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Original article

Synthesis and anti-inflammatory evaluation of some condensed [4-(3,4-dimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl]acetic acid hydrazide

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ABSTRACT

Some new 1,3,4-triazolo-, 1,3,4-oxadiazolo-, 1,3,4-thiadiazol-, and pyrazolo-3,4-dimethylphenyl-1(2*H*)-oxo-phthalazine derivatives were synthesized and identified by IR, ^{1}H NMR, MS and elemental analysis. Most of the newly synthesized products were tested for their anti-inflammatory activities. Among them, compounds **11**, **17b**, **20**, **21** and **22** are active compare to the activity of indomethacin®.

1. Introduction

Most of the current nonsteroidal anti-inflammatory drugs (**NSAIDs**) show serious side effects including gastrointestinal disorders and kidney damage. Therefore, studies for developing safer **NSAIDs** lacking the gastrointestinal and renal side effects of currently used ones have recently been of interest for many researchers. Most of the classical **NSAIDs** exert their side effects by inhibition of **COX-1** enzyme. Since the **COX-1** isoform is the constitutive one that is responsible for regulation of physiological processes, and the **COX-2** isoform is discovered to be the enzyme induced by a inflammatory stimuli, selective inhibition of **COX-2** provides a rationale for developing anti-inflammatory and analgesic agents that lack the **GI** liabilities exhibited by currently marketed **NSAIDs**. Although the diarylheterocyclic compounds are mainly studied as new class of **NSAIDs** without gastric side effects, many studies have also focused on a different type of compounds to develop safer **NSAIDs** [1].

In the last few years, the attention was oriented towards the synthesis and biological evaluation of phthalazine derivatives as they exhibit a board spectrum of biological activities. Indeed, several phthalazine derivatives have been reported to possess antitumor [2–5], antihypertensive [6,7], antithrombotic [8], anticonvulsant [9,10], antidiabetic [11,12], antimicrobial [13], anti-trypanosomal [14], and anti-inflammatory activities [1,15–20].

In view of the aforementioned facts, it seemed most interesting to synthesize some condensed [4-(3,4-dimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl]acetic acid hydrazides with the aim to evaluate their anti-inflammatory activities.

2. Results and discussion

2.1. Chemistry

4-(3,4-Dimethylphenyl)phthalazin-1(2*H*)-one (**3**) was obtained from the reaction of 2-(3,4-Dimethylbenzoyl)benzoic acid (**2**) with hydrazine hydrate. The IR spectrum showed a characteristic absorption band at $\nu = 1664\ cm^{-1}$ corresponding to CO group. The ^{1}H NMR spectrum showed NH at $\delta = 11.17$ ppm. Compound **3** was treated with ethyl chloroacetate to afford the corresponding phthalazine acetic acid ethyl ester **4** (Scheme 1). The structure of compound **4** was confirmed by spectral data (IR, ^{1}H NMR, and MS). Its mass spectrum shows the prominent ion peak at *m*/*z* 336 (M^+, 60). The ^{1}H NMR showed a triplet signal at δ 1.20 assigned for CH_3CH_2, a quartet signal at 4.20 assigned for CH_2CH_3, a singlet

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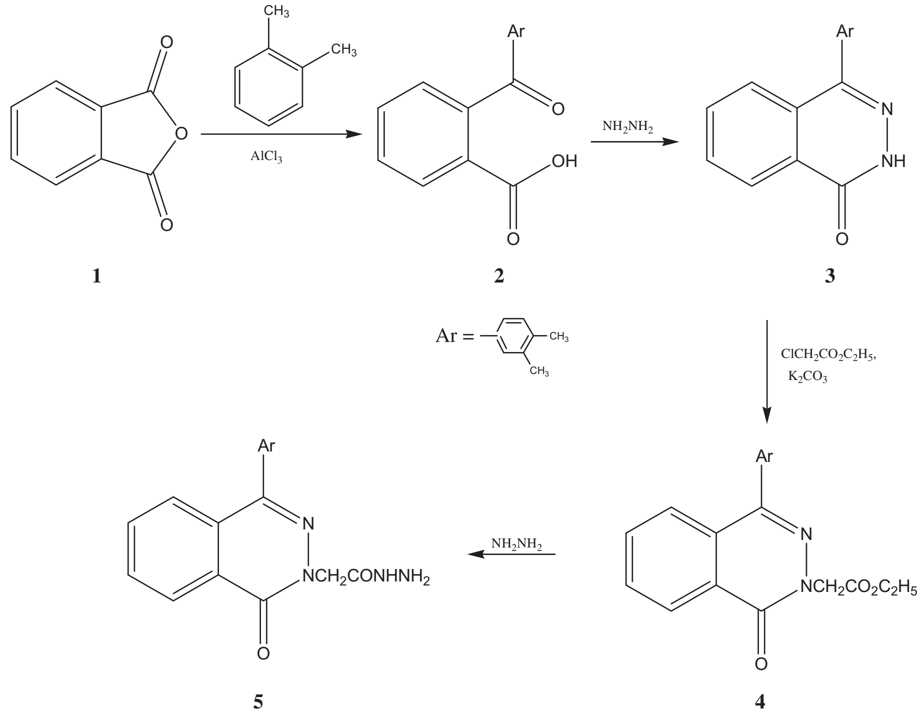


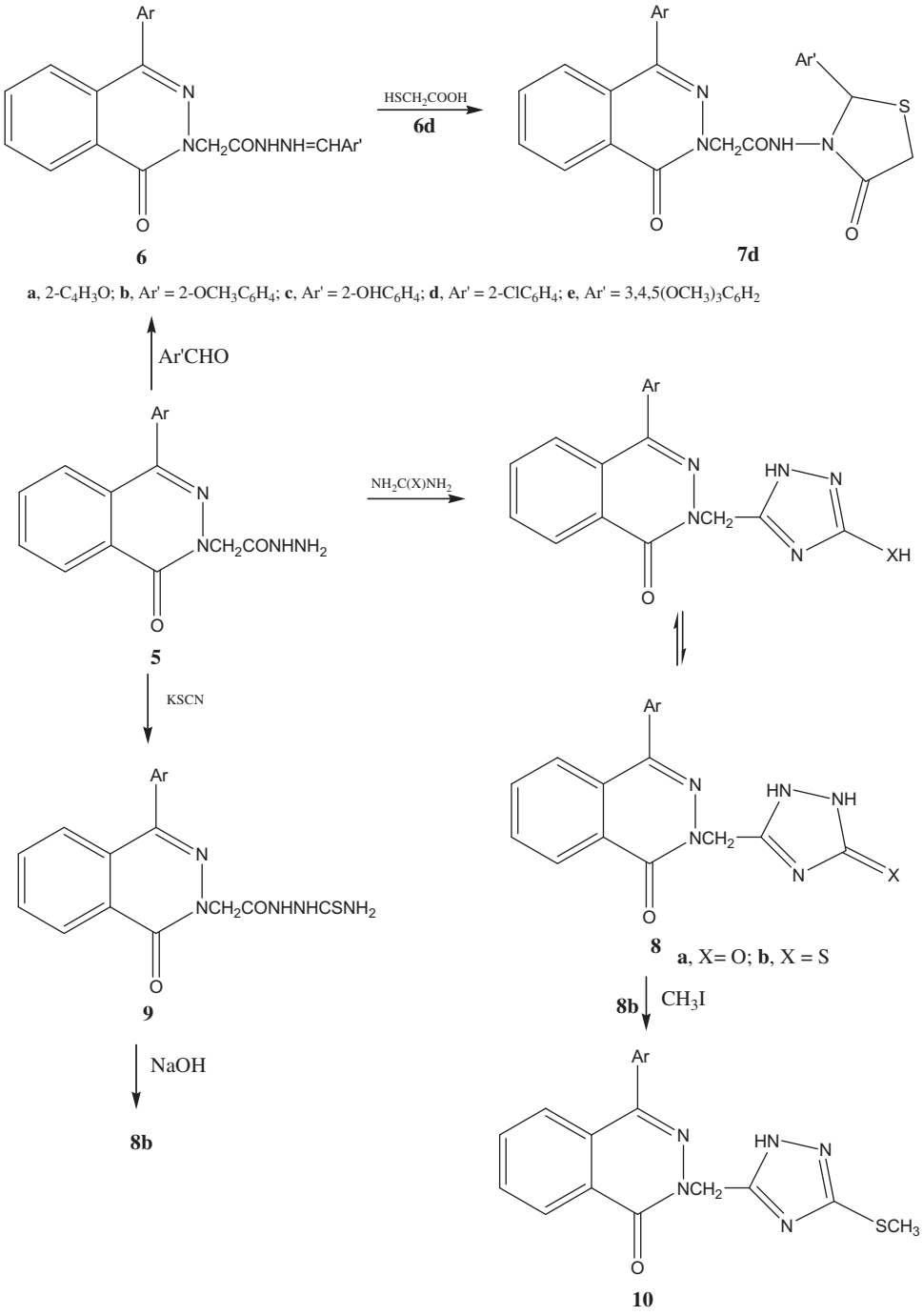
Scheme 1.

signal at 4.97 assigned for CH_2CO, besides 2 CH_3 and aromatic protons. The phthalazine acetic acid ethyl ester **4** was converted to the corresponding hydrazide **5** in high yield by the reaction with hydrazine hydrate (Scheme 1). The ^1H NMR of compound 5 shows $\delta = 4.40$ (s, 2H, NH_2 exchangeable with D_2O), 4.90 (s, 2H, CH_2CO), 9.30 (s, 1H, NH exchangeable with D_2O) ppm and aromatic protons. Whereas, the hydrazide derivatives constitute a class of compounds that have served as useful intermediates towards construction of different heterocyclic compounds [21]. The hydrazide derivative **5** reacted with some aromatic aldehydes namely, furfural, 2-methoxybenazldehyde, 2-hydroxybenzaldehyde, 2-chlorobenzaldehyde, and 3,4,5-trimethoxybenzaldehyde to obtain the corresponding phthalazinyl acetic acid methylidene hydrazides **6a**–**e** (Scheme 2). The ^1H NMR spectra of compounds **6a**–**e** showed the methylidene protones at $\delta = 8.96$–9.33 ppm. On the other hand, [4-(3,4-dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid [1-(2-chlorophenyl)methylidenehydrazide] (**6d**) reacted with thioglycolic acid to afford the corresponding thiazolidine derivative **7d** in moderate yield (Scheme 2). The ^1H NMR of compound **7d** shows $\delta = 3.02$ for assigned SCH_2, 5.50 for assigned CH, 8.80 ppm for assigned NH, and the aromatic protons.

However, fusion of the hydrazide derivative **5** with urea and/or thiourea afforded the corresponding triazolo phthalazines **8a** and **8b**, respectively. The ^1H NMR of compound **8a** showed $\delta = 8.57$, 10.77 ppm assigned for 2 NH which are exchangeable with D_2O. The IR spectrum of compound **8a** revealed the absence of hydroxyl group. So, the structure of compound **8a** exists in the keto-form. The ^1H NMR of compound **8b** revealed that the structure exists predominantly in the enol-form, whereas SH appears at $\delta = 4.35$ and NH at $\delta = 8.56$ ppm. The mass spectrum of compound **8b** showed the prominent ion peak at m/z (%) = 263 (M^+, 70). However, triazolo phthalazine **8b** could be obtained, in higher yield than that obtained previously, through cyclization at $\delta = 4.35$ and NH at $\delta = 8.56$ ppm. The mass spectrum of compound **8b** showed the prominent ion peak at m/z (%) = 263 (M^+, 70). However, triazolo phthalazine **8b** could be obtained, in higher yield than that obtained previously, through cyclization at $\delta = 4.35$ and NH at $\delta = 8.56$ ppm. The mass spectrum of compound **8b** showed the prominent ion peak at m/z (%) = 263 (M^+, 70). However, triazolo phthalazine **8b** could be obtained, in higher yield than that obtained previously, through cyclization of phthalazine thiosemicarbazide **9** with sodium hydroxide (Scheme 2). The treatment of mercapto triazolo phthalazine **8b** with methyl iodide gave the corresponding methylthio triazolo phthalazine **10**. Its ^1H NMR showed SCH_3 at $\delta = 2.53$ ppm.

Accordingly, the hydrazide **5** reacted with carbon disulfide to obtain the corresponding oxadiazole thione **11** which is converted to the methylthio oxadiazolo phthalazine **12** (Scheme 3). The ^1H NMR of compound **11** revealed the signal at $\delta = 7.06$ ppm assigned for NH. Whatever, the ^1H NMR of compound **12** showed the absence of NH and showed the signal at $\delta = 2.55$ ppm assigned for SCH_3. Also, the hydrazide **5** reacted with benzoyl chloride and acetyl chloride to give the compounds **13a**,**b**, respectively. The ^1H NMR of compound **13a** showed 2 NH at $\delta = 10.08$ and 10.21 ppm, and the ^1H NMR of compound **13b** showed $COCH_3$ at $\delta = 2.04$ ppm. The derivatives **13a**,**b** are cyclized to the corresponding oxadiazolo phthalazines **14a**,**b**, respectively by the reaction with phosphorus oxychloride (Scheme 3). The required structures **14a**,**b** were confirmed by spectral data (see the experimental part). However, The derivatives **13a**,**b** are cyclized to the corresponding pyrazolo phthalazines **15a**,**b**, respectively by the reaction with sodium ethoxide (Scheme 3). The ^1H NMR of compounds **15a**,**b** showed the pyrazolo protons at $\delta = 7.10$ and at $\delta = 7.00$ ppm, respectively.

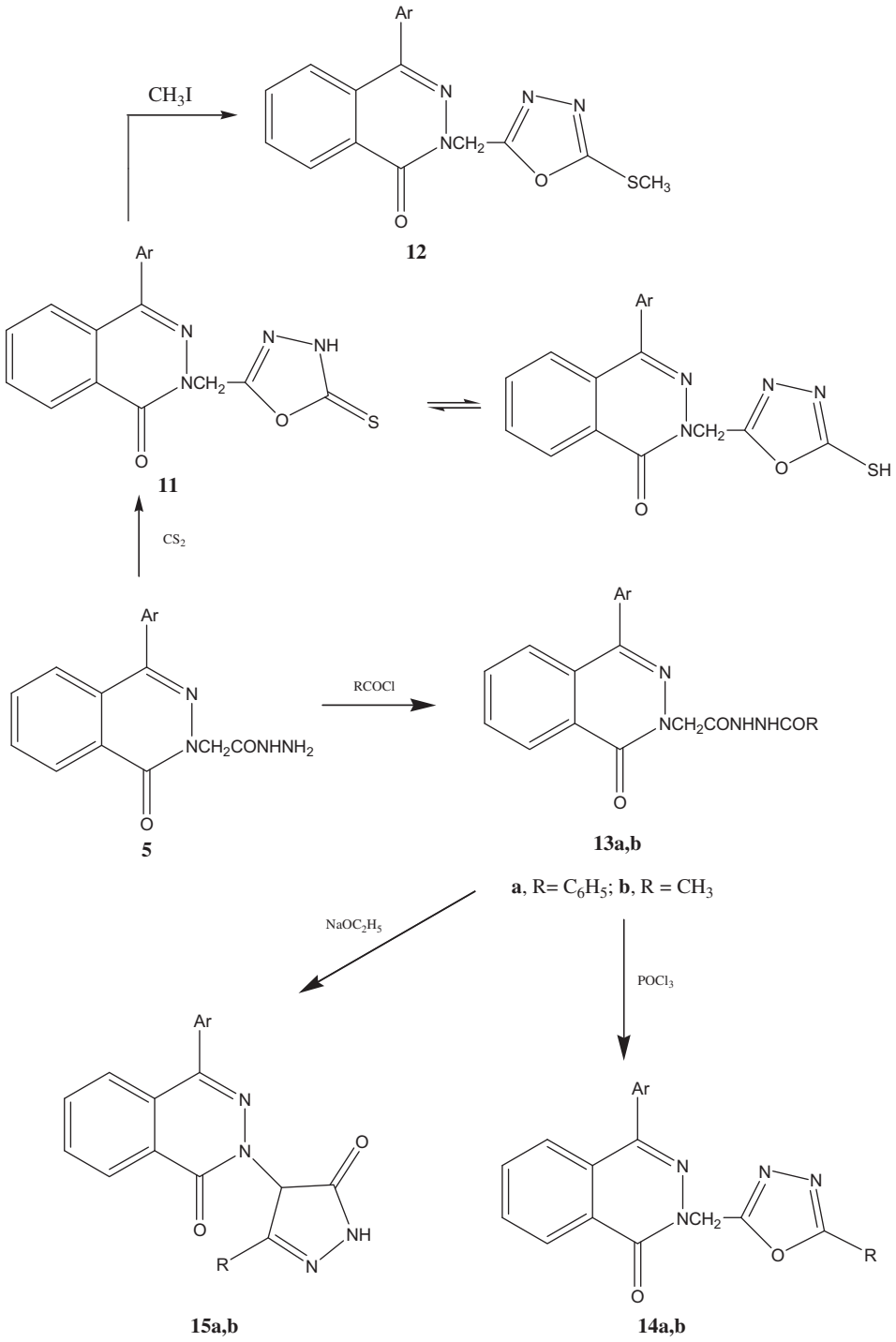
The hydrazide derivative **5** reacted with acrylonitrile to afford the corresponding cyanoethyl hydrazide **16** in good yield. The structure of compound **16** was confirmed by the ^1H NMR, where the ethyl protones appear at $\delta = 3.02$, and 3.17. Also, the hydrazide derivative **5** reacted with three isothiocyanate derivatives, namely phenyl, benzyl, and methyl to give the corresponding thiocarbamate derivatives **17a**–**c**, respectively. Similarly, the hydrazide derivative **5** reacted with phenyl and ethyl isocyanate to produce the corresponding carbamate derivatives **18a**,**b**, respectively. The



Scheme 2.

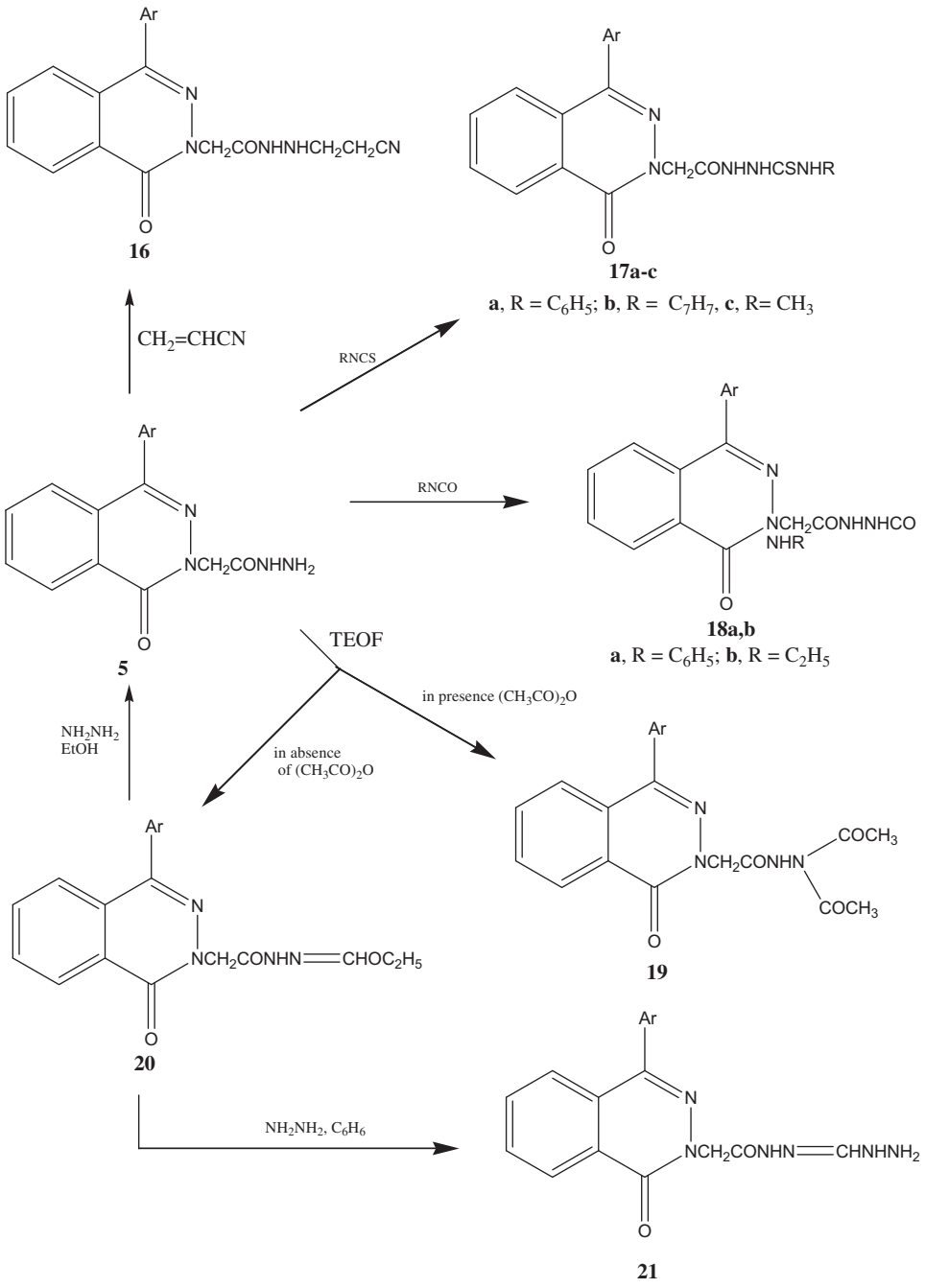
^{1}H NMR of compound **17a** showed $\delta = 10.08$ (s, 1H, CONH, exchangeable with D_2O), 12.52 (s, 1H, NHPh, exchangeable with D_2O) ppm. The ^{1}H NMR of compound **18a** showed $\delta = 9.26$ assigned for NHPh (exchangeable with D_2O), and 10.14 ppm assigned for CONH (exchangeable with D_2O). However, the hydrazide derivative reacted with triethyl orthoformate in the presence of acetic anhydride gave 2-*N*,*N*-diacetyl hydrazide derivative **19** rather than ethyl imidate derivative **20** which is obtained via the reaction of the hydrazide **5** with triethyl orthoformate only. The ^{1}H NMR of compound **19** showed $\delta = 2.48$ ppm assigned for 2 $COCH_3$ whereas the ^{1}H NMR of compound **20** showed $\delta = 1.30$ (t, J, 10 Hz, 3H, CH_3CH_2), 4.10 (q, J, 10 Hz, 3H, CH_3CH_2), and 7.40 (s, 1H, CH=N). The hydrazino hydrazide derivative **21** was obtained from the reaction of ethyl imidate derivative **20** with hydrazine hydrate in benzene, whereas the hydrazide **5** was obtained when the ethyl imidate derivative **20** treated with hydrazine hydrate in ethanol. The ^{1}H NMR of compound **21** showed the absence of ethyl protons and showed 4.35 assigned for NH_2, 4.51 assigned for NH, and 10.58 ppm assigned for CONH. The mass spectrum of compound **21** shows the prominent ion peak at m/z (%) = 364 (M^+, 2) (Scheme 4).

The hydrazide derivative **5** reacted with ethyl acetoacetate to afford the corresponding pyrazolo phthalazine derivative **22** in keto–enol form, which turn cyclized to pyrazolo triazolo phthalazine derivative **23** by the reaction with hydrazine hydrate. The ^{1}H NMR of



Scheme 3.

compound **22** showed $\delta = 2.30$ assigned for CH_3, 4.97 assigned CH_2, and 6.10 ppm assigned for 4-CH_2. The mass spectrum of compound **22** showed the prominent ion peak at m/z (%) = 386 (M^+-2, 4). The structure of compound **23** was verified by the spectral data. Where, the IR showed the absorption band at $\nu = 1664$ cm^{-1} assigned for CO, and the ^1H NMR showed $\delta = 1.94$ (s, 3H, CH_3), 2.46 (s, 6H, 2 CH_3), 2.60 (s, 2H,CH_2), 5.30 (s, 2H,CH_2), 7.28–8.50 (m, 7H, Ar–H) ppm. Accordingly, the hydrazide derivative **5** condensed with acetyl acetone to produce the required derivative **24** which was cyclized to the corresponding pyrazol derivative **25** via treatment with sodium ethoxide (Scheme 5). The ^1H NMR of compound **24** showed $\delta = 1.96$ (s, 3H, N=CCH_3), 2.29 (s, 3H, OCCH_3), 2.54 (s, 2H,N=CCH_2), 10.60 (s, 1H, NH, exchangeable with D_2O) ppm. While, the ^1H NMR of compound **25** showed $\delta = 2.30$ (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 4.64 (s, 2H, CH_2), 6.18 (s, H, CH), ppm. On the other hand, on heating of the hydrazide derivative **5** on oil bath gave the corresponding carboxylic acid **26** (Scheme 5). The structure of acid **26** was confirmed by spectral data. The IR showed the absorption band at $\nu = 2542$–3500 assigned for (OH), 1701, 1667 assigned for (2 CO) cm^{-1}. The ^1H NMR showed $\delta = 2.34$ (s, 6H, 2 CH_3), 4.82(s, 2H, CH_2), 7.30–8.20 (m, 7H, Ar–H) ppm. The mass spectrum showed the prominent ion peak at m/z (%) = 307 (M^+-1, 41).



Scheme 4.

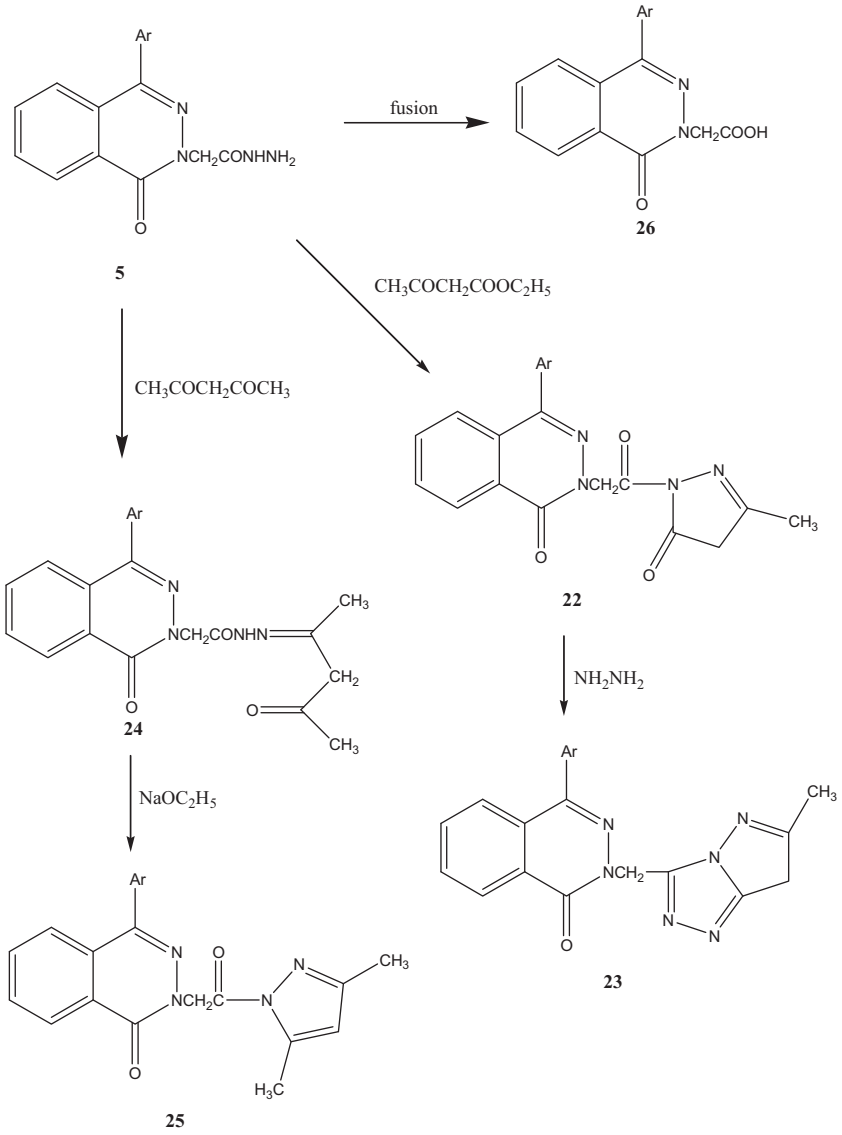
The thiocarbamate derivatives **17a–c** were cyclized to the corresponding triazole derivatives **27a–c** by treatment with sodium hydroxide, while they were cyclized to the corresponding thiadiazolo phthalazine derivatives **28a–c** by using conc. Sulfuric acid (Scheme 6). The required structures were verified by spectral data.

2.2. Anti-inflammatory testing

A series of phthalazin-4-yl acetic acid derivatives [22] was reportedly prepared and showed anti-inflammatory activity in the carrageenin and edema test. Recently, several groups have studied the structure-activity relationship of novel series of 4-aryl-substituted phthalazinones [17,23] and showed that the presence of 4-aryl substituted on the phthalazinone nucleus contributes to a good anti-inflammatory and nociceptive activities. It is also well-known that some heteroaryl acetic acids possess anti-inflammatory activities [24,25]. Substitution of the acetamide side chain on the lactam nitrogen in the phthalazinone derivatives [1] resulted in compounds having considerably high antinociceptive and anti-inflammatory activities without gastric lesion and bleeding at the given dose.

It was of interest to prepare a number of 4-aryl-oxophthalazine-2-yl acetic acid derivatives for pharmacological screening.

Various pyrazole derivatives including the drugs butazolidin, celebrex were reported as effective anti-inflammatory agents [1,26]. Consequently, it was though worthwhile to incorporate



Scheme 5.

pyrazole moieties in the 2 position of phthalazinone nucleus in a hope to yield safe and potent anti-inflammatory compounds.

Compounds **11**, **17b**, **20**, **21** and **22** showed the higher anti-inflammatory activities than that of the reference drug (Table 1). The presence of: oxadiazole thione ring in compound **11**, tolyl thiocarbamate group in compound **17b**, ethoxy imidine moiety in derivative **20**, hydrazino imidine function in derivative **21**, and pyrazolone ring in compound **22**, it might be, increased the anti-inflammatory activity. The most potent anti-inflammatory activities were recorded for compound **17b**.

3. Conclusion

We reported here the synthesis of different 4-(3,4-dimethylphenyl)phthalazin-1(2*H*)-ones. Most of the newly synthesized compounds were tested for their anti-inflammatory activity. The results in Table 1 revealed that compounds **11**, **17b**, **20**, **21** and **22** are active compared to the activity of indomethacin®, while all other compounds showed no significant difference compared to that of reference drug.

4. Experimental

4.1. Chemistry

All melting points are uncorrected and measured using Electrothermal IA 9100 apparatus (Shimadzu, Japan). IR spectra were recorded as potassium bromide pellets on Bruker FT-IR spectrophotometer and the data are given in ν_{max} (cm^{-1}), Faculty of Science, Cairo University, Cairo, Egypt. ^1H NMR spectra were determined on a Jeol-Ex-300 NMR spectrometer and chemical shifts were expressed as part per million; ppm (δ values) against TMS as internal reference (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were recorded on 3 and EI + Q1 MSLMR UPLR, National Research Centre, Cairo, Egypt. Microanalyses were operated at the Microanalytical Center, Cairo University, Cairo, Egypt and the results were within the accepted range (±0.4%) of the calculated values. 2-(3,4-Dimethybenzoyl)benzoic acid (**2**) was obtained as reported [27].

4.1.1. 4-(3,4-Dimethylphenyl)phthalazin-1(2H)-one (**3**)

Hydrazine hydrate (98%) 0.5 mL was added to a solution of 5 g **2** (0.02 mol) in 20 mL absolute ethanol. The reaction mixture was

Table 1
Anti-inflammatory activity of new [4-(3,4-dimethylphenyl)-1(2*H*)-oxo-phthalazin-2-yl] acetic acid hydrazides in the sponge implantation test.

Groups	Mean differences	*q*-Value	*p*-Value	Conclusion
Control (c) *vs* indomethacin	14.47	8.339	$P < 0.001$	Significant
c vc **6a**	7.81	3.921	$P > 0.05$	Non significant
c vc **6b**	7.42	4.639	$P > 0.05$	Non significant
c vc **6c**	7.91	3.737	$P > 0.05$	Non significant
c vc **6d**	8.72	2.246	$P > 0.05$	Non significant
c vc **8b**	8.44	2.761	$P > 0.05$	Non significant
c vc **9**	7.54	4.418	$P > 0.05$	Non significant
c vc **11**	15.23	9.738	$P < 0.001$	Significant
c vc **13a**	7.26	4.934	$P > 0.05$	Non significant
c vc **13b**	8.93	1.859	$P > 0.05$	Non significant
c vc **16**	8.61	2.448	$P > 0.05$	Non significant
c vc **17a**	8.12	3.350	$P > 0.05$	Non significant
c vc **17b**	17.15	13.273	$P < 0.001$	Significant
c vc **17c**	7.45	4.584	$P > 0.05$	Non significant
c vc **18a**	7.74	4.050	$P > 0.05$	Non significant
c vc **18b**	8.67	2.338	$P > 0.05$	Non significant
c vc **19**	8.24	3.871	$P > 0.05$	Non significant
c vc **20**	15.12	9.536	$P < 0.001$	Significant
c vc **21**	14.37	10.351	$P < 0.001$	Significant
c vc **22**	15.532	9.862	$P < 0.001$	Significant
c vc **24**	7.94	3.985	$P > 0.05$	Non significant
c vc **26**	6.87	4.538	$P > 0.05$	Non significant

refluxed for 2 h, after cooling the obtained solid was filtered off and crystallized from ethanol to give 4.6 g **3** in 92% yield as colorless crystals. Mp 264–266 °C. IR (KBr, ν cm^{-1}): 3157(NH), 1664(CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.46 (s, 6H, 2 CH_3), 7.28–8.50 (m, 7H, Ar–H), 11.17 (s, 1H, NH, exchangeable with D_2O). MS (*m*/*z*, %): 250 (M^+, 81), 249 (100). Anal. ($C_{16}H_{14}N_2O$).

*4.1.2. [4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid ethyl ester (**4**)*

A mixture of 3.0 g **3** (0.012 mol), 5.8 g ethyl chloroacetate (0.048 mol) and 6.7 g potassium carbonate (0.048 mol) in 30 mL dry acetone was heated under reflux for 30 h, cooled at room temperature and poured into water. The obtained solid was filtered off and crystallized from petroleum ether 40–60 °C to give 2.7 g **4** in yield 70% as yellowish white crystals. Mp 140–143 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.20 (t, $J = 10$ Hz, 3H, CH_3CH_2), 2.46 (s, 6H, 2 CH_3), 4.20 (q, $J = 10$ Hz, 2H, CH_2CH_3), 4.97 (s, 2H, CH_2CO), 7.28–8.36 (Ar–H). MS (*m*/*z*, %): 336 (M^+, 60), 263 (100). Anal. ($C_{20}H_{20}N_2O_3$).

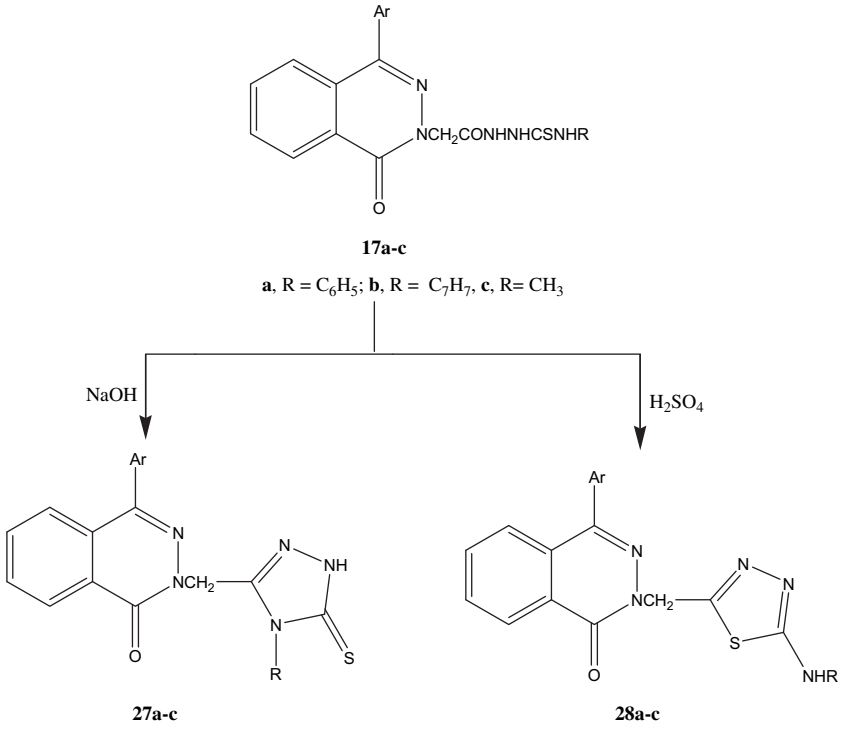
*4.1.3. [4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid hydrazide (**5**)*

A mixture of 3.2 g **4** (0.01 mol) and 2 mL hydrazine hydrate in 50 mL absolute ethanol was refluxed for 3 h and cooled at room temperature. The resultant solid was filtered and crystallized from ethanol to give 2.5 g **5** in yield 79% as colorless crystals. Mp 238–240 °C. IR (KBr, ν cm^{-1}): 3335,3300 (NH_2), 3191 (NH), 1652(CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.30 (s, 6H, 2 CH_3), 4.40 (s, 2H, NH_2 exchangeable with D_2O), 4.90 (s, 2H, CH_2CO), 7.31–8.35 (Ar–H), 9.30 (s, 1H, NH exchangeable with D_2O). Anal. ($C_{18}H_{18}N_4O_2$).

*4.1.4. Reaction of phthalazine hydrazide derivative **5** with some aromatic aldehydes*

A mixture of 0.3 g the acetic acid hydrazide **5** (0.001 mol) and an appropriate aromatic aldehyde (0.001 mol) was refluxed in 20 mL absolute ethanol for 3 h. After cooling the separated solid was collected by filtration, dried and crystallized from the proper solvent to give aryl methylidene hydrazide derivatives **6a–e**.

*4.1.4.1. [4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid (1-furylmethylidene)hydrazide (**6a**).* >0.32 g **6a** in yield 80% as faint brown crystals from toluene. Mp 232–234 °C. IR (KBr, cm^{-1}): 3198 (NH), 1655 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.35 (s, 6H, 2 CH_3), 5.45 (s, 2H, CH_2), 5.70(s, 1H, NH, exchangeable with D_2O), 6.39–7.97 (m, 10 H, Ar–H), 8.97 (s, 1H, CH). Anal. ($C_{23}H_{20}N_4O_3$).



Scheme 6.

*4.1.4.2. [4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid [1-(2-methoxyphenyl)methylidenehydrazide] (**6b**).* 0.34 g **6b** in yield 78% as colorless crystals from ethanol. Mp 152–154 °C. IR (KBr, ν cm^{-1}): 3282 (NH), 1649 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.30 (s, 6H, 2 CH_3), 3.81 (s, 3H, OCH_3), 5.27(s, 1H, NH, exchangeable with D_2O), 5.50 (s, 2H, CH_2), 6.80–7.85 (m, 11H, Ar–H), 8.96(s, 1H, CH). Anal. ($C_{26}H_{24}N_4O_3$).

*4.1.4.3. [4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid [1-(2-hydroxyphenyl)methylidenehydrazide] (**6c**).* 0.32 g **6**c in yield 75% as colorless crystals from toluene. Mp 220–224 °C. IR (KBr, ν cm^{-1}): 4200 (OH), 1619 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.33 (s, 6H, 2 CH_3), 4.93 (s, 2H, CH_2), 6.16(s, 1H, NH, exchangeable with D_2O), 6.85–7.99 (m, 11H, Ar–H), 8.74 (s, 1H, CH), 11.26 (s, 1H, OH, exchangeable with D_2O). Anal. ($C_{25}H_{22}N_4O_3$).

*4.1.4.4. [4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid [1-(2-chlorophenyl)methylidenehydrazide] (**6d**).* 0.36 g **6d** in yield 82% as yellow crystals from toluene. Mp 240–244 °C. IR (KBr, ν cm^{-1}): 3207 (NH), 1659 (CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.35 (s, 6H, 2 CH_3), 5.01 (s, 2H, CH_2), 6.09 (s, 1H, NH, exchangeable with D_2O), 6.75–8.00 (m, 11H, Ar–H), 8.99(s, 1H, CH). MS (m/z, %): 446 (M^+Cl^{37}, 1), 444 (M^+Cl^{35}, 4), 263 (100). Anal. ($C_{25}H_{21}ClN_4O_2$).

*4.1.4.5. [4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetic acid [1-(3,4,5-trimethoxyphenyl)methylidenehydrazide] (**6e**).* 0.43 g **6e** in yield 85% as colorless crystals from toluene. Mp 232–236 °C. IR (KBr, ν cm^{-1}): 3177 (MH), 1665 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.32 (s, 6H, 2 CH_3), 3.34 (s, 9H, 3 CH_3), 4.88 (s, 2H, CH_2), 6.33 (s, 1H, NH, exchangeable with D_2O), 7.31–8.66 (m, 9H, Ar–H), 8.54(s, 1H, CH). Anal. ($C_{28}H_{28}N_4O_5$).

*4.1.5. 2-[4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]N-[4-oxo-2-(2-chlorophenyl)thiazolidin-3-yl]acetamide (**7d**)*

A mixture of 0.6 g chloromethylidene hydrazide derivative **6d** (0.0013 mol), 0.1 mL thioglycolic acid (0.0013 mol), 0.5 g anhydrous zinc chloride and few drops of piperidine was refluxed in 20 mL dry *N*,*N*-dimethylformamide for 24 h. The reaction mixture was then poured onto water and the precipitated solid was filtered off, washed several times with water, dry to give 0.32 g **7d** in yield 53% as yellowish brown crystals from toluene. Mp 298–301 °C. IR (KBr, ν cm^{-1}): 3177 (NH), 1715, 1663 (2 CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.31 (s, 6H, 2 CH_3), 3.02 (s, 2H, SCH_2), 5.00 (s, 2H, CH_2), 5.50 (s, 1H, CH), 6.67–8.50 (m, 11H, Ar–H), 8.80 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{25}H_{23}ClN_4O_2$).

*4.1.6. Reaction of phthalazine hydrazide derivative **5** with urea and/or thiourea*

A mixture of 0.3 g the acetic acid hydrazide **5** (0.001 mol) and urea or thioure (0.001 mol) was heated in oil bath at 195 °C for 8 h. After cooling, the sticky mass was dissolved in 30 mL 8% sodium hydroxide, filtered, and the filtrate was acidified with 2 N HCl. The solid was collected by filtration, dried and crystallized from the proper solvent to give triazolo phthalazine derivatives **8a**,**b**, respectively.

*4.1.6.1. 5-[4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]methyl-1,2,4-triazol-3-one (**8a**).* 0.15 g **8a** in yield 43% as colorless crystals from ethyl acetate. Mp 156–158 °C. IR (KBr, ν cm^{-1}): 3290 (NH), 1650, 1634 (2CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.0(s, 1H, NH, exchangeable with D_2O), 2.34 (s, 6H, 2 CH_3), 4.46(s, 2H, CH_2), 7.20–8.10 (m, 7H, Ar–H), 8.0 (s, 1H, CONH, exchangeable with D_2O). Anal. ($C_{19}H_{17}N_5O_2$).

*4.1.6.2. 2-[(5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-4-(3,4-dimethylphenyl)-phthalazin-1(2H)-one (**8b**).* 0.18 g **8b** in yield 51% as pale yellow crystals from ethanol. Mp 178–181 °C. IR (KBr, ν cm^{-1}): 3205,3154 (2 NH), 1675 (CO), 1255 (CS). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.0 (s, 2H, NH, exchangeable with D_2O), 2.35 (s, 6H, 2 CH_3), 4.46 (s, 2H, CH_2), 7.35–8.18 (m, 7H, Ar–H). MS (m/z, %): 263 (M^+, 70), 249 (21), 236 (100). Anal. ($C_{19}H_{17}N_5OS$).

*4.1.7. 1-{2-[4-(3,4-Dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]acetyl}thiosemicarbazide (**9**)*

A mixture of 0.3 g the acetic acid hydrazide **5** (0.001 mol), 0.09 g potassium thiocyanate (0.00 mol), 0.8 mL conc. HCl, and water 20 mL was refluxed for 12 h. After cooling the precipitated solid was filtered off to give 0.27 g **9** in yield 70% as colorless crystals from ethanol. Mp 184–186 °C. IR (KBr, ν cm^{-1}): 3444 (NH_2), 3290, 3159 (2 NH), 1661 (CO), 1240 (CS). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.96(s, 1H, NHCS, exchangeable with D_2O), 2.36 (s, 6H, 2 CH_3), 5.80 (s, 2H, CH_2), 6.01(s, 1H, CONH, exchangeable with D_2O), 7.26–7.80 (m, 7H, Ar–H), 8.55(s, 2H, NH_2, exchangeable with D_2O). MS (m/z, %) = 265 (M^+–NH_2, 4), 291 (47), 263 (100). Anal. ($C_{19}H_{19}N_5O_2S$).

*4.1.8. Reaction of **9** with NaOH*

A mixture of NaOH (8%, 15 mL) and thiosemicarbazide **9** (0.3 g, 0.001 mol) in 20 mL absolute ethanol was refluxed for 10 h. The solution was then cooled and neutralized with 2 *N* HCl. The solid obtained was filtered, washed with water and crystallized from ethanol to give a product which is identical in Mp, TLC to **8b**; in yield 71%.

*4.1.9. Reaction of **8b** with methyl iodide*

To a suspension of the triazole **8b** (0.36 g, 0.001 mol) in ethanol (15 mL), sodium hydroxide solution (1 mL, 15%) was added followed by the addition of methyl iodide (0.001 mol) and the reaction mixture was refluxed on a water bath for 5 h then left overnight, diluted with water and cooled. The solid product that formed was filtered off to give 0.33 g 2-[(5-methythio-4H-1,2,4-triazol-3-yl) methyl]-4-(3,4-dimethylphenyl)-phthalazin-1(2H)-one (**10**) in yield 87% as pale yellow crystals from dilute ethanol. Mp 159–161 °C. IR (KBr, ν cm^{-1}): 3195 (NH), 1668 (CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, 2 CH_3), 2.53 (s, 3H, SCH_3), 5.00 (s, 2H, CH_2), 7.01–8.10 (m, 7H, Ar–H), 14.05 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{20}H_{19}N_5OS$).

*4.1.10. 4-(3,4-Dimethylphenyl)-2-[(4,5-dihydro-5-thiooxo-1,3,4-oxadiazol-2-yl)methyl] phthalazin-1(2H)-one (**11**)*

0.3 g Hydrazide **5** (0.001 mol) was added to 0.14 g potassium hydroxide solution in 20 mL absolute ethanol. Then, 3 mL carbon disulfide was added portionwise and the reaction mixture was refluxed till no odour of hydrogen sulfide evolved (14 h). The reaction mixture was poured onto ice water and rendered acidic with hydrochloric acid. The precipitated solid was filtered, dried and crystallized from ethanol to give 0, 21 g **6** in yield 58% as yellow crystals. Mp 178–180 °C. IR (KBr, ν cm^{-1}): 3191 (NH), 1655 (CO), 1240 (CS). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, 2 CH_3), 5.02 (s, 2H, CH_2CO), 7.06 (s, 1H, NH exchangeable with D_2O), 7.26–7.76 (Ar–H). MS (m/z, %): 264 (M^+–C_2N_2OS, 23), 103 (41), 64 (100). Anal. ($C_{19}H_{16}N_4O_2S$).

*4.1.11. Reaction of **11** with CH_3I*

To a mixture of absolute ethanol 20 mL containing KOH (0.05 g, 0.001 mol), compound **11** (0.36 g, 0.001 mol) methyl iodide (0.001 mol) was added. The reaction mixture was stirred overnight and poured onto water. The precipitated solid was collected by filtration, dried and crystallized from ethanol to give 1,2-dihydro-4-(3,4-dimethylphenyl)-2-[(5-methylthio)-1,3,4-oxadiazol-2-yl]phthalazine (**12**); 0.19 g in yield 50% as colorless crystals. Mp 120–124 °C. ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, 2

CH_3), 2.55 (s, 3H, SCH_3), 5.10 (s, 2H, CH_2), 7.18–7.80 (m, 7H, Ar–H). Anal. ($C_{20}H_{18}N_4O_2S$).

*4.1.12. Reaction of phthalazine hydrazide derivative **5** with acid chlorides*

A mixture of 0.5 g the acetic acid hydrazide **5** (0.002 mol) and benzoyl chloride or acetyl chloride (0.004 mol) in 20 mL dry *N,N*-dimethylformamide containing few drops of triethylamine was refluxed for 14 h. After cooling, the reaction mixture poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from the proper solvent to give **13a**,**b**, respectively.

*4.1.12.1. N-Benzoyl-1-{2-[4-(3,4-dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]aceto}hydrazide (**13a**).* 0.7 g **13a** in yield 82% as colorless crystals from toluene. Mp > 350 °C. IR (KBr, ν cm^{-1}): 3295, 3200 (2 NH), 1658 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.37 (s, 6H, 2 CH_3), 5.10 (s, 2H, CH_2), 7.27–8.50 (m, 12H, Ar–H), 10.08(s, 1H, CONH, exchangeable with D_2O), 10.21 (s, 1H, NHCOPh, exchangeable with D_2O). MS (*m*/*z*, %): 263 (100), 105 (30), 77 (72). Anal. ($C_{25}H_{22}N_4O_3$).

*4.1.12.2. N-Acetyl-1-{2-[4-(3,4-dimethylphenyl)-1(2H)-oxo-phthalazin-2-yl]aceto}hydrazide (**13b**).* 0.62 g **13b** in yield 85% as colorless crystals from toluene. Mp 300–302 °C. IR (KBr, ν cm^{-1}): 3298, 3165 (2 NH), 1661 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.04 (s, 3H, $COCH_3$), 2.32 (s, 6H, 2 CH_3), 5.33 (s, 2H, CH_2), 7.26–7.74 (m, 10H, Ar–H), 10.10 (s, 2H, 2 NH, exchangeable with D_2O). Anal. ($C_{20}H_{20}N_4O_3$).

*4.1.13. Reaction **13a**,**b** with phosphorus oxychloride*

To 5 mL phosphorus oxychloride, **13a** and/or **13b** (0.001 mol) was added portionwise with stirring at 0 °C. The temperature was elevated gradually to 150 °C and the temperature was kept for 6 h. After cooling, the reaction mixture poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from the proper solvent to give **14a**,**b**, respectively.

*4.1.13.1. 4-(3,4-Dimethylphenyl)-2-[(5-phenyl-1,3,4-oxadiazol-2-yl) methyl] phthalazin-1(2H)-one (**14a**).* 0.24 g **14a** in yield 58% as dark brown crystals from ethanol. Mp 201–204 °C. IR (KBr, ν cm^{-1}): 1649 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.32 (s, 6H, 2 CH_3), 5.47 (s, 2H, CH_2), 7.45–8.19 (m, 12H, Ar–H). Anal. ($C_{25}H_{20}N_4O_2$).

*4.1.13.2. 4-(3,4-Dimethylphenyl)-2-[(5-methyl-1,3,4-oxadiazol-2-yl) methyl] phthalazin-1(2H)-one (**14b**).* 0.24 g **14b** in yield 69% as reddish brown crystals from ethanol. Mp 130–132 °C. IR (KBr, ν cm^{-1}): 1657 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.29 (s, 6H, 2 CH_3), 2.50 (s, 3H, CH_3), 5.40 (s, 2H, CH_2), 7.10–8.01 (m, 7H, Ar–H). MS (*m*/*z*, %): 346 (M^+, 12), 263 (100), 249 (6). Anal. ($C_{20}H_{18}N_4O_2$).

*4.1.14. Reaction **13a**,**b** with sodium ethoxide*

To a solution of sodium ethoxide (0.23 g Na in 20 mL abs. ethanol), **13a** and/or **13b** (0.001 mol) was added. The reaction mixture was refluxed for 5 h. After cooling, the reaction mixture poured onto ice/HCl. The precipitated solid was collected by filtration, dried and crystallized from the proper solvent to give **15a**,**b**, respectively.

*4.1.14.1. 2-(4,5-Dihydro-3-phenyl-5-oxo-1H-pyrazol-4-yl)-4-(3,4-dimethylphenyl)phthalazin-1(2H)-one (**15a**).* 0.3 g **15a** in yield 75% as colorless crystals from ethanol. Mp 238–240 °C. IR (KBr, ν cm^{-1}): 3190 (NH), 1694, 1653 (CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.38 (s, 6H, 2 CH_3), 4.40 (s, 1H, CH), 7.10 (s, 1H, NH, exchangeable with D_2O), 7.20–8.50 (m, 12H, Ar–H). Anal. ($C_{24}H_{19}N_4O_2$).

*4.1.14.2. 2-(4,5-Dihydro-3-methyl-5-oxo-1H-pyrazol-4-yl)-4-(3,4-dimethylphenyl)phthalazin-1(2H)-one (**15b**).* 0.24 g **15b** in yield 72% as colorless crystals from petroleum ether 40–60 °C. Mp 191–194 °C. IR (KBr, ν cm^{-1}): 3219 (NH), 1701, 1632 (CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.94(s, 3H, CH_3), 2.40 (s, 6H, 2 CH_3), 4.48 (s, 1H, CH), 7.0(s, 1H, NH, exchangeable with D_2O), 7.20–8.00 (m, 7H, Ar–H). Anal. ($C_{19}H_{17}N_4O_2$).

*4.1.15. N′-(2-cyanoethyl)-2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide (**16**)*

A mixture of 0.3 g the acetic acid hydrazide **5** (0.001 mol) and 0.1 g acrylonitrile (0.001 mol) in 20 mL pyridine was refluxed for 13 h. After cooling, the reaction mixture poured onto ice water and HCl. The precipitated solid was collected by filtration, dried and crystallized from methanol to give 0.24 g **16** in yield 63% as pale yellow crystals. Mp 160–163 °C. IR (KBr, ν cm^{-1}): 3203 (NH), 2200 (CN), 1630 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.0 (s, 1H, $NHCH_2$, exchangeable with D_2O), 2.30 (s, 6H, 2 CH_3), 3.02 (t, J = 10.0 Hz, 2H, $NHCH_2$), 3.17 (t, J = 10.0 Hz, 2H, CH_2CN), 5.00 (s, 2H, CH_2CO), 7.20–8.30 (m, 7H, Ar–H), 8.0 (s, 1H, CONH, exchangeable with D_2O). Anal. ($C_{21}H_{21}N_5O_2$).

*4.1.16. Reaction of phthalazine hydrazide derivative **5** with isothiocyanate derivatives*

A mixture of 0.5 g the acetic acid hydrazide **5** (0.002 mol) and isothiocyanate derivatives namely, phenyl, benzyl, and/or methyl (0.002 mol) in 20 mL dry *N,N*-dimethylformamide containing few drops of triethylamine was refluxed for 24 h. After cooling, the reaction mixture poured onto ice water and HCl. The precipitated solid was collected by filtration, dried and crystallized from the proper solvent to give **17a**–**c**, respectively.

*4.1.16.1. 1-{2-[4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl] acetyl}-4-phenylthiosemicarbazide (**17a**).* 0.65 g **17a** in yield 72% as colorless crystals from toluene. Mp > 350 °C. IR (KBr, ν cm^{-1}): 3338, 3294, 3154 (3 NH), 1663 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.0 (s, 1H, NHCS, exchangeable with D_2O), 2.34 (s, 6H, 2 CH_3), 5.10 (s, 2H, $COCH_2$), 6.81–8.19 (m, 12H, Ar–H), 10.08 (s, 1H, CONH, exchangeable with D_2O), 12.52 (s, 1H, NHPh, exchangeable with D_2O). Anal. ($C_{25}H_{23}N_5O_2S$).

*4.1.16.2. 1-{2-[4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl] acetyl}-4-benzylthiosemicarbazide (**17b**).* 0.64 g **17b** in yield 68% as colorless crystals from toluene. Mp 290–294 °C. IR (KBr, ν cm^{-1}): 3294, 3228, 3154 (3 NH), 1653 (CO), 1274 (CS). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.35 (s, 6H, 2 CH_3), 5.10(s, 2H, $COCH_2$), 5.80(s, 2H, CH_2Ph), 7.20–8.53 (m, 12H, Ar–H), 10.08(s, 1H, CONH, exchangeable with D_2O), 12.10 (s, 1H, NHCS, exchangeable with D_2O), 12.15 (s, 1H, $CSNHCH_2$, exchangeable with D_2O). Anal. ($C_{26}H_{25}N_5O_2S$).

*4.1.16.3. 1-{2-[4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl] acetyl}-4-methylthiosemicarbazide (**17c**).* 0.55 g **17c** in yield 70% as colorless crystals from ethanol. Mp 234–238 °C. IR (KBr, v cm^{-1}): 3298, 3221, 3153 (3 NH), 1660 (CO), 1234 (CS). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.00 (s, 3H, $NHCH_3$), 2.10 (s, 1H, NHCS, exchangeable with D_2O), 2.34 (s, 6H, 2 CH_3), 5.08 (s, 2H, $COCH_2$), 7.34–8.12 (m, 7H, Ar–H), 10.10 (s, 1H, CONH, exchangeable with D_2O). Anal. ($C_{20}H_{21}N_5O_2S$).

*4.1.17. Reaction of phthalazine hydrazide derivative **5** with isocyanate derivatives*

A mixture of 0.5 g the acetic acid hydrazide **5** (0.002 mol) and isocyanate derivatives namely, phenyl, and/or ethyl (0.004 mol) in 20 mL dry *N,N*-dimethylformamide was refluxed for 18 h. After cooling, the reaction mixture poured onto ice water. The precipitated solid was collected by filtration, dried and crystallized from the proper solvent to give **18a**,**b**, respectively.

*4.1.17.1. 1-{2-[4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl] acetyl}-4-phenylsemicarbazide (**18a**).* 0.64 g **18a** in yield 73% as colorless crystals from toluene. Mp 196–200 °C. IR (KBr, ν cm^{-1}): 3204 (NH), 1664 (CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.33 (s, 6H, 2 CH_3), 4.92 (s, 2H, $COCH_2$), 6.01 (s, 1H, NHCO, exchangeable with D_2O), 7.26–8.40 (m, 12H, Ar–H), 9.26 (s, 1H, NHPh, exchangeable with D_2O), 10.14 (s, 1H, CONH, exchangeable with D_2O). Anal. ($C_{25}H_{23}N_5O_3$).

*4.1.17.2. 1-{2-[4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl] acetyl}-4-ethylsemicarbazide (**18b**).* 0.60 g **18b** in yield 76% as colorless crystals from toluene. Mp 250–252 °C. IR (KBr, ν cm^{-1}): 3291, 3262, 3157 (3 NH), 1692 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.00 (t, J, 10 Hz, 3H, CH_3CH_2), 2.30 (s, 6H, 2 CH_3), 3.03 (q, J, 10 Hz, 3H, CH_3CH_2), 4.8 (s, 2H, $COCH_2$), 7.30–8.30 (m, 7H, Ar–H), 9.90(s, 1H, CONH, exchangeable with D_2O), 6.37 (s, 1H, NHCO, exchangeable with D_2O), 6.19(s, 1H, $NHCH_2$, exchangeable with D_2O). Anal. ($C_{21}H_{23}N_5O_3$).

*4.1.18. Reaction of phthalazine hydrazide derivative **5** with triethyl orthoformate in acetic anhydride*

A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 0.5 mL triethyl orthoformate were refluxed in 5 mL acetic anhydride for 6 h. The solution was allowed to cool and then the formed solid was collected, dried and crystallized from ethanol to give *N′*,*N′*-diacetyl-2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetohydrazide (**19**); 0.66 g in yield 80.5% as colorless crystals. Mp 180–184 °C. IR (KBr, ν cm^{-1}): 3194 (NH), 1667, 1619 (4CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.30 (s, 6H, 2 CH_3), 2.48 (s, 6H, 2 $COCH_3$), 5.30 (s, 2H, $COCH_2$), 7.30–8.30 (m, 7H, Ar–H), 8.32 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{24}H_{22}N_4O_4$).

*4.1.19. Reaction of phthalazine hydrazide derivative **5** with triethyl orthoformate*

A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 5 mL triethyl orthoformate were refluxed for 13 h on steam bath. The formed solid was collected, dried and crystallized from ethyl acetate to give *N′*-(ethoxymethylene)-2(4-(3,4-dimethylphenyl)-1-oxophthalazine-2(1H)-yl)acetohydrazide (**20**); 0.54 g in yield 71.1% as colorless crystals. Mp 218–222 °C. IR (KBr, ν cm^{-1}): 3350, 3300 (NH_2), 3250 (NH), 1670 (2CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.30 (t, J, 10 Hz, 3H, CH_3CH_2), 2.30 (s, 6H, $2CH_3$), 4.10 (q, J, 10 Hz, 3H, CH_3CH_2), 5.30 (s, 2H, $COCH_2$), 7.40 (s, 1H, CH═N), 7.30–8.30 (m, 7H, Ar–H), 10.32 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{21}H_{19}N_4O_3$).

*4.1.20. Reaction of N′-(ethoxymethylene)-2(4-(3,4-dimethylphenyl)-1-oxophthalazine-2(1H)-yl)acetohydrazide (**20**) with hydrazine hydrate in benzene*

To a solution of 0.76 g **20** (0.002 mol) in 10 mL benzene, 0.2 mL hydrazine hydrate 100% was added. The reaction mixture was refluxed for 9 h. After cooling the obtained solid was collected, dried and crystallized from ethyl acetate to give compound **21**; 0.62 g in yield 86.1% as colorless crystals. Mp 248–250 °C. IR (KBr, ν cm^{-1}): 3190 (NH), 1640 (2 CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, 2 CH_3), 4.35 (s, 2H, NH_2, exchangeable with D_2O), 4.51 (s, 1H, CHNH, exchangeable with D_2O), 5.21 (s, 2H, $COCH_2$), 7.50 (s, 1H, CH═N), 7.5–8.6 (m, 7H, Ar–H), 10.58 (s, 1H, CONH, exchangeable with D_2O). MS (*m*/*z*, %): 364 (M^+, 2), 291 (60), 262 (100). Anal. ($C_{19}H_{20}N_6O_2$).

*4.1.21. 5-methyl-3-oxo-2-[1′(2H)-oxo-4′-(3,4-dimethylphenyl) phthalazin-2′-ylmethylcarbonyl]-3,4-dihydropyrazol (**22**)*

A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 0.39 g ethylacetoacetate (0.003 mol) in 20 mL ethanol was refluxed for 7 h. After cooling the obtained solid was collected, dried and crystallized from diethyl ether to give compound **22**; 0.55 g in yield 70.5% as faint yellow crystals. Mp 268–280 °C. IR (KBr, ν cm^{-1}): 1668, 1660, 1649 (3 CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.30 (s, 3H, CH_3), 2.34 (s, 6H, 2 CH_3), 2.50 (s, 2H,CH_2), 4.97 (s, 2H, CH_2), 7.5–8.6 (m, 7H, Ar–H). MS (*m*/*z*, %): 386 (M^+–2, 4), 263 (100). Anal. ($C_{22}H_{20}N_4O_3$).

*4.1.22. 2-[(6-methyl-7H-pyrazolo[5,1-c][1,2,4]triazol-3-yl)methyl]-4-(3,4-dimethylphenyl)phthalazin-1(2H)-one (**23**)*

Hydrazine hydrate (98%) 0.5 cm^3 was added to a solution of 0.4 g **22** (0.001 mol) in 10 cm^3 absolute ethanol. The reaction mixture was refluxed for 6 h, after cooling the obtained solid was filtered off and crystallized from ethanol to give 0.32 g **23** in yield 84.2% as colorless crystals. Mp 240–244 °C; IR (KBr): $\bar{\nu}$ = 1664(CO) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.94 (s, 3H, CH_3), 2.46 (s, 6H, $2CH_3$), 2.60 (s, 2H,CH_2), 5.30 (s, 2H,CH_2), 7.28–8.50 (m, 7H, Ar–H) ppm; MS: *m*/*z* (%) = 250 (M^+, 81), 249 (100). Anal. ($C_{22}H_{20}N_6O$).

*4.1.23. Reaction of phthalazine hydrazide derivative **5** with acetyl acetone*

A mixture of 0.6 g the acetic acid hydrazide **5** (0.002 mol) and 0.30 g acetyl acetone (0.003 mol) in 20 mL ethanol was refluxed for 13 h. After cooling the obtained solid was collected, dried and crystallized from ethanol to give 2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)-*N′*-(4-oxopentan-2-ylidene)acetohydrazide **24**; 0.62 g in yield 77.5% as faint yellow crystals. Mp 206–209 °C. IR (KBr, ν cm^{-1}): 3191(NH), 1655, 1630 (2CO); ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.96 (s, 3H, N = CCH_3), 2.29 (s, 3H, $OCCH_3$), 2.36 (s, 6H, 2 CH_3), 2.54 (s, 2H, N═CCH_2), 4.83 (s, 2H,CH_2), 7.50–8.20 (m, 7H, Ar–H), 10.60 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{23}H_{24}N_4O_3$).

*4.1.24. 2-(2-(3,5-Dimethyl-1H-pyrazol-1-yl)-2-oxoethyl)-4-(3,4-dimethylphenyl)phthalazin-1(2H)-one (**25**)*

To a solution of sodium ethoxide (0.23 g Na in 20 mL abs. ethanol), 0.39 g **24** (0.001 mol) was added. The reaction mixture was refluxed for 11 h. After cooling, the reaction mixture poured onto ice/HCl. The precipitated solid was collected by filtration, dried and crystallized from ethyl acetate to give compound **25**; 0.35 g in yield 89.7% as faint brown crystals. Mp 102–106 °C. IR (KBr, ν cm^{-1}): 1648 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.30 (s, 3H, CH_3), 2.36 (s, 6H, $2CH_3$), 2.48 (s, 3H, CH_3), 4.64 (s, 2H, CH_2), 6.18 (s, H, CH), 7.50–8.20 (m, 7H, Ar–H). Anal. ($C_{23}H_{22}N_4O_2$).

*4.1.25. Fusion of the hydrazide derivative **5***

The hydrazide derivative **5** (0.6 g, 0.002 mol) was heated in an oil bath at 200 °C for 1 h. After cooling, crystallize from ethanol to give 2-(4-(3,4-dimethylphenyl)-1-oxophthalazin-2(1H)-yl)acetic acid **26**; 0.40 g in yield 65.57% as brown crystals. Mp 138–141 °C. IR (KBr, ν cm^{-1}): 2542–3500 (OH), 1701, 1667 (2 CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, $2CH_3$), 4.82(s, 2H, CH_2), 7.30–8.20 (m, 7H, Ar–H). MS (*m*/*z*, %): 307 (M^+−1, 41), 263 (100), 294 (43). Anal. ($C_{18}H_{16}N_2O_3$).

*4.1.26. Reaction of thiocarbamate derivatives **17a**–**c** with NaOH*

(0.001 mol) of thiocarbamate derivatives **17a**, **17b**, or **17c** was dissolved in sodium hydroxide solution (2 N, 20 mL). The clear solution was heated on a water bath for 2 h, filtered after cooling and neutralized with dilute hydrochloric acid. The precipitated solid was collected by filtration, dried and crystallized from the proper solvent to give the corresponding triazole derivatives **27a**–**c**, respectively.

*4.1.26.1. 2-[(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl]-4-(3,4-dimethylphenyl)phthalazin-1(2H)one (**27a**).* 0.38 g **27a** in yield 86% as colorless crystals from ethanol. Mp > 350 °C. IR (KBr, ν cm^{-1}): 3204 (NH), 1670 (CO). ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm):

2.30 (s, 6H, $2CH_3$), 4.20 (s, 2H, CH_2), 7.00–8.20 (m, 12H, Ar–H), 13.80 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{25}H_{21}N_5OS$).

*4.1.26.2. 2-[(5-mercapto-4-benzyl-4H-1,2,4-triazol-3-yl)methyl]-4-(3,4-dimethylphenyl)phthalazin-1(2H)one (**27b**).* 0.36 g **27b** in yield 80% as colorless crystals from ethanol. Mp 256–269 °C. IR (KBr, ν cm^{-1}): 3204 (NH), 1663 (CO). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.30 (s, 6H, $2CH_3$), 4.42 (s, 2H,CH_2), 5.74 (s, 2H,CH_2Ph), 7.20–8.19 (m, 12H, Ar–H), 13.45(s, 1H, NH, exchangeable with D_2O). Anal. ($C_{26}H_{23}N_5OS$).

*4.1.26.3. 2-[(5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl)methyl]-4-(3,4-dimethylphenyl)phthalazin-1(2H)one (**27c**).* 0.30 g **27c** in yield 78.9% as colorless crystals from ethanol. Mp 250–252 °C. IR (KBr, ν cm^{-1}): 3195 (NH), 1671 (CO). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, $2CH_3$), 3.0 (s, 3H, CH_3), 4.21 (s, 2H, CH_2), 13.50 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{20}H_{19}N_5OS$).

*4.1.27. Reaction of thiocarbamate derivatives **17a–c** with H_2SO_4*

(0.001 mol) of of thiocarbamate derivatives **17a**, **17b**, or**17c** was mixed with conc. H_2SO_4 (5 mL), left overnight. The reaction mixture poured onto ice water, the precipitated solid was collected by filtration, dried and crystallized from the proper solvent to give thiadiazol derivatives **28a–c**, respectively.

*4.1.27.1. 4-(3,4-Dimethyl)-2-[(5-phenylamino-1,3,4-thiadiazol-2-yl) methyl]phthalazin-1-(2H)-one (**28a**).* 0.32 g **28a** in yield 72.73% as colorless crystals from ethanol. Mp 142–146 °C. IR (KBr, ν cm^{-1}): 3215 (NH), 1670 (CO). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.30 (s, 6H, $2CH_3$), 5.10 (s, 2H, CH_2), 6.81–8.19 (m, 12H, Ar–H), 10.10 (s, 1H, NH, exchangeable with D_2O). Anal. ($C_{25}H_{21}N_5OS$).

*4.1.27.2. 4-(3,4-Dimethyl)-2-[(5-benzylamino-1,3,4-thiadiazol-2-yl) methyl]phthalazin-1-(2H)-one (**28b**).* 0.38 g **28b** in yield 84.4% as colorless crystals from ethanol. Mp 101–104 °C. IR (KBr, ν cm^{-1}): 3215 (NH), 1664 (CO). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, $2CH_3$), 4.2 (s, 1H, NH, exchangeable with D_2O), 4.35(s, 2H, CH_2), 5.10 (s, 2H,CH_2Ph), 7.20–8.19 (m, 12H, Ar–H). Anal. ($C_{25}H_{21}N_5OS$).

*4.1.27.3. 4-(3,4-Dimethyl)-2-[(5-methylamino-1,3,4-thiadiazol-2-yl) methyl]phthalazin-1-(2H)-one (**28c**).* 0.32 g **28c** in yield 84.2% as colorless crystals from ethanol. Mp 138–140 °C. IR (KBr, ν cm^{-1}): 3190 (NH), 1669 (CO). ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 2.34 (s, 6H, $2CH_3$), 2.78 (s, 3H, 2 CH_3), 5.10(s, 2H, CH_2), 7.34 (s, 1H, NH, exchangeable with D_2O), 7.45–8.19 (m, 12H, Ar–H). Anal. ($C_{25}H_{21}N_5OS$).

4.2. Anti-inflammatory testing

4.2.1. Animals

Male rats (180–200 g) were used taking into account the international principles and local, regulations concerning the care and the use of laboratory animals [28]. The animal had free access to a standard commercial diet and water, keeping at rooms maintained at about 25 °C.

4.2.2. Sponge implantation technique

The sponge implantation technique was described previously [28]. Sponges used are prepared from polyvinyl foam sheets (thickness 5 mm). Discs are punched out to a standard size and weight (2.5 ± 0.05 mg) using a 5.0 mm cork borer. The sponges are then soaked in 70%v/v ethanol for 30 min, rinsed four times with distilled water and heated at 80 °C for 2 h. Sponges are implanted in rats under diethyl ether anesthesia. Four 5 mm ventral incisions are made, 2 in both groins and axellae on both sides, and the dermis separated from the underlying muscle layer by insertion of blunt forceps to form separate cavities into which sponges are inserted. Four sponges are implanted per rat and each incision is closed by two stitches.

The sponges were lefted for 10 days during which the tested compounds or Indomethacin (reference standard) was injected intraperitoneally. Indomethacin was given at a dose level 20 mg/kg and tested compounds were given at equimolar dose levels. Animals in the control group were given the solvent (10% aqueous solution of Tween 80) at dose volume comparable to the test doses. The animals are sacrificed, the sponges prepared and dried until the weight remain constant. The net dry weight after subtracting the weight of the sponge is determined.

4.2.3. Anti-Inflammatory activity

One-way Analysis of Variance (ANOVA): $F = 2.985$

The P value is <0.0001, considered extremely significant. Variation among column means it is significantly greater than expected by chance.

Tukey-Kramer Multiple Comparisons Test: If the value of q is greater than 5.132 then the P value is less than 0.05.

References

[1] D.S. Dogruer, E. Kupeli, E. Yesilada, M.F. Sahin, Arch. Pharmacol. 337 (2004) 303–310.
[2] X. Cockcroft, K.J. Dillon, L. Dixon, J. Drzewiecki, P. Eversley, S. Gomes, J. Hoare, F. Kirrigan, I. Natthews, K.A. Menear, N.M.B. Martin, R. Newton, J. Paul, G.C.M. Smith, J. Vile, A.J. Whittlr, Bioorg. Med. Chem. Lett. 16 (2006) 1040–1044.
[3] X. Cockcroft, K.J. Dillon, L. Dixon, J. Drzewiecki, F. Kirrigan, V.M. Loh, N.M.B. Martin, K.A. Menear, G.C.M. Smith, Bioorg. Med. Chem. Lett. 15 (2005) 2235–2239.
[4] J.S. Kim, H. Lee, M. Suh, H.P. Choo, S.K. Lee, H.J. Park, C. Kim, S.W. Park, C. Lee, Bioorg. Med. Chem. 12 (2004) 3683–3686.
[5] A. Haikal, E. El-Ashery, J. Banoub, Carbohydr. Res. 338 (2003) 2291–2299.
[6] S. Demirayak, A. Karaburun, R. Beis, Eur. J. Med. Chem. 39 (2004) 1089–1095.
[7] N. Watanabe, Y. Kabasawa, Y. Takase, M. Matsukura, K. Miyazaki, H. Ishihara, K. Kodama, H. Adashi, J. Med. Chem. 41 (1998) 3367–3372.
[8] M. Johnsen, K. Rehse, H. Petz, J. Stasch, E. Bischoff, Arch. Pharmacol. 336 (2003) 591–597.
[9] M. Kornet, G. Shackleford, J. Heterocycl. Chem. 36 (1999) 1095–1096.
[10] O.M. Nassar, Indian. J. Heterocycl. Chem. 7 (1997) 105–108.
[11] G.R. Madhavan, R. Chakrabarti, S.K. Kumar, P. Misra, R.N. Mamidi, V. Balraju, K. Ksiram, R.K. Babu, J. Suresh, B.B. Lohray, V.B. Lohray, J. Iqbal, R. Rajagopalan, Eur. J. Med. Chem. 36 (2001) 627–637.
[12] E.M. Lenz, I.D. Wilson, B. Wright, E.A. Partidge, C.T. Roddgers, P.R. Haycock, J.C. Lindon, J.K. Nicholson, J. Pharm. Biomed. Anal. 28 (2002) 31–43.
[13] M.C. Cardia, E. Distinto, E. Maccioni, A. Delogu, J. Heterocycl. Chem. 40 (2003) 1011.
[14] E.D. Olmo, M.G. Armas, J. Lopez-perez, G. Ruiz, F. Vargas, A. Gimenez, E. Deharo, A.S. Feliciano, Bioorg. Med. Chem. Lett. 11 (2001) 2755–2757.
[15] M. Napoletano, G. Norcini, F. Pellacini, F. Marchini, G. Moraazzoni, P. Ferlenga, L. Pradella, Bioorg. Med. Chem. Lett. 10 (2000) 2235–2238.
[16] A.K. Chakraborti, B. Gopalakrishnan, E. Sobhia, A. Malde, Bioorg. Med. Chem. Lett. 13 (2003) 2473–2479.
[17] M. Van der Mey, A. Hatzelmann, G.P. Van Klink, I.J. Van der Lann, G.J. Sterk, U. Thibaut, H. Timmerman, J. Med. Chem. 44 (2001) 2511–2522.
[18] M. Van der Mey, B. Hildegard, C. Dennis, A. Hatzelmann, H. Timmerman, J. Med. Chem. 45 (2002) 2526–2533.
[19] K.M. Shubin, V.A. Kuznetsov, V.A. Galishev, Tetrahedron Lett. 45 (2004) 1407–1408.
[20] H. Brad, C. Guo-Qiang, P.C. Partha, R.F. James, P. Liping, M.R. Robert, B.R. Anthony, R. Andreas, S. Kelvin, T. Maya, P.W. Ryan, X. Shimin, Z. Dawei, H. Faye, R.L. Matthew, S. Rashed, L. Vivian, G. Daved, H.P. Matthew, H. Bradley, S. Lisa, M. Scot, M.W. Lu, S.T. Andrew, J. Med. Chem. 51 (2008) 6271–6279.
[21] A.E. Wael, I.H. Mohamed, E.M.T. Hala, A.H.A. Adel, Monatsh. Chem. 139 (2008) 1055–1060.
[22] B.D. Robert, and W. Robin, Eur. Patent APPL. 2, 895 (1979); Chem. Abstr.. 92(1980), 76533v.
[23] M. Van der Mey, A. Hatzelmann, G.P. Van Klink, I.J. Van der Lann, G.J. Sterk, U. Thibaut, H. Timmerman, J. Med. Chem. 44 (2001) 2523–2535.
[24] W.O. Foye, "Principle of Medicinal Chemistry", Philadeliphia, London, third ed. (1989) p. 516.
[25] W.O. Foye, T.L. Lemke, D.A. Williams, "Principle of Medicinal Chemistry", a Waverly Company, fourth ed. (1989) p. 554.
[26] T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, A.W. Veenhuizen, P.C. Isakson, J. Med. Chem. 40 (1997) 1347–1365.
[27] J.P. Danial, R.J. Carl, Laboratory Text for Organic Chemistry, 1979, pp 363.
[28] P.N. Sexena, Arch. Int. Pharmacodyn. Ther. 126 (1960) 228.