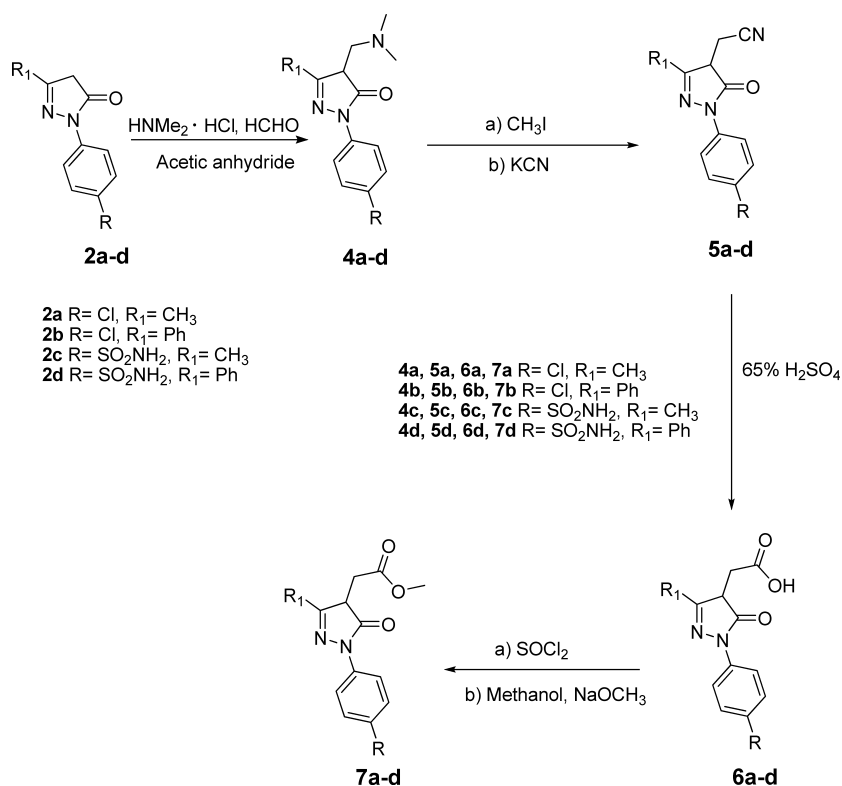


Chart 2. Synthesis of Target Compounds 4–7

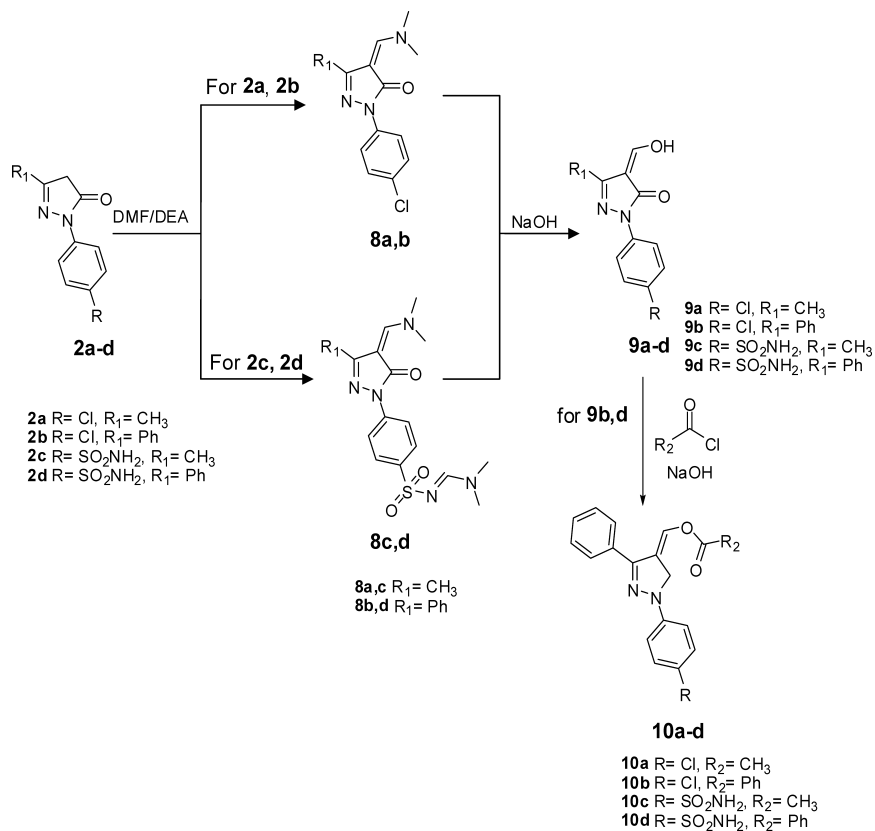


Chart 3. Synthesis of Target Compounds 8–10

tion of **2a** with Vilsmeier–Haack reagent (DMF-POCl<sub>3</sub>).<sup>36</sup> Alkaline hydrolysis of **8a–d** afforded the corresponding 4-hydroxymethylene pyrazolone derivatives **9a–d** from which **9b**

and **9d** were subjected to Schotten–Baumann reaction<sup>37</sup> using acetyl chloride or benzoyl chloride in the presence of sodium hydroxide to give the corresponding ester derivatives **10a–d**

Table 1. Anti-inflammatory Effect and Percentage Inhibition of Phenylbutazone, and Synthesized Compounds on Carrageenan Induced Edema of the Hind Paw in Rats ( $n=5$ )

Cpd. No.	Dose (mmol/kg)	Edema (mm)±S.E.M.		% Inhibition	
		2h	3h	2h	3h
Control	0	3.24±0.39	3.60±0.24	0	0
Phenylbutazone	0.32	1.38±0.09***	1.06±0.19***	57.28	70.55
2a	0.32	2.39±0.25	2.60±0.38	26.23	27.70
2b	0.32	2.20±0.25	2.33±0.29**	32.09	35.27
2c	0.32	1.14±0.18***	1.52±0.12***	64.80	57.80
2d	0.32	1.33±0.17***	1.81±0.08***	59.04	49.70
3a	0.32	2.16±0.14*	2.50±0.17*	33.18	30.69
3b	0.32	1.83±0.21***	3.06±0.19	43.50	15.00
3d	0.32	1.30±0.21***	1.31±0.18***	60.34	63.69
3e	0.32	2.03±0.19**	3.07±0.17	37.19	14.72
4a	0.32	1.99±0.30**	2.20±0.08***	38.67	38.88
4b	0.32	2.54±0.21	2.08±0.22***	21.73	42.33
4c	0.32	2.60±0.33	3.03±0.26	19.90	15.80
4d	0.32	2.96±0.19	2.76±0.16	8.58	23.44
5a	0.32	1.77±0.19***	2.33±0.18**	45.37	35.28
5b	0.32	1.22±0.24***	1.58±0.18***	62.34	56.11
5c	0.32	1.72±0.24***	2.41±0.16*	46.90	33.05
5d	0.32	1.65±0.03***	2.72±0.19	48.90	24.44
6a	0.32	1.54±0.14***	0.79±0.13***	52.34	78.05
6b	0.32	0.48±0.06***	0.48±0.07***	85.20	86.67
6c	0.32	1.41±0.05***	1.42±0.24***	56.60	60.50
6d	0.32	1.57±0.17***	1.97±0.16***	51.50	45.28
7a	0.32	3.07±0.14	3.07±0.10	5.25	14.58
7b	0.32	0.45±0.07***	1.22±0.12***	86.10	66.19
7c	0.32	2.05±0.28**	2.11±0.28***	36.79	41.40
7d	0.32	2.10±0.20*	2.33±0.22**	35.20	35.30
8a	0.32	1.73±0.08***	2.39±0.26**	46.60	33.70
8b	0.32	1.08±0.04***	1.23±0.07***	66.67	65.80
8c	0.32	1.76±0.08***	2.32±0.12**	45.52	35.40
8d	0.32	1.43±0.16***	1.96±0.23***	55.86	45.56
9a	0.32	0.60±0.11***	1.40±0.17***	81.50	61.11
9b	0.32	0.75±0.05***	0.61±0.13***	76.85	82.90
9c	0.32	1.55±0.18***	2.11±0.16***	52.00	40.38
9d	0.32	1.23±0.17***	2.22±0.20***	62.09	38.33
10a	0.32	1.88±0.06***	2.66±0.08	42.06	25.97
10b	0.32	0.41±0.05***	1.34±0.07***	87.30	62.78
10c	0.32	1.62±0.16***	2.33±0.17**	50.00	35.14
10d	0.32	1.64±0.29***	2.04±0.16***	49.23	43.33

Statistical analysis was carried out by one-way ANOVA test. \*Significance difference from the control value at  $p<0.05$ . \*\*Significance difference from the control value at  $p<0.01$ . \*\*\*Significance difference from the control value at  $p<0.001$ .

(Chart 3).

#### Pharmacological Screening. Anti-inflammatory Activity

All the targeted compounds were evaluated for their anti-inflammatory activity *via* carrageenan-induced rat paw edema method of Winter *et al.*<sup>38)</sup> using phenylbutazone as a reference drug. The results were recorded in Table 1.  $IC_{50}$  values results of the compounds **3a, b, d, e**, **4a–d**, **5a–d**, **6a–d**, **9a–d**, and phenylbutazone were calculated and recorded in Table 2.

Results summarized in Table 1 revealed that all tested compounds showed anti-inflammatory activity. After 2h the activity of compounds **2c, d**, **3d**, **5b**, **6b**, **7b**, **8b**, **9a, b, d**, and **10b** exhibited higher activity (58.95–87.35% inhibition) than the reference drug phenylbutazone (57.41% inhibition). After 3h only compounds **6a, b**, and **9b** showed higher activity (78.06 to 86.67% inhibition) than the reference drug (70.56% inhibition).

**Structure–Activity Correlation** Considering the anti-

inflammatory activity of the synthesized pyrazolones unsubstituted at the 4-position, compounds **2c, d**, and **3d** bearing a benzenesulfonamide moiety in the 1-position showed superior activity than their 1-(4-chlorophenyl) analogues **2a, b**, and **3a**. The only exception was compound **3e** which showed lower potency than **3b**. Also, *N*-methylated compounds **3a** and **3d** showed better activity than *O*-methylated ones **3b** and **3e**. In the synthesized compounds, the 3-position was substituted either with  $CH_3$  or phenyl group. Generally, it was observed in derivatives carrying 1-(4-chlorophenyl) group that the 3-phenyl derivatives **2b**, **3b**, **4b**, **5b**, **6b**, **7b**, **8b**, and **9b** were more active than their corresponding 3-methyl analogues **2a**, **3a**, **4a**, **5a**, **6a**, **7a**, **8a**, and **9a**. However, the case was different with the series carrying 1-(benzenesulfonamide) moiety *i.e.*, those with 3-methyl group **2c**, **3d**, **4c**, **5c**, **6c**, **7c**, and **9c** exhibited higher activity than the 3-phenyl analogues **2d**, **3e**, **4d**, **5d**, **6d**, **7d**, and **9d**.

Table 2. IC<sub>50</sub> Values of the Chosen Compounds and Reference Drug Phenylbutazone

Cpd. No.	IC <sub>50</sub> (mmol/kg) after 3 h
Phenylbutazone	0.22
<b>3a</b>	0.52
<b>3b</b>	1.08
<b>3d</b>	0.25
<b>3e</b>	1.09
<b>4a</b>	0.41
<b>4b</b>	0.38
<b>4c</b>	1.00
<b>4d</b>	0.69
<b>5a</b>	0.45
<b>5b</b>	0.28
<b>5c</b>	0.49
<b>5d</b>	0.66
<b>6a</b>	0.20
<b>6b</b>	0.19
<b>6c</b>	0.27
<b>6d</b>	0.35
<b>7b</b>	0.23
<b>9a</b>	0.26
<b>9b</b>	0.19
<b>9c</b>	0.50
<b>9d</b>	0.42

Table 3. Analgesic Activity of Phenylbutazone, and 17 Synthesized Compounds in Mice

Cpd. No.	Dose (mmol/kg)	No. of animals	No. of protected animals	% Protection
Control	0	6	0	0
Phenylbutazone	0.32	6	2	33.33
<b>2c</b>	0.32	6	1	16.67
<b>3b</b>	0.32	6	1	16.67
<b>3d</b>	0.32	6	4	66.67
<b>6a</b>	0.32	6	2	33.33
<b>6b</b>	0.32	6	2	33.33
<b>6c</b>	0.32	6	1	16.67
<b>7a</b>	0.32	6	1	16.67
<b>7b</b>	0.32	6	0	0
<b>7c</b>	0.32	6	1	16.67
<b>8b</b>	0.32	6	0	0
<b>9a</b>	0.32	6	1	16.67
<b>9b</b>	0.32	6	6	100
<b>9d</b>	0.32	6	3	50
<b>10a</b>	0.32	6	4	66.67
<b>10b</b>	0.32	6	1	16.67
<b>10c</b>	0.32	6	1	16.67
<b>10d</b>	0.32	6	0	0

Table 4. Ulcerogenic Effect of Phenylbutazone and 6 Synthesized Compounds in Rats

Cpd. No.	Dose (mmol/kg)	No. of animals	% Incidence divided by 10	Average No. of ulcer	Average severity	Ulcer index
Control	0	5	0	0	0	0
Phenylbutazone	0.32	5	10	19.0	1.20	30.20
<b>2c</b>	0.32	5	6	1.4	1.14	8.50
<b>3d</b>	0.32	5	8	3.0	1.06	12.06
<b>6b</b>	0.32	5	10	6.2	1.06	17.26
<b>7b</b>	0.32	5	6	3.6	1.13	10.70
<b>9b</b>	0.32	5	8	3.8	1.00	12.80
<b>10b</b>	0.32	5	6	3.4	1.06	10.46

The 4-position of the pyrazolone ring tended to be tolerant of a variety of functionality. Introduction of dimethylamino-methyl moiety **4a–d** in this position greatly decreased the anti-inflammatory activity. Conversion of dimethylamino-methyl into cyanomethyl analogues **5a–d** improved the activity. Also, rigidification of the dimethylaminomethyl moiety in **4a–d** by double bond to give dimethylaminomethylene derivatives **8a–d** greatly improved the activity. The presence of acidic center as COOH group in **6a–d** or enolic group in **9a–d** resulted in a significant increase in the activity. Blocking of these acidic centers by esterification as in **7a–d** and **10a–d**, respectively led to decrease of the activity at least at one time interval. Also, it was observed specifically for 1-(4-chlorophenyl) derivatives that aryl ester **10b** was more active than the corresponding alkyl ester **10a**.

**Analgesic Activity** Compounds that exhibited good anti-inflammatory activity and phenylbutazone were screened for their analgesic activity using the reported method of *p*-benzoquinone induced writhing in mice by Okun *et al.*<sup>39)</sup> From the obtained results in Table 3, it was noticed that almost all tested compounds which showed good anti-inflammatory activity showed also good analgesic activity equal to or higher than

phenylbutazone except compounds **7b**, **8b**, and **10d** that exhibited only good anti-inflammatory activity but couldn't protect any animal from writhing. Moreover, the acidic pyrazolone **9b** found to be the best derivative that showed analgesic activity. Ester formation resulted in decreasing of analgesic activity as shown in compounds **7a, d**, and **10a–d** when compared with their acidic counterparts **6a, b** and **9b, d**.

**Ulcerogenic Effect** The ulcerogenic effect of six active compounds and phenylbutazone was evaluated by the reported method of Meshali *et al.*,<sup>40)</sup> and the ulcer index was calculated according to the method of Robert *et al.*<sup>41)</sup> and recorded in Table 4. From the obtained results, it was observed that all the tested compounds for ulcerogenic potential showed better GIT tolerance than phenylbutazone and compound **2c** was showed the least GIT side effects with ulcer index 8.54. Ester derivatives **7b** and **10b** exhibited better GIT tolerance with ulcer indices 10.73 and 10.46, respectively than their corresponding acidic analogues **6b** and **9b** that having ulcer indices 17.26 and 12.80, respectively.

**Acute Toxicity** LD<sub>50</sub> of some representative compounds **3d**, **6b, c**, **7b, c**, **9b, d**, and **10a, b** was determined using Finney's method.<sup>42)</sup> The tested compounds showed a high

Table 5. COX-1/COX-2 Percentage Inhibition Ratio of Compounds **6b**, **7b**, **9b** and Indomethacin

Cpd. No.	COX-1/COX-2
Indomethacin	3.94
<b>6b</b>	1.22
<b>7b</b>	0.74
<b>9b</b>	1.14

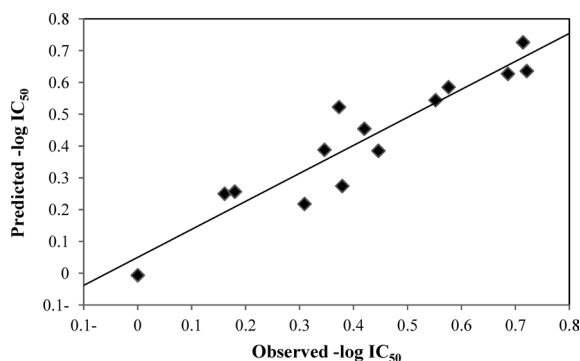


Fig. 3. Relation between Experimentally Observed and Predicted Activity

safety margin since intraperitoneal injection of doses less than 1.62 mmol/kg body weight of the tested compounds failed to kill any mouse after their observation for 24h. LD<sub>50</sub> of compounds **7b**, **c**, and **9d** was 3.25 mmol/kg body weight, while of compounds **6a**, **c**, **9b** and **10a**, **b** was 2.44 mmol/kg body weight and of compound **5a** was 1.62 mmol/kg body weight.

**In Vitro COX Inhibition Assay** Compounds **6b**, **7b**, **9b** and indomethacin were evaluated for their selectivity to inhibit COX-1 and/or COX-2 isoenzymes using Cayman's COX-Activity Assay kit according to the manufacturer instruction.<sup>43</sup> The ratio of percentage inhibition of COX-1/COX-2 was calculated and recorded in Table 5. Results revealed that the tested compounds were non-selective as they have almost equal inhibitory effect on both isoform of COX-enzyme ranging from 42–57% inhibition; also it was found that the ester derivative **7b** showed COX-2 inhibition which indicates that intact ester was active as acidic analogue **6b**.

**2D-QSAR Study** 2D-QSAR study was performed in order to find a mathematical correlation between the structures of twenty compounds **3a**, **b**, **d**, **e**, **4a–d**, **5a–d**, **6a–d**, and **9a–d** and their anti-inflammatory activity<sup>44,45</sup> that expressed as  $-\log IC_{50}$  values, using Molecular Operating Environment (MOE) software package. The most relevant descriptors derived for modeling the anti-inflammatory activity are listed in Table 6 and the relation between the obtained results of experimentally observed and predicted values of anti-inflammatory activity were presented in Table 7 and illustrated by Fig. 3. 2D-QSAR models were validated with the leave one out (LOO) method. The best derived QSAR model for the twenty pyrazolone derivatives was presented by the following triparametric equation with correlation coefficient ( $R^2$ )=0.88 and root mean square (RMSE)=0.072.

$$P IC_{50} = -0.14428 + 1.71350 \cdot FASA\_H - 0.04094 \cdot \text{dipole} \\ - 0.05496 \cdot AM1\_IP \\ n = 14, \quad RMSE = 0.072, \quad R^2 = 0.88$$

Table 6. The Most Relevant Descriptors Derived for Modeling the Anti-inflammatory Activity

Cpd. No.	P IC <sub>50</sub>	Dipole	FASA_H	AM1_IP
<b>3a</b>	0.2790	1.2776	0.7647	9.4780
<b>3b</b>	-0.0330	1.1271	0.8191	11.1909
<b>3d</b>	0.6000	1.5005	0.6058	9.9810
<b>3e</b>	0.0370	1.3553	0.6570	10.2922
<b>4a</b>	0.3790	1.3059	0.5880	9.7443
<b>4b</b>	0.4200	0.9460	0.6700	9.2869
<b>4c</b>	0.0000	1.8596	0.4598	10.4407
<b>4d</b>	0.1610	1.3212	0.5542	9.1167
<b>5a</b>	0.3460	0.2782	0.6384	10.0057
<b>5b</b>	0.5520	1.4235	0.7396	9.4651
<b>5c</b>	0.3090	1.9773	0.5503	9.0965
<b>5d</b>	0.1800	1.7702	0.6011	10.1263
<b>6a</b>	0.6860	0.5800	0.7966	10.3644
<b>6b</b>	0.7210	0.6779	0.8044	10.3734
<b>6c</b>	0.5680	0.9918	0.6206	9.4124
<b>6d</b>	0.4460	1.6806	0.6866	10.5202
<b>9a</b>	0.5760	0.9654	0.7999	10.9451
<b>9b</b>	0.7140	1.3182	0.8936	11.0372
<b>9c</b>	0.2950	1.3242	0.7101	9.9182
<b>9d</b>	0.3730	1.4027	0.7535	10.3089

Table 7. Experimentally Observed, Predicted Activity of 20 Compounds (SPRED) and Their Residual (SRES)

Cpd. No.	Activity P IC <sub>50</sub>	\$PRED	\$RES
<b>3a</b>	0.2790		
<b>3b</b>	-0.0330		
<b>3d</b>	0.6000		
<b>3e</b>	0.0370		
<b>4a</b>	0.3790	0.2743	0.1047
<b>4b</b>	0.4200	0.4546	-0.0346
<b>4c</b>	0.0000	-0.0064	0.0064
<b>4d</b>	0.1610	0.2501	-0.0891
<b>5a</b>	0.3460	0.3882	-0.0422
<b>5b</b>	0.5520	0.5445	0.0075
<b>5c</b>	0.3090	0.2178	0.0912
<b>5d</b>	0.1800	0.2566	-0.0766
<b>6a</b>	0.6860	0.6274	0.0586
<b>6b</b>	0.7210	0.6361	0.0849
<b>6c</b>	0.5680		
<b>6d</b>	0.4460	0.3851	0.0609
<b>9a</b>	0.5760	0.5853	-0.0093
<b>9b</b>	0.7140	0.7264	-0.02124
<b>9c</b>	0.2950		
<b>9d</b>	0.3730	0.5228	-0.1498

From this model, it was observed that FASA\_H (i3D fractional hydrophobic surface area) was positively correlated with anti-inflammatory activity but dipole (i3D The dipole moment calculated using the MNDO Hamiltonian [MOPAC]) and AM1\_IP (i3D The ionization potential (kcal/mol) calculated using the AM1 Hamiltonian [MOPAC]) were negatively correlated with activity indicated that increasing in the values of FASA\_H is beneficial for the anti-inflammatory activity and this matched with the experimental data where the most active compounds **6b** and **9b** which are having P IC<sub>50</sub> 0.72 and 0.71 possessed relatively higher values of FASA\_H 0.80 and 0.89, respectively. Also, Compounds with lower activity **4c** and **4d** with P IC<sub>50</sub> 0.00 and 0.16 possessed lower FASA\_H values



0.45 and 0.55, respectively.

## Conclusion

From this study, we conclude that 1-(4-chlorophenyl or benzenesulfonamide)-2,3- and/or 4-substituted-1*H*-pyrazol-5(4*H*)-ones could be considered to be useful building blocks for future research to synthesis of potential anti-inflammatory and analgesic agents with minimal side effects. Pharmacological screening and biological evaluation revealed that all the tested compounds have good anti-inflammatory activity with respect to the newly synthesized compounds **6b**, **7b**, and **9b** that showed the best anti-inflammatory activity. Also, they have good analgesic activity except for **7b**. Furthermore, they are non-selective towards COX-1 or COX-2 but they exhibited better GIT tolerance than the reference drug phenylbutazone. Analysis of 2D-QSAR model provided details on fine relationship linking structure and activity which indicated that the fractional hydrophobic surface area (FASA<sub>H</sub>), the dipole moment (dipole), and the ionization potential (AM1\_IP) influence the anti-inflammatory activity of pyrazolone derivatives. From SAR point of view, it was found that the presence of acidic center as COOH or enolic group in pyrazolone derivatives improves anti-inflammatory activity and formation of ester derivatives found to be safer than their acidic counterparts but with lower anti-inflammatory and analgesic activities.

## Experimental

**Chemistry** All chemicals were purchased from VWR International Merck, Germany or Sigma-Aldrich and used without further purification. Compound (4-chlorophenyl)hydrazine hydrochloride **1a** was purchased from Sigma-Aldrich. Melting Points were carried out by open capillary tube method using Stuart SMP3 Melting Point apparatus and they are uncorrected. Elemental Microanalysis was carried out at the Micro Analytical Center, Cairo University or at The Regional Center for Mycology and Biotechnology, Al-Azhar University. Infrared spectra were recorded on Shimadzu Infrared spectrometer IR Affinity-1 (FTIR-8400S, Kyoto, Japan) or Jasco Infrared spectrometer (FTIR-4100, Japan), and expressed in wave number (cm<sup>-1</sup>), using potassium bromide discs. <sup>1</sup>H- and <sup>13</sup>C-NMR Spectra were recorded on a Varian Mercury VX-300MHz, <sup>13</sup>C, 70MHz NMR spectrometer, or JEOL-ECA500, 500MHz Japan, NMR spectrometer, the spectra were run at 300MHz or 500MHz in deuterated chloroform (CDCl<sub>3</sub>) or dimethylsulfoxide (DMSO-*d*<sub>6</sub>). Chemical shifts were expressed in  $\delta$  units and were related to that of the solvents. As for the proton magnetic resonance, D<sub>2</sub>O was carried out for NH and OH exchangeable protons. Mass Spectra were recorded using Shimadzu Gas Chromatograph Mass spectrometer-Qp 2010 plus (Japan) or JEOL JMS-AX 500 mass spectrometer (Japan). All the reactions were followed by TLC using silica gel F254 plates (Merck), using chloroform: methanol 9:1 or chloroform as eluting system and were visualized by UV-lamp. Compounds **1b**,<sup>25)</sup> and **3a**<sup>31)</sup> were prepared according to reported methods, while compounds **8a**,<sup>36)</sup> **8b**,<sup>46)</sup> **2a**,<sup>26–28)</sup> **2c**,<sup>29,30)</sup> **2b**,<sup>33–35)</sup> and **3d**<sup>32)</sup> were prepared according to modified procedures from the reported methods.

**General Procedure for the Synthesis of Compounds 2a–d**  
Method A; A mixture of equimolar amounts of **1a** (1.78g, 10mmol) and ethyl acetoacetate (1.30g, 10mmol) or ethyl benzoylacetate (1.92g, 10mmol) in glacial acetic acid (15mL)

and fused sodium acetate (0.08g, 1mmol) was stirred at room temperature for 24h, the reaction mixture was poured onto ice water, the solid was collected, dried and crystallized from ethanol.

Method B; A mixture of **1b** (11.15g, 50mmol) and ethyl acetoacetate (6.50g, 50mmol) or ethyl benzoylacetate (9.60g, 50mmol) was refluxed in methanol (75mL) for 15h. The resulting solution was then concentrated and poured onto ice water. The separated solid was filtered, washed with water, dried and crystallized from methanol.

1-(4-Chlorophenyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**2a**): Yield 95% (from method A). mp 169–171°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$ : 2.19 (3H, s, CH<sub>3</sub>), 3.43 (2H, s, CH<sub>2</sub>), 7.34 (2H, d, Ar-H, *J*=7.0Hz), 7.84 (2H, d, Ar-H, *J*=7.3Hz). IR (KBr) cm<sup>-1</sup>: 3047 (CH aromatic), 2962, 2924 (CH aliphatic), 1680 (C=O). MS *m/z*: 210 (M<sup>+</sup>+2), 208 (M<sup>+</sup>). Anal. Calcd for C<sub>10</sub>H<sub>9</sub>ClN<sub>2</sub>O (208.64): C, 57.57; H, 4.35; N, 13.43. Found: C, 57.74; H, 4.04; N, 13.60.

1-(4-Chlorophenyl)-3-phenyl-1*H*-pyrazol-5(4*H*)-one (**2b**): Yield 91% (from method A). mp 163–164°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$ : 6.05 (1H, s, CH of pyrazole), 7.3–7.92 (9H, m, Ar-H), 12.02 (1H, s, ex, OH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>)  $\delta$ : 85.3, 122.2, 125.1, 127.9, 128.5, 128.8, 129.6, 133.1, 137.7, 150.0, 154.0. IR (KBr) cm<sup>-1</sup>: 3050 (CH aromatic), 2962, 2912 (CH aliphatic), 1712 (C=O). MS *m/z*: 272 (M<sup>+</sup>+2), 270 (M<sup>+</sup>). Anal. Calcd for C<sub>15</sub>H<sub>11</sub>ClN<sub>2</sub>O (270.71): C, 66.55; H, 4.10; N, 10.35. Found: C, 66.68; H, 4.13; N, 10.26.

4-(3-Methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**2c**): Yield 88% (from method B). mp 237–238°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$ : 2.12 (3H, s, CH<sub>3</sub>), 3.42 (2H, s, CH<sub>2</sub>), 7.35 (2H, s, ex, NH<sub>2</sub>), 7.84 (2H, d, Ar-H, *J*=7.8Hz), 7.91 (2H, d, Ar-H, *J*=7.1Hz). IR (KBr) cm<sup>-1</sup>: 3317, 3244 (NH<sub>2</sub>), 3043 (CH aromatic), 2981, 2958 (CH aliphatic), 1627 (C=O), 1334, 1161 (SO<sub>2</sub>). MS *m/z*: 253 (M<sup>+</sup>). Anal. Calcd for C<sub>10</sub>H<sub>11</sub>N<sub>3</sub>O<sub>3</sub>S (253.27): C, 47.42; H, 4.38; N, 16.59. Found: C, 47.70; H, 4.63; N, 16.74.

4-(5-Oxo-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**2d**): Yield 75% (from method B). mp 211–213°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>)  $\delta$ : 6.05 (1H, s, CH of pyrazole), 6.51 (2H, s, ex, NH<sub>2</sub>), 7.36–8.06 (9H, m, Ar-H), 12.00 (1H, s, ex, OH). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>)  $\delta$ : 86.0, 120.8, 125.2, 126.7, 128.1, 128.3, 128.5, 140.5, 141.2, 150.5, 154.5. IR (KBr) cm<sup>-1</sup>: 3329, 3244 (NH<sub>2</sub>), 3066 (CH aromatic), 2981, 2958 (CH aliphatic), 1732 (C=O), 1323, 1153 (SO<sub>2</sub>). MS *m/z*: 315 (M<sup>+</sup>). Anal. Calcd for C<sub>15</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S (315.34): C, 57.13; H, 4.16; N, 13.33. Found: C, 57.37; H, 4.40; N, 13.08.

**General Procedure for the Synthesis of Compounds 3b–e**  
A solution of sodium hydroxide (0.50g, 12.50mmol) in water (1mL) was added to a solution of **2a–d** (5.73mmol) in methanol (5mL). The mixture was warmed on a water bath and dimethyl sulfate (0.70g, 7.50mmol) was added. The mixture was refluxed for 1h and allowed to cool, with continuous stirring. Methanol was distilled off, hot water was added to the residue, the mixture was filtered, the filtrate was extracted with benzene, calcium oxide was added to remove excess dimethyl sulfate and filtered. The filtrate was evaporated under vacuum and the residue was recrystallized from benzene.

Separation of the mixture **3b** and **3c** by preparative TLC. TLC plates are prepared by mixing silica gel, with a small amount of calcium sulfate (gypsum) and water. This mixture is spread as thick slurry on glass plates (20cm×20cm). The

resultant plate is dried and activated by heating in an oven for thirty minutes at 110°C. The mixture (about 0.5 g was dissolved in chloroform) is applied to the plate as a thin even layer horizontally to and just above the solvent level. When developed with solvent (chloroform–methanol, 9.5:0.5), the compounds separate in horizontal bands with light to deep yellow color. Lower band (*R<sub>f</sub>* is 0.18) and upper band (*R<sub>f</sub>* is 0.90) were scraped off the backing material. The backing material is then extracted with chloroform and filtered to give the isolated material upon removal of the solvent. Lower bands yielded compounds **3c** (yield 23%) while, upper bands yielded compounds **3b** (yield 71%).

1-(4-Chlorophenyl)-5-methoxy-3-phenyl-1*H*-pyrazole (**3b**): Yield 71%. mp 56–58°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 4.01 (3H, s, OCH<sub>3</sub>), 6.02 (1H, s, CH of pyrazole), 7.36–7.89 (9H, m, Ar-H). IR (KBr) cm<sup>-1</sup>: 3061, 3026 (CH aromatic), 2926, 2854 (CH aliphatic). MS *m/z*: 286 (M<sup>+</sup>+2), 284 (M<sup>+</sup>). *Anal.* Calcd for C<sub>16</sub>H<sub>13</sub>ClN<sub>2</sub>O (284.74): C, 67.49; H, 4.60; N, 9.84. Found: C, 67.49; H, 4.62; N, 9.97.

2-(4-Chlorophenyl)-1-methyl-5-phenyl-1*H*-pyrazol-3(2*H*)-one (**3c**): Yield 23%. mp 211–213°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.13 (3H, s, N–CH<sub>3</sub>), 6.02 (1H, s, CH of pyrazolone), 7.15–7.54 (9H, m, Ar-H). IR (KBr) cm<sup>-1</sup>: 3000 (CH aromatic), 2926, 2852 (CH aliphatic), 1762 (C=O); MS *m/z*: 286 (M<sup>+</sup>+2), 284 (M<sup>+</sup>). *Anal.* Calcd for C<sub>16</sub>H<sub>13</sub>ClN<sub>2</sub>O (284.74): C, 67.49; H, 4.60; N, 9.84. Found: C, 67.53; H, 4.58; N, 9.97.

4-(2,3-Dimethyl-5-oxo-2,5-dihydro-1*H*-pyrazol-1-yl)-benzenesulfonamide (**3d**): Yield 60%. mp 252–253°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.27 (3H, s, CH<sub>3</sub>), 2.85 (3H, s, N–CH<sub>3</sub>), 5.00 (1H, s, CH of pyrazolone), 7.18–8.27 (6H, m, Ar-H+ex, NH<sub>2</sub>). IR (KBr) cm<sup>-1</sup>: 3410, 3279 (NH<sub>2</sub>), 3100 (CH aromatic), 2924, 2860 (CH aliphatic), 1735 (C=O). MS *m/z*: 267 (M<sup>+</sup>). *Anal.* Calcd for C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S (267.30): C, 49.43; H, 4.90; N, 15.72. Found: C, 49.51; H, 4.88; N, 15.84.

4-(5-Methoxy-3-phenyl-1*H*-pyrazol-1-yl)benzenesulfonamide (**3e**): Yield 48%. mp 143–145°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 4.07 (3H, s, OCH<sub>3</sub>), 6.51 (1H, s, CH of pyrazole), 7.26–8.09 (11H, m, Ar-H+ex, NH<sub>2</sub>). <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>) δ: 59.6, 85.0, 120.7, 127.2, 127.8, 128.5, 128.7, 132.5, 141.1, 141.2, 126.7, 156.6. IR (KBr) cm<sup>-1</sup>: 3410, 3279 (NH<sub>2</sub>), 3060 (CH aromatic), 2924, 2854 (CH aliphatic). MS *m/z*: 329 (M<sup>+</sup>). *Anal.* Calcd for C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>S (329.37): C, 58.34; H, 4.59; N, 12.76. Found: C, 58.36; H, 4.50; N, 12.89.

**General Procedure for the Synthesis of Compounds 4a–d**  
A solution of dimethylamine hydrochloride (0.81 g, 10 mmol) in 37% HCHO (0.30 g, 10 mmol) was stirred at room temperature for 30 min; acetic anhydride (3.20 mL) was added dropwise at (70–90°C). After being stirred for 30 min, compound **2a–d** (6.70 mmol) was added and the mixture was stirred for 12 h at (70–75°C). After cooling to room temperature, the reaction mixture was poured onto ice water, neutralized with ammonia solution. The solid was collected, dried and crystallized from ethanol.

1-(4-Chlorophenyl)-4-((dimethylamino)methyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**4a**): Yield 82%. mp 203–204°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.09 (3H, s, CH<sub>3</sub>), 2.24 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 2.31–2.35 (1H, m, CH), 2.42–2.45 (1H, m, CH), 3.40–3.50 (1H, m, CH), 7.37–7.45 (4H, m, Ar-H). IR (KBr) cm<sup>-1</sup>: 3062 (CH aromatic), 2924, 2854 (CH aliphatic), 1712 (C=O). MS *m/z*: 266 (M<sup>+</sup>+1), 264 (M–1). *Anal.* Calcd for C<sub>13</sub>H<sub>16</sub>ClN<sub>3</sub>O (265.73): C, 58.76; H, 6.07; N, 15.81. Found: C, 58.81; H, 6.12; N,

16.04.

1-(4-Chlorophenyl)-4-((dimethylamino)methyl)-3-phenyl-1*H*-pyrazol-5(4*H*)-one (**4b**): Yield 81%. mp 112–114°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 1.92 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 2.79–2.85 (1H, m, CH), 2.89–3.05 (1H, m, CH), 3.30–3.40 (1H, m, CH), 7.10–7.92 (9H, m, Ar-H); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 29.6, 30.2, 61.2, 126.1, 128.1, 128.8, 129.1, 130.3, 133.0, 136.0, 142.6, 151.8, 166.3. IR (KBr) cm<sup>-1</sup>: 3059, 3032 (CH aromatic), 2924, 2850 (CH aliphatic), 1718 (C=O). MS *m/z*: 329 (M<sup>+</sup>+2), 327 (M<sup>+</sup>). *Anal.* Calcd for C<sub>18</sub>H<sub>18</sub>ClN<sub>3</sub>O (327.80): C, 65.95; H, 5.53; N, 12.82. Found: C, 66.03; H, 5.51; N, 12.98.

4-(4-((Dimethylamino)methyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**4c**): Yield 80%. mp 194–196°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.89 (3H, s, CH<sub>3</sub>), 2.07 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 2.70–2.80 (1H, m, CH), 2.88–3.00 (1H, m, CH), 3.38–3.44 (1H, m, CH), 7.37 (2H, s, ex, NH<sub>2</sub>), 7.83–7.67 (4H, m, Ar-H). IR (KBr) cm<sup>-1</sup>: 3414, 3232 (NH<sub>2</sub>), 3082 (CH aromatic), 2920, 2850 (CH aliphatic), 1716 (C=O), 1334, 1161 (SO<sub>2</sub>). MS, *m/z*: 310 (M<sup>+</sup>). *Anal.* Calcd for C<sub>13</sub>H<sub>18</sub>N<sub>4</sub>O<sub>3</sub>S (310.37): C, 50.31; H, 5.85; N, 18.05. Found: C, 50.28; H, 5.91; N, 18.24.

4-(4-((Dimethylamino)methyl)-5-oxo-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**4d**): Yield 93%. mp 128–130°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 1.95 (6H, s, N(CH<sub>3</sub>)<sub>2</sub>), 2.75–2.78 (1H, m, CH), 2.90–2.94 (1H, m, CH), 3.38–3.44 (1H, m, CH), 6.99 (2H, s, ex, NH<sub>2</sub>), 7.30–8.12 (9H, m, Ar-H). IR (KBr) cm<sup>-1</sup>: 3425, 3221 (NH<sub>2</sub>), 3062 (CH aromatic), 2924, 2854 (CH aliphatic), 1720 (C=O), 1369, 1161 (SO<sub>2</sub>); MS *m/z*: 372 (M<sup>+</sup>). *Anal.* Calcd for C<sub>18</sub>H<sub>20</sub>N<sub>4</sub>O<sub>3</sub>S (372.44): C, 58.05; H, 5.41; N, 15.04. Found: C, 58.12; H, 5.38; N, 15.22.

#### General Procedure for the Synthesis of Compounds 5a–d

To a solution of **4a–d** (10 mmol) in acetone (50 mL), iodomethane (2.31 g, 16.30 mmol) was added below 5°C in an ice bath. Then the mixture was stirred at room temperature over night. The product was concentrated under vacuum and the obtained residue was dissolved in methanol then a solution of KCN (1.57 g, 24.20 mmol) in water (1 mL) was added. The solution was stirred at room temperature over night and poured onto ice water. The resulting mixture was extracted with CHCl<sub>3</sub>. The extract was washed with water, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum.

2-(1-(4-Chlorophenyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-4-yl)acetonitrile (**5a**): Yield 65% as oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.06 (3H, s, CH<sub>3</sub>), 2.19–2.24 (1H, m, CH), 2.71–2.74 (1H, m, CH), 2.89–2.92 (1H, m, CH), 7.80–7.95 (4H, m, Ar-H). IR (KBr) cm<sup>-1</sup>: 3060 (CH aromatic), 2926, 2856 (CH aliphatic), 2343 (CN), 1762 (C=O). MS *m/z*: 251 (M<sup>+</sup>+4), 249 (M<sup>+</sup>+2). *Anal.* Calcd for C<sub>12</sub>H<sub>10</sub>ClN<sub>3</sub>O (247.68): C, 58.19; H, 4.07; N, 16.97. Found: C, 58.27; H, 4.12; N, 17.26.

2-(1-(4-Chlorophenyl)-5-oxo-3-phenyl-4,5-dihydro-1*H*-pyrazol-4-yl)acetonitrile (**5b**): Yield 88% as oil. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.02–2.19 (1H, m, CH), 2.71–2.74 (1H, m, CH), 2.92–2.96 (1H, m, CH), 7.19–7.90 (9H, m, Ar-H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 25.8, 43.8, 119.9, 126.9, 128.4, 128.5, 128.6, 128.7, 129.7, 136.5, 138.0, 148.9, 162.5. IR (KBr) cm<sup>-1</sup>: 3060 (CH aromatic), 2921, 2853 (CH aliphatic), 2253 (CN), 1716 (C=O). MS *m/z*: 311 (M<sup>+</sup>+2), 309 (M<sup>+</sup>). *Anal.* Calcd for C<sub>17</sub>H<sub>12</sub>ClN<sub>3</sub>O (309.74): C, 65.92; H, 3.90; N, 13.57. Found: C, 65.97; H, 3.88; N, 13.71.

4-(4-(Cyanomethyl)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**5c**): Yield 88% as oil.



$^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 2.06 (3H, s,  $\text{CH}_3$ ), 2.16–2.22 (1H, m, CH), 2.68–2.72 (1H, m, CH), 2.88–2.92 (1H, m, CH), 7.68–8.06 (6H, m, Ar-H+ex,  $\text{NH}_2$ ). IR (KBr)  $\text{cm}^{-1}$ : 3421, 3201 ( $\text{NH}_2$ ), 3066, 3047 (CH aromatic), 2924, 2845 (CH aliphatic), 2260 (CN), 1705 (C=O), 1338, 1161 ( $\text{SO}_2$ ). MS  $m/z$ : 294 ( $\text{M}^+ + 2$ ). *Anal.* Calcd for  $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_3\text{S}$  (292.31): C, 49.31; H, 4.14; N, 19.17. Found: C, 49.29; H, 4.31; N, 19.43.

4-(4-(Cyanomethyl)-5-oxo-3-phenyl-4,5-dihydro-1H-pyrazol-yl)benzenesulfonamide (**5d**): Yield 61% as oil.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 2.23–2.29 (1H, m, CH), 2.52–2.57 (1H, m, CH), 2.90–2.93 (1H, m, CH), 7.35–8.00 (11H, m, Ar-H+ex,  $\text{NH}_2$ ). IR (KBr)  $\text{cm}^{-1}$ : 3050 (CH aromatic), 2900, 2850 (CH aliphatic), 2260 (CN), 1707 (C=O), 1371, 1159 ( $\text{SO}_2$ ). MS  $m/z$ : 356 ( $\text{M}^+ + 2$ ). *Anal.* Calcd for  $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_3\text{S}$  (354.38): C, 57.62; H, 3.98; N, 15.81. Found: C, 57.71; H, 4.07; N, 16.12.

#### General Procedure for the Synthesis of Compounds 6a–d

A solution of **5a–d** (10 mmol) in 65% sulfuric acid (40 mL) was refluxed 3 h and poured into ice water. The solid was filtered, washed with water, dried and crystallized from ethanol.

2-(1-(4-Chlorophenyl)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)acetic Acid (**6a**): Yield 47%. mp 168–170°C.  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ )  $\delta$ : 2.18–2.34 (4H, m,  $\text{CH}_3 + \text{CH}$ ), 2.72–2.77 (1H, m, CH), 2.90–2.95 (1H, m, CH), 7.48–7.65 (4H, m, Ar-H), 7.27 (1H, s, ex, OH). IR (KBr)  $\text{cm}^{-1}$ : 3406–2503 (OH carboxylic), 2924, 2850 (CH aliphatic), 1716 (C=O). MS  $m/z$ : 268 ( $\text{M}^+ + 2$ ), 266 ( $\text{M}^+$ ). *Anal.* Calcd for  $\text{C}_{12}\text{H}_{11}\text{ClN}_2\text{O}_3$  (266.68): C, 54.05; H, 4.16; N, 10.50. Found: C, 54.12; H, 4.19; N, 10.77.

2-(1-(4-Chlorophenyl)-5-oxo-3-phenyl-4,5-dihydro-1H-pyrazol-4-yl)acetic Acid (**6b**): Yield 71%. mp 220–223°C.  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ )  $\delta$ : 2.72–2.77 (1H, m, CH), 2.85–2.88 (1H, m, CH), 3.29–3.33 (1H, m, CH), 7.41–7.99 (10H, m, Ar-H+ex, OH). IR (KBr)  $\text{cm}^{-1}$ : 3402–2495 (OH carboxylic), 2927 (CH aliphatic), 1716 (C=O). MS  $m/z$ : 330 ( $\text{M}^+ + 2$ ), 328 ( $\text{M}^+$ ). *Anal.* Calcd for  $\text{C}_{17}\text{H}_{13}\text{ClN}_2\text{O}_3$  (328.74): C, 62.11; H, 3.99; N, 8.52. Found: C, 62.19; H, 4.04; N, 8.68.

2-(3-Methyl-5-oxo-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-4-yl)acetic Acid (**6c**): Yield 65%. mp above 350°C.  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ )  $\delta$ : 2.04–2.30 (6H, m,  $\text{CH}_3 + \text{CH}_2 + \text{CH}$ ), 7.30–7.87 (6H, m, Ar-H+ex,  $\text{NH}_2$ ), 8.08 (1H, s, ex, OH). IR (KBr)  $\text{cm}^{-1}$ : 3298–2669 (OH carboxylic), 3298, 3240 ( $\text{NH}_2$ ), 3101 (CH aromatic), 2920, 2850 (CH aliphatic), 1720 (C=O), 1330, 1157 ( $\text{SO}_2$ ). MS  $m/z$ : 312 ( $\text{M}^+ + 1$ ); *Anal.* Calcd for  $\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_5\text{S}$  (311.31): C, 46.30; H, 4.21; N, 13.50. Found: C, 46.71; H, 4.42; N, 13.69.

2-(5-Oxo-3-phenyl-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-4-yl)acetic Acid (**6d**): Yield 69%. mp 235–236°C.  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ )  $\delta$ : 2.05–2.31 (3H, m,  $\text{CH}_2 + \text{CH}$ ), 7.32 (2H, s, ex,  $\text{NH}_2$ ), 7.42–8.06 (9H, m, Ar-H), 8.08 (1H, s, ex, OH). IR (KBr)  $\text{cm}^{-1}$ : 3352–2550 (OH carboxylic), 3352, 3251 ( $\text{NH}_2$ ), 3066 (CH aromatic), 2920, 2850 (CH aliphatic), 1720 (C=O), 1330, 1161 ( $\text{SO}_2$ ). MS  $m/z$ : 373 ( $\text{M}^+$ ). *Anal.* Calcd for  $\text{C}_{17}\text{H}_{15}\text{N}_3\text{O}_5\text{S}$  (373.38): C, 54.68; H, 4.05; N, 11.25. Found: C, 54.97; H, 4.18; N, 11.38.

#### General Procedure for the Synthesis of Compounds 7a–d

A suspension of **6a–d** (5 mmol) in thionyl chloride (5 mL) was heated gently under reflux until a homogenous solution was obtained then for further 45 min. The solution was then evaporated to dryness under vacuum in a water bath to remove excess thionyl chloride. The residue was azeotroped three times with dry benzene (5 mL) each, where the last traces of

thionyl chloride were removed. The residue was then refluxed in sodium methoxide (10 mL) for 3 h. The reaction mixture poured onto ice water, then extracted with chloroform. The extract was washed with water, dried over anhydrous  $\text{Na}_2\text{SO}_4$ , and concentrated under vacuum, dried and crystallized from ethanol.

Methyl 2-(1-(4-Chlorophenyl)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-yl)acetate (**7a**): Yield 40%. mp 115–116°C (decompose).  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 1.84 (3H, s,  $\text{CH}_3$ ), 2.38–2.52 (1H, m, CH), 2.67–2.72 (1H, m, CH), 3.10–3.16 (1H, m, CH), 3.88 (3H, s,  $\text{OCH}_3$ ), 7.42–7.79 (4H, m, Ar-H). IR (KBr)  $\text{cm}^{-1}$ : 2924, 2850 (CH aliphatic), 1770, 1631 (2C=O). MS  $m/z$ : 282 ( $\text{M}^+ + 2$ ), 280 ( $\text{M}^+$ ). *Anal.* Calcd for  $\text{C}_{13}\text{H}_{13}\text{ClN}_2\text{O}_3$  (280.70): C, 55.62; H, 4.67; N, 9.98. Found: C, 55.67; H, 4.72; N, 10.13.

Methyl 2-(1-(4-Chlorophenyl)-5-oxo-3-phenyl-4,5-dihydro-1H-pyrazol-4-yl)acetate (**7b**): Yield 45%. mp above 350°C.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 2.05–2.08 (1H, m, CH), 2.86–2.88 (1H, m, CH), 2.94–2.96 (1H, m, CH), 3.71 (3H, s,  $\text{OCH}_3$ ), 7.44–8.04 (9H, m, Ar-H). IR (KBr)  $\text{cm}^{-1}$ : 3059 (CH aromatic), 2924, 2854 (CH aliphatic), 1774, 1716 (2C=O). MS  $m/z$ : 343 ( $\text{M}^+ + 1$ ), 341 ( $\text{M} - 1$ ). *Anal.* Calcd for  $\text{C}_{18}\text{H}_{15}\text{ClN}_2\text{O}_3$  (342.77): C, 63.07; H, 4.41; N, 8.17. Found: C, 63.16; H, 4.47; N, 8.31.

Methyl 2-(3-Methyl-5-oxo-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-4-yl)acetate (**7c**): Yield 70%. mp 222–223°C.  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ )  $\delta$ : 2.14 (3H, s,  $\text{CH}_3$ ), 2.05–2.11 (1H, m, CH), 2.40–2.43 (1H, m, CH), 2.65–2.70 (1H, m, CH), 3.16 (3H, s,  $\text{OCH}_3$ ), 7.32–8.08 (6H, m, Ar-H+ex,  $\text{NH}_2$ ). IR (KBr)  $\text{cm}^{-1}$ : 3344, 3251 ( $\text{NH}_2$ ), 3105, 3078 (CH aromatic), 2924, 2854 (CH aliphatic), 1724, 1620 (2C=O), 1327, 1157 ( $\text{SO}_2$ ). MS  $m/z$ : 327 ( $\text{M}^+ + 2$ ). *Anal.* Calcd for  $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_5\text{S}$  (325.34): C, 47.99; H, 4.65; N, 12.92. Found: C, 48.13; H, 4.42; N, 13.16.

Methyl 2-(5-Oxo-3-phenyl-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-4-yl)acetate (**7d**): Yield 67%. mp 229–230°C.  $^1\text{H-NMR}$  ( $\text{DMSO-}d_6$ )  $\delta$ : 2.22–2.44 (2H, m,  $\text{CH}_2$ ), 2.65–2.70 (1H, m, CH), 3.16 (3H, s,  $\text{OCH}_3$ ), 7.29–8.20 (11H, m, Ar-H+ex,  $\text{NH}_2$ ). IR (KBr)  $\text{cm}^{-1}$ : 3363, 3259 ( $\text{NH}_2$ ), 3105, 3066 (CH aromatic), 2924, 2854 (CH aliphatic), 1728, 1620 (2C=O), 1330, 1161 ( $\text{SO}_2$ ). MS  $m/z$ : 387 ( $\text{M}^+$ ). *Anal.* Calcd for  $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_5\text{S}$  (387.40): C, 55.80; H, 4.42; N, 10.85. Found: C, 56.24; H, 4.63; N, 11.22.

#### General Procedure for the Synthesis of Compounds 8a–d

To a suspension of **2a–d** (6 mmol) in isopropanol (12 mL), *N,N*-dimethylformamide diethylacetal (2.87 mL, 19.50 mmol) was added and stirred for 2 h at room temperature. An additional amount of *N,N*-dimethylformamide diethylacetal (0.95 mL, 6.50 mmol) was further added and the reaction mixture was stirred at room temperature for another 20 h. The formed precipitate was filtered off, washed with isopropanol, dried and recrystallized from isopropanol.

1-(4-Chlorophenyl)-4-((dimethylamino)methylene)-3-methyl-1H-pyrazol-5(4H)-one (**8a**): Yield 77%. mp 203–204°C.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 2.14 (3H, s,  $\text{CH}_3$ ), 3.21 (3H, s,  $\text{N}(\text{CH}_3)_2$ ), 3.78 (3H, s,  $\text{N}(\text{CH}_3)_2$ ), 6.95 (1H, s,  $\text{CHN}(\text{CH}_3)_2$ ), 7.28 (2H, d, Ar-H,  $J=8.7\text{ Hz}$ ), 7.98 (2H, d, Ar-H,  $J=9.0\text{ Hz}$ ).  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ )  $\delta$ : 13.7, 43.3, 48.0, 99.2, 120.1, 128.4, 128.6, 138.2, 152.2, 162.0. IR (KBr)  $\text{cm}^{-1}$ : 3032 (CH aromatic), 2920 (CH aliphatic), 1674 (C=O). MS  $m/z$ : 265 ( $\text{M}^+ + 2$ ), 263 ( $\text{M}^+$ ). *Anal.* Calcd for  $\text{C}_{13}\text{H}_{14}\text{ClN}_3\text{O}$  (263.72): C, 59.21; H, 5.35; N, 15.93. Found: C, 59.36; H, 5.48; N, 16.26.

1-(4-Chlorophenyl)-4-((dimethylamino)methylene)-3-phenyl-1*H*-pyrazol-5(4*H*)-one (**8b**): Yield 81%. mp 180–182°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.29 (3H, s, N(CH<sub>3</sub>)), 3.81 (3H, s, N(CH<sub>3</sub>)), 7.26 (1H, s, CHN(CH<sub>3</sub>)<sub>2</sub>), 7.31–8.07 (9H, m, Ar-H). IR (KBr) cm<sup>-1</sup>: 3055 (CH aromatic), 2924 (CH aliphatic), 1670 (C=O). MS *m/z*: 327 (M<sup>+</sup>+2), 325 (M<sup>+</sup>). Anal. Calcd for C<sub>18</sub>H<sub>16</sub>ClN<sub>3</sub>O (325.79): C, 66.36; H, 4.95; N, 12.90. Found: C, 66.45; H, 4.89; N, 13.12.

*N'*-(4-(4-((Dimethylamino)methylene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)phenylsulfonyl)-*N,N*-dimethylformimidamide (**8c**): Yield 79%. mp 234–236°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.20 (3H, s, CH<sub>3</sub>), 3.00 (3H, s, NCHN(CH<sub>3</sub>)), 3.11 (3H, s, NCHN(CH<sub>3</sub>)), 3.34 (3H, s, N(CH<sub>3</sub>)), 3.83 (3H, s, N(CH<sub>3</sub>)), 7.05 (1H, s, CHN(CH<sub>3</sub>)<sub>2</sub>), 7.85 (2H, d, Ar-H, *J*=8.7Hz), 8.09 (1H, s, (NCHN(CH<sub>3</sub>)<sub>2</sub>)), 8.13 (2H, d, Ar-H, *J*=8.7Hz). IR (KBr) cm<sup>-1</sup>: 3078, 3032 (CH aromatic), 2924, 2854 (CH aliphatic), 1674 (C=O), 1330, 1145 (SO<sub>2</sub>). MS *m/z*: 363 (M<sup>+</sup>). Anal. Calcd for C<sub>16</sub>H<sub>21</sub>N<sub>5</sub>O<sub>3</sub>S (363.43): C, 52.88; H, 5.82; N, 19.27. Found: C, 52.91; H, 5.87; N, 19.35.

*N'*-(4-(4-((Dimethylamino)methylene)-5-oxo-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)phenylsulfonyl)-*N,N*-dimethylformimidamide (**8d**): Yield 77%. mp 183–185°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 3.01 (3H, s, NCHN(CH<sub>3</sub>)), 3.11 (3H, s, NCHN(CH<sub>3</sub>)), 3.33 (3H, s, N(CH<sub>3</sub>)), 3.78 (3H, s, N(CH<sub>3</sub>)), 6.01 (1H, s, CHN(CH<sub>3</sub>)<sub>2</sub>), 7.42–8.26 (10H, m, Ar-H+NCHN(CH<sub>3</sub>)<sub>2</sub>). IR (KBr) cm<sup>-1</sup>: 3055, 3028 (CH aromatic), 2927 (CH aliphatic), 1678 (C=O), 1330, 1141 (SO<sub>2</sub>). MS *m/z*: 425 (M<sup>+</sup>). Anal. Calcd for C<sub>21</sub>H<sub>23</sub>N<sub>5</sub>O<sub>3</sub>S (425.50): C, 59.28; H, 5.45; N, 16.46. Found: C, 59.32; H, 5.48; N, 16.63.

#### General Procedure for the Synthesis of Compounds 9a–d

A mixture of **8a–d** (3.80 mmol), NaOH (0.56 g, 14 mmol), isopropanol (15 mL) and water (15 mL) was refluxed 7 h. The isopropanol was removed under vacuum, ice water (10 mL) was added to the residue, then acidified with 10% hydrochloric acid till pH=1, the solid formed was collected by filtration, washed with water, dried and crystallized from isopropanol.

1-(4-Chlorophenyl)-4-(hydroxymethylene)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**9a**): Yield 67%. mp 316–318°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 2.42 (3H, s, CH<sub>3</sub>), 4.70 (1H, s, ex, OH), 7.42 (2H, d, Ar-H, *J*=9.0Hz), 7.79 (2H, d, Ar-H, *J*=9.0Hz), 9.43 (1H, s, CH–OH). <sup>13</sup>C-NMR (CDCl<sub>3</sub>) δ: 29.6, 106.0, 121.0, 126.0, 129.0, 132.0, 149.0, 159.0, 183.4. IR (KBr) cm<sup>-1</sup>: 3414 (OH), 3050 (CH aromatic), 2924, 2850 (CH aliphatic), 1658 (C=O). MS *m/z*: 238 (M<sup>+</sup>+2), 236 (M<sup>+</sup>). Anal. Calcd for C<sub>11</sub>H<sub>9</sub>ClN<sub>2</sub>O<sub>2</sub> (236.65): C, 55.83; H, 3.83; N, 11.84. Found: C, 55.91; H, 3.87; N, 12.15.

1-(4-Chlorophenyl)-4-(hydroxymethylene)-3-phenyl-1*H*-pyrazol-5(4*H*)-one (**9b**): Yield 76%. mp 126–127°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 5.00 (1H, s, ex, OH), 7.45–7.92 (9H, m, Ar-H), 9.72 (1H, s, CH–OH). IR (KBr) cm<sup>-1</sup>: 3251 (OH), 3062 (CH aromatic), 2924, 2850 (CH aliphatic), 1643 (C=O). MS *m/z*: 300 (M<sup>+</sup>+2), 298 (M<sup>+</sup>). Anal. Calcd for C<sub>16</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>2</sub> (298.72): C, 64.33; H, 3.71; N, 9.38. Found: C, 64.48; H, 3.82; N, 9.62.

4-(4-(Hydroxymethylene)-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**9c**): Yield 57%. mp 144–146°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.34 (3H, s, CH<sub>3</sub>), 4.93 (1H, s, ex, OH), 7.38 (2H, s, ex, NH<sub>2</sub>), 7.90 (2H, d, Ar-H, *J*=8.7Hz), 7.98 (2H, d, Ar-H, *J*=9.0Hz), 9.28 (1H, s, CH–OH). IR (KBr) cm<sup>-1</sup>: 3468 (OH), 3309, 3205 (NH<sub>2</sub>), 3039 (CH aromatic), 2800 (CH aliphatic), 1701 (C=O), 1323, 1157 (SO<sub>2</sub>). MS *m/z*: 281 (M<sup>+</sup>). Anal. Calcd for C<sub>11</sub>H<sub>11</sub>N<sub>3</sub>O<sub>4</sub>S (281.28): C,

46.97; H, 3.94; N, 14.94. Found: C, 47.11; H, 4.03; N, 15.18.

4-(4-(Hydroxymethylene)-5-oxo-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzenesulfonamide (**9d**): Yield 58%. mp 237–239°C (decomposed). <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 6.10 (s, 1H, ex, OH), 7.43 (2H, s, ex, NH<sub>2</sub>), 7.44–8.16 (9H, m, Ar-H), 9.35 (1H, s, CH–OH). IR (KBr) cm<sup>-1</sup>: 3564 (OH), 3356, 3267 (NH<sub>2</sub>), 3062 (CH aromatic), 2924, 2854 (CH aliphatic), 1627 (C=O), 1307, 1161 (SO<sub>2</sub>). MS *m/z*: 343 (M<sup>+</sup>). Anal. Calcd for C<sub>16</sub>H<sub>13</sub>N<sub>3</sub>O<sub>4</sub>S (343.35): C, 55.97; H, 3.82; N, 12.24. Found: C, 56.34; H, 4.08; N, 12.68.

#### General Procedure for the Synthesis of Compounds 10a–d

To a cold stirred solution of **9b** or **9d** (2.50 mmol) and NaOH (0.20 g, 5 mmol) in distilled water, acetyl chloride or benzoyl chloride (4 mmol) was added at 5°C portion wise. The reaction mixture was stirred at room temperature for 2h, and then acidified with 10% hydrochloric acid till neutralization; the solid formed was filtered, washed with water, dried and crystallized from ethanol.

(1-(4-Chlorophenyl)-5-oxo-3-phenyl-1*H*-pyrazol-4(5*H*)-ylidene)methyl Acetate (**10a**): Yield 58%. mp 290–291°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.49 (3H, s, CH<sub>3</sub>), 7.44–8.00 (10H, m, Ar-H+C=CH). IR (KBr) cm<sup>-1</sup>: 3055 (CH aromatic), 2924, 2850 (CH aliphatic), 1700 (2C=O). MS *m/z*: 340 (M<sup>+</sup>), 338 (M-2). Anal. Calcd for C<sub>18</sub>H<sub>13</sub>ClN<sub>2</sub>O<sub>3</sub> (340.76): C, 63.44; H, 3.85; N, 8.22. Found: C, 63.42; H, 3.89; N, 8.45.

(1-(4-Chlorophenyl)-5-oxo-3-phenyl-1*H*-pyrazol-4(5*H*)-ylidene)methyl Benzoate (**10b**): Yield 65%. mp 286–288°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>) δ: 7.42–8.06 (15H, m, Ar-H+C=CH). IR (KBr) cm<sup>-1</sup>: 3055 (CH aromatic), 1693 (2C=O). MS *m/z*: 404 (M<sup>+</sup>+2), 402 (M<sup>+</sup>). Anal. Calcd for C<sub>23</sub>H<sub>15</sub>ClN<sub>2</sub>O<sub>3</sub> (402.82): C, 68.58; H, 3.75; N, 6.95. Found: C, 68.63; H, 3.72; N, 7.08.

(5-Oxo-3-phenyl-1-(4-sulfamoylphenyl)-1*H*-pyrazol-4(5*H*)-ylidene)methyl Acetate (**10c**): Yield 66%. mp 336–337°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 2.46 (3H, s, CH<sub>3</sub>), 7.32–8.04 (12H, m, Ar-H+C=CH+ex, NH<sub>2</sub>). IR (KBr) cm<sup>-1</sup>: 3329, 3271 (NH<sub>2</sub>), 3070 (CH aromatic), 2981, 2924 (CH aliphatic), 1732 (2C=O). MS *m/z*: 385 (M<sup>+</sup>). Anal. Calcd for C<sub>18</sub>H<sub>15</sub>N<sub>3</sub>O<sub>5</sub>S (385.39): C, 56.10; H, 3.92; N, 10.90. Found: C, 56.18; H, 4.03; N, 11.04.

(5-Oxo-3-phenyl-1-(4-sulfamoylphenyl)-1*H*-pyrazol-4(5*H*)-ylidene)methyl Benzoate (**10d**): Yield 78%. mp 274–276°C. <sup>1</sup>H-NMR (DMSO-*d*<sub>6</sub>) δ: 7.23–8.16 (17H, m, Ar-H+C=CH+ex, NH<sub>2</sub>). IR (KBr) cm<sup>-1</sup>: 3344, 3251 (NH<sub>2</sub>), 3070, 3028 (CH aromatic), 2962, 2924 (CH aliphatic), 1685 (2C=O). MS *m/z*: 447 (M<sup>+</sup>). Anal. Calcd for C<sub>23</sub>H<sub>17</sub>N<sub>3</sub>O<sub>5</sub>S (447.46): C, 61.74; H, 3.83; N, 9.39. Found: C, 61.81; H, 3.79; N, 9.52.

**Pharmacological Screening** The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval, Faculty of Pharmacy, Cairo University.

**Anti-inflammatory (in Vivo Screening)** All the targeted compounds were evaluated for their anti-inflammatory activity using carrageenan-induced rat paw edema method of Winter *et al.*<sup>38</sup> The employed technique is based on the ability of the tested compounds to inhibit the edema produced in the hind paw of the rat after injection of carrageenan. Male Wistar albino rats (obtained from the animal house of Faculty of Pharmacy, Cairo University) weighing 120–180 g were used to investigate the carrageenan-induced rat paw edema. The rats were kept in animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into 38 groups of five rats each.

The initial hind paw volume of rats was determined volumetrically by means of plethysmometer 7150 (UGO, Basile, Italy). Phenylbutazone (reference standard) and the tested compounds suspended in 2% Tween 80 were administered intraperitoneally at a dose of 0.32 mmol/kg body weight, while the control group received only 2% Tween 80, 1 h before induction of inflammation. The paw edema was induced by subplantar injection of (0.1 mL) of 1% carrageenan solution in saline (0.9%). Paw edema volume was measured after 2 and 3 h using the plethysmometer and compared with the initial hind paw volume of each rat. The difference of average values between treated and control group is calculated for each time interval and evaluated statistically. Quantitative variables from normal distribution were expressed as means  $\pm$  standard error (S.E.M.). The anti-inflammatory activity was expressed as percentage inhibition of edema volume in treated animals in comparison with the control group according to the following equation:

$$\% \text{ inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where  $V_c$  is the mean of edema volume of rat paw after administration of carrageenan in the control group,  $V_t$  is the mean of edema volume of rat paw after administration of the tested compounds or the reference drugs.

The  $IC_{50}$  values of the compounds **3a, b, d, e**, **4a–d**, **5a–d**, **6a–d**, **9a–d**, and phenylbutazone were determined according to the previous reported procedure<sup>38)</sup> using different doses ranged from 0.08 to 1.30 mmol/kg body weight

**Analgesic Activity** Compounds that exhibited good anti-inflammatory activity and phenylbutazone were screened for their analgesic activity using the reported method of *p*-benzoquinone-induced writhing in mice by Okun *et al.*<sup>39)</sup> Adult male albino mice weighing 20–25 g were used in this study. Phenylbutazone (reference standard) and the tested compounds were prepared as suspension in 2% Tween 80. A sensitivity test was carried out one day before drug administration,<sup>47)</sup> were the animals were injected intraperitoneally with 0.20–0.25 mL of 0.02% freshly prepared solution of *p*-benzoquinone in distilled water. Animals showing writhing to *p*-benzoquinone within 30 min were chosen for studying the analgesic activity. On the next day, mice were divided into 19 groups of six animals each. The control group received only 2% Tween 80 while the rest of the groups received the reference standards and the tested compounds at a dose of 0.32 mmol/kg body weight. After one hour, 0.02% solution of *p*-benzoquinone was administered intraperitoneally and the animals were observed for 30 min after injection of the irritant, the animals showing writhing were counted in each group. Writhing is known as stretch, torsion to one side, drawing up of hind leg, retraction of the abdomen, so that the belly of mouse touches the floor. The mice showing any of the previous signs are counted as positive responses and this method depend on the ability of tested compounds to protect the animals from writhing signs made by *p*-benzoquinone. The analgesic activity was evaluated as the percentage protection of tested animals against irritant *p*-benzoquinone induced writhing response compared with the control group according the following equation:

$$\% \text{ protection} = \frac{\text{number of protected animals}}{\text{total number of animals}} \times 100$$

**Ulcerogenic Effect** The ulcerogenic effect of the six most

active compounds as anti-inflammatory agents and phenylbutazone was evaluated by the reported method of Meshali *et al.*<sup>40)</sup> Adult male albino rats weighing 120–180 g were used in this study. Animals were fasted eighteen hours before the drug administration, then divided into 8 groups each of five animals and received the drug orally. The first group received 2% tween 80 and kept as control, the second group received phenylbutazone in a dose of 0.32 mmol/kg body weight and the rest of the groups were received 6 tested compounds in the same dose.

Food was allowed two hours after administration of the drugs and rats were received the same dose orally for three successive days. Two hours after the last dose, rats were sacrificed, the stomach of each rat were removed, opened along greater curvature and cleaned by washing with cold saline. The stomach was stretched on a corkboard using pins and examined with a magnifying lens (10 $\times$ ) for the presence of ulcers and erosions. Ulcer index was calculated according to the method of Robert *et al.*<sup>41)</sup> The degree of ulcerogenic effect was expressed in term of percentage incidence of ulcer in each group of animals divided by 10, the average number of ulcers per stomach and the average severity of ulcers by visual observation. The ulcer index was expressed as summation value of the above three values.

**Acute Toxicity**  $LD_{50}$  of some representative compounds was determined using Finney's method.<sup>42)</sup> Adult male mice weighing 20–25 g were divided into groups each of six animals. Minimal dose that killed all animals and the maximal dose that failed to kill any animal were determined *via* several increasing intraperitoneal doses. Animals were kept under observation for 24 h during which any mortality in each group were recorded.

**In Vitro COX Inhibition Study** The most active compounds as anti-inflammatory **6b**, **7b**, **9b**, and indomethacin were tested for their ability to inhibit COX-1 and/or COX-2 using Cayman's COX-Activity Assay kit (catalogue No. 760151, Cayman Chemicals, Ann Arbor, MI, U.S.A.) which measure the peroxidase activity of COX by the method of Kulmacz and Lands.<sup>43)</sup> COX-1/COX-2 percentage inhibition activity ratio of the tested compounds and indomethacin was calculated and recorded.

**2D-QSAR Study. Data Set** A data set of twenty compounds was used for the present QSAR study. All the molecular modeling calculations and docking simulation studies were performed using Molecular Operating Environment (MOE<sup>®</sup>) version 10.2010, Chemical Computing Group Inc., Montreal, Canada. The computational software operated under "Windows XP" installed on an Intel Pentium IV PC with a 1.6 GHz processor and 512 MB memory. All the interaction energies and different calculations were automatically calculated. The biological activity values  $IC_{50}$  were converted to negative logarithmic ( $P\ IC_{50}$ ) and used as the dependant variable for the QSAR analysis. Thirteen different molecular descriptors (independent variables) were selected and calculated for the submitted structures aiming cover a wide range of different electronic, hydrophobic and topological characters. The correlation matrix was calculated to avoid multicollinearity between the calculated descriptors. The correlation matrix indicated that some of the descriptors used are highly correlated which suggests avoiding the combinations between such intercorrelated descriptors ( $|r| \geq 0.80$ , where  $r$  is the simple linear



coefficient). QSAR model was then constructed after ensuring reasonable correlation of anti-inflammatory activity with individual descriptor and minimum inter-correlation among the descriptors used in derived model.

**Statistical Analysis** A mathematical structure–activity equation established a statistical relationship between a dependent variable (biological activity) and a set of independent variables (descriptors).<sup>48,49</sup> Stepwise linear regression analysis (SLRA) technique was used to test the best structural predictors for activity. For the current dataset of 20 compounds, the QSAR model development was restricted to a maximum of three variables in accordance to the general accepted rule for the compounds: descriptors ratio to be around 5 : 1. The developed QSAR models are evaluated using the following statistical measures as root mean square error (RMSE) and correlation coefficient ( $R^2$ ) and validated by the leave-out technique (LOO technique), where each object of the data set is taken away, one at a time. In this case, given  $n$  objects,  $n$  reduced models are developed. The predicted activities (P IC<sub>50</sub>) for the tested compounds calculated using multi-linear regression (\$\$PRED) technique.

**Acknowledgments** The Authors would like to thank Prof. Dr. Ashraf B. El-Den, Department of Pharmacology, Faculty of Pharmacy, Ain-Shams University for carrying out the *in vitro* screening and Prof. Dr. Nadia Mahfouz, Department of Medicinal Chemistry, Faculty of Pharmacy, Assuit University for carrying out the 2D-QSAR study. Also, thank Dr. Walaa Wadie, Department of Pharmacology, Faculty of Pharmacy, Cairo University for her helping during carrying the *in vivo* study.

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