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Original article

Design and synthesis of some new thiophene, thienopyrimidine and thienothiadiazine derivatives of antipyrine as potential antimicrobial agents

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ABSTRACT

4-acetamide pyrazolone **2** was synthesized by acetylation of 4-amino antipyrine **1** in excellent yield. 4-acetamide pyrazolone **2** was exploited as a starting material for the syntheses of hitherto unknown different types of new heterocyclic compounds incorporating the antipyrine moiety which expect highly biological activity against various microorganisms. Thus, Claisen condensation of 4-acetamide pyrazolone **2** with diethyl oxalate have been utility to afford new 4-oxaloacetyl antipyrine **3,** which upon hydrazinolysis of the ester function to obtain the acetohydrazide derivative **18** which used as starting material to synthesize 1,2,4-triazol **19** and hydrazone **20** derivatives. 4-aminothiophene carboxylate derivatives **6, 7** were synthesized by utility of Gewald reaction. On the other hand, Michael type addition of the enolate ion of acetyl functions in acetamide pyrazolone **2** to the activated double bond in arylidenemalonoester to furnish pyrane derivative **9** was done. Finally, 4-acetamide pyrazolone **2** was treated with aromatic substituted aldehyde to exhibit thiophenacrylamide derivative **10**. Compound **6** gave characteristic reaction for enaminonitriles, thus, the behavior of *o*-aminoester of 4-aminothiophene carboxylate derivative **6** toward electrophilic reagent, one carbon donars, amide and acid was also investigated to afford the correspondence thiophene derivatives **11,12,13,15** and **16**. In addition, treatment of carboxamide derivative **16** with thionyl chloride afforded the thienothiadiazine derivative **17**. The characterization of all synthesized compounds was done by elemental analysis and spectral studies. Moreover, all the synthesized compounds were tested against antimicrobial activities by the disc diffusion method, which exhibited higher promising biological activities.

1. Introduction

Since the antipyrine (AP) was first synthesized by Knorr [1] in 1883, there has been a continued interest in the studies of antipyrine derivatives (APDs). Up to now, broad properties of APDs have been investigated and reported in many fields. In biofunctional compounds, broad bioactivities of APDs as antitumor [2–4], antimicrobial [5–7], antiviral [8,9], analgesic, and anti-inflammatory drugs [10–12], etc. have been investigated, and their bioactivity diversities have been reported in these contexts. Now, APDs have been accepted as important biomodel compounds in the biological systems [13], Inspired from these facts, this paper deals with the synthesis of novel thiophene, pyrane and thienopyrimidine derivatives incorporating antipyrine moiety, where, the nitrogen of the 4-aminoantipyrine moiety is substituted by thiophene, pyrane or thienopyrimidine moieties due to the well-documented antimicrobial and antitumor activity of these biologically active moieties [14,15]. The newly synthesized compounds were evaluated as antimicrobial agents against gram positive and gram negative bacteria and fungi.

2. Results and discussion

2.1. Chemistry

The synthetic strategies adopted for the synthesis of the target compounds are depicted in Schemes 1–5. In Scheme 1, the starting compound *N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-*1H*-pyrazol-4-yl) acetamide **2** was prepared by acetylation of 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one **1** with acetic anhydride in excellent yield.

Structure of the product **2** was confirmed on the basis of its correct elemental analysis and spectral data. IR spectra of compound **2** revealed the presence of characteristic bands for two carbonyl functional groups and disappearance the amino group. The site of acetylation in compound **2** was supported by ^{1}H NMR spectrum, whereas, recorded a signal at δ 2.1 (s, 3H, $COCH_3$), 1.9 (s,

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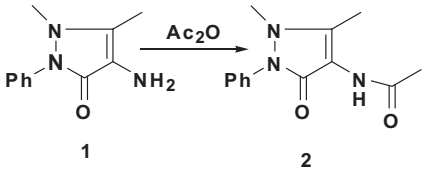
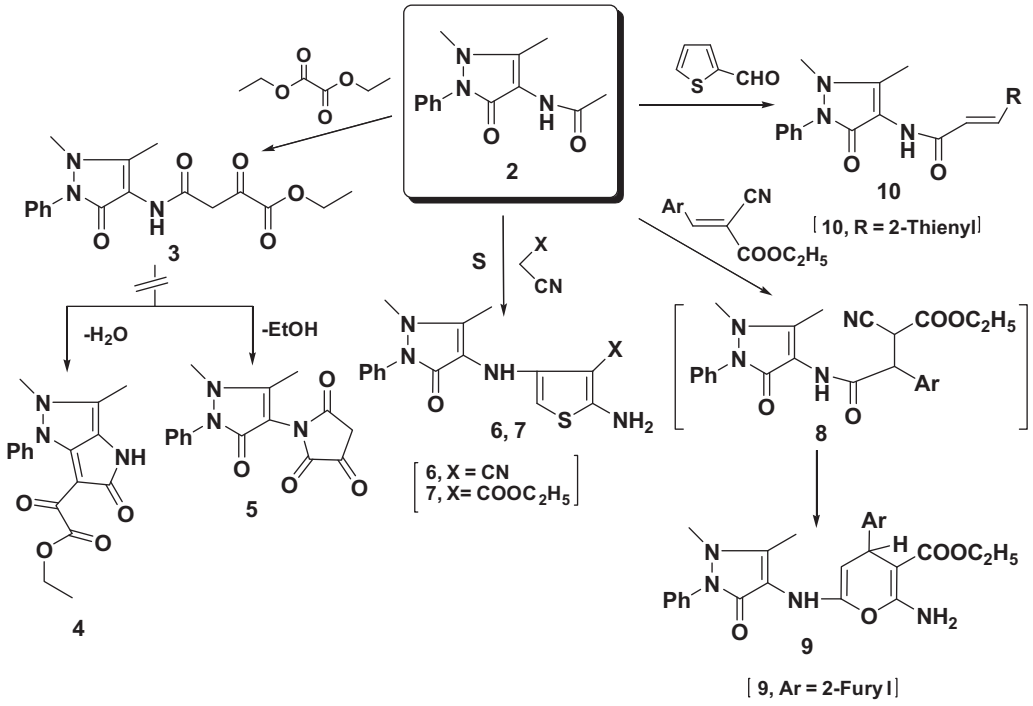


Scheme 1. The synthetic route for 4-acetylantipyrine.

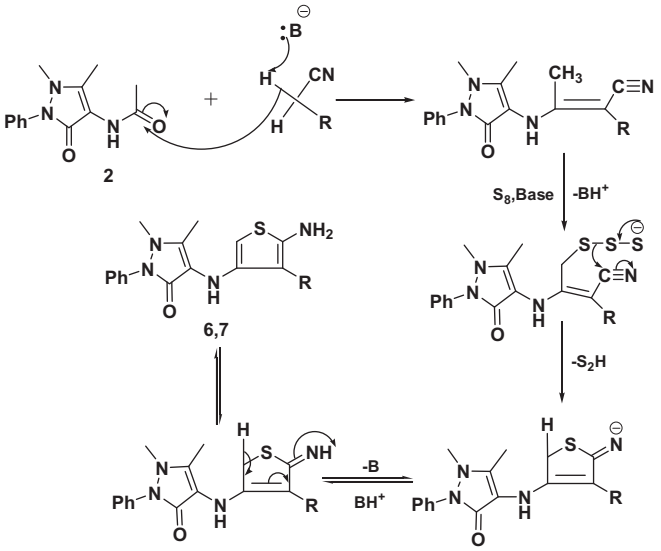
3H, CH_3), 3.4 (s, 3H, N–CH_3), 7.2–7.5 (m, 5H, Ar–H), 9.0 ppm (s, H, NH). In addition, the structure of **2** was supported by its mass spectrum which revealed a molecular ion peak at *m/z* 247[M + 2] (1.44%) corresponding to the molecular formula $C_{13}H_{15}N_3O_2$. In the present study the Claisen condensation at this stage, it was attempted to prepare the proposed 1,2,4,5-tetrahydropyrrolo[3,2-*c*] pyrazol-6-yl)-2-oxoacetate **4** or pyrrolidine-2,3,5-trione **5** derivatives of antipyrine by the Claisen condensation of 4-acetylantipyrine **2** with diethyl oxalate in molar ratio 1:1 in the presence of sodium ethoxide. Surprisingly, 4-oxaloacetylantipyrine **3** was obtained instead. The structure of such unexpected compound **3** was substantiated on basis of their IR, Mass and ^{1}H NMR spectral data. The IR spectra were characterized by the presence of 4C=O absorption band at 1750–1669 cm^{-1} corresponding to the ketone and ester group. Also, the ^{1}H NMR spectrum indicated the presence of a singlet at δ 3.4 ppm which could be assigned to active methylene and the signal for acetyl proton collapsed from a singlet in compound **2** to a triplet and quartet assigned to ester in compound **3** at δ 1.6 and 4.1 ppm (Scheme 2). The synthesis of the key intermediate thiophene derivatives **6** and **7** could be achieved according to the method described by Gewald [16], by reacting acetylantipyrine **2** with sulfur and the appropriate active nitrile bearing an electron withdrawing groups such as malononitrile and/or ethyl cyanoacetate respectively, in ethanol in the presence of a basic catalyst such as piperidine. Structures of the latter products were based on analytical and spectral data. The infrared spectrum of compound **6** indicated the absence of the C=O absorption band for the acetyl group and contains characteristic absorption bands at 3390, 3226 and 2206 cm^{-1} for the NH_2/NH and C≡N functional groups. The molecular ion peak of compound **6** was found in the mass spectrum at *m/z* 345[M^+] (10.17) corresponding to the molecular formula $C_{16}H_{15}N_5OS$, with a base peak at 63(100%). Also, the infrared spectrum of compound **7** indicated the presence of characteristic absorption bands for NH_2 and ester functional groups. In addition, the molecular structure of compound **7** was established by ^{1}H NMR spectrum which exhibited lack of the characteristic signal of $COCH_3$ group and the presence of triplet at 1.2 ppm, quartet at 4.1 ppm assigned for ethyl ester moiety in addition to amino protons at 9.6 ppm. The molecular ion peak of compound **7** was found in the mass spectrum at *m/z* 372(M^+) (100%).

The formation of **6** and **7** is assumed to proceed via Gewald reaction that an activated nitrile first condenses with a ketone yielding a Knoevenagel-Cope condensation product which is then thiolated at the active methyl function group with elemental sulfur followed by ring closure (Scheme 3). In contrast to the behavior of acetyl antipyrine **2** towards arylidenemalonoester, acetyl antipyrine **2** reacted with ethyl 2-cyano-3-(furan-2-yl) acrylate in ethanol/piperidene in a molar ratio (1:1) to yield pyrane derivative **9**. Elemental analysis and spectral data are in full agreement with the proposed structure **9** (See Experimental Section). Compound **9** are likely formed via Michael type addition of the enolate ion of the acetyl function in **2** to activated double bond in arylidenemalonoester to give the acyclic adducts **8**, which cyclized into the pyrane derivative **9** [17]. In conjunction of our investigation on the synthesis of thiophene acrylamide derivative incorporating antipyrine moiety **10** of potential interest by using aromatic substituted aldehyde which reacts with acetyl antipyrine **2**. Structure of the thiophen acrylamide derivative **10** was confirmed on the basis of its correct elemental analysis and spectral data (Scheme 2). The ^{1}H NMR spectrum of compound **10** in DMSO-d_6 revealed the absence of acetyl moiety. In addition, the structure of **10** was supported by its mass spectrum which revealed a molecular ion peak at *m/z* 339 [M + 2] (2.04), 121(100%).

In Scheme 4, the presence of reactive group in thiophene ring, namely 2-amino and 3-cyano/carboxyethoxy group give access to

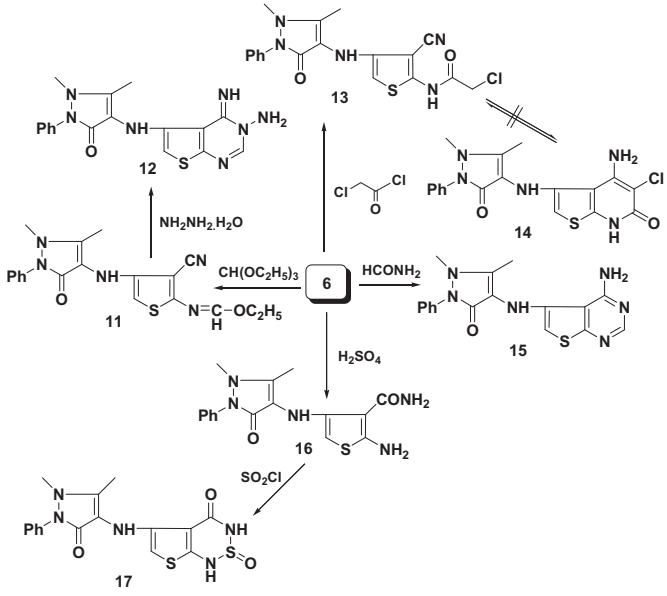


Scheme 2. Modification of Claisen, Gewald Condensation and Michael addition.

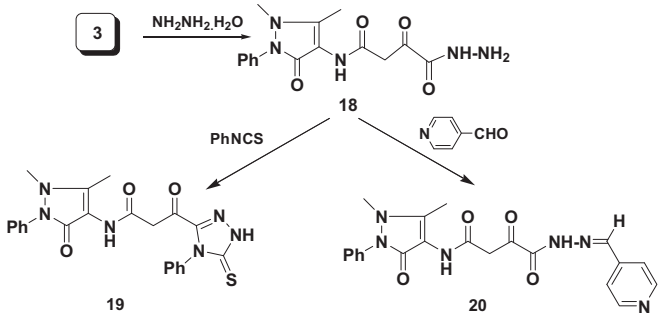


Scheme 3. Reaction mechanism for the synthetic route for *o*-aminothiophene derivative.

compounds having fused rings. On the other hand, *o*-aminocarbonitrile derivative **6** gave characteristic reaction for enaminonitriles. Thus, it used as a key precursor for the synthesis of condensed heterocyclic compounds of expected biological activity. The behavior of *o*-aminocarbonitrile derivative **6** toward electrophilic reagent, one carbon donars, acid and amide was also investigated. Thus, the alkylation reaction of compound **6** with excess triethyl orthoformate at 200 °C under solvent free condition yielded the ethoxymethyleneamino derivative **11**. In addition to, the reactivity of compound **11** toward hydrazine hydrate was also investigated. Thus, hydrazinolysis of compound **11** with hydrazine hydrate furnished 4-(3-amino-4-imino-3,4-dihydrothieno[2,3-*d*] pyrimidin-5-ylamino)-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one **12.** Moreover, 2-chloro-*N*-(3-cyano-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-ylamino)thiophen-2-yl) acetamide **13** were prepared by reacting the thiophene derivative **6** with chloroacetyl chloride. The expected thieno[2,3-*b*]pyridin



Scheme 4. Investigation the behavior of enaminonitrile.



Scheme 5. The synthetic route for hydrazide, 1,2,4-triazole and hydrazone derivatives.

derivative **14** formation was ruled out on the basis of analytical and spectral data. The IR spectrum of **13** showed the presence of 3248 (2NH), 3091 (CH-arom.), 2960 (CH-aliph.), 2168 (C≡N), 1724, 1669 (2C=O). The molecular ion peak of compound **13** was found in the mass spectrum at *m*/*z* 401 [M + 2] (22.07) corresponding to the molecular formula $C_{18}H_{16}ClN_5O_2S$. Also, compound **6** was cyclocondensed with formamide under reflux and afforded the thienopyrimidine **15**. The IR spectrum of **15** showed the absence of (C≡N) and presence of (br, NH_2/NH) at 3340, 3230, (CH arom.) at 3104, (CH aliph.) at 2893, (C=O) at 1669 cm^{-1}. The mass spectrum of **15** exhibited a molecular ion peak *m*/*z* at 352 (M^+, 3.14%), with a base peak at 148. Its ^{1}H NMR spectrum in (DMSO-d_6) revealed signals at δ 1.6 (s, 3H, CH_3), 2.6 (s, 3H, N–CH_3), 4.8 (s, 1H, NH), 6.5 (s, 2H, NH_2), 7.5–8.4 (m, 7H, Ar–H). Hydrolysis of compound **6** with sulfuric acid at room temperature furnished the novel carboxamide derivative **16**; via partially hydrolysis of cyano group. The IR spectrum of **16** showed bands at 3390, 3159 (br, $2NH_2$/NH), 3031 (CH-arom.), 2896 (CH-aliph.), 1728, 1660 cm^{-1} (2C=O). It's ^{1}H NMR spectrum in (DMSO-d_6) revealed signals at δ 1.9 (s, 3H, CH_3), 2.9 (s, 3H, N–CH_3), 4.5(s, 1H, NH), 4.9 (s, 2H, NH_2), 7.2–7.7 (m, 8H, Ar–H+ NH_2). Mass spectrum of **16** exhibited a molecular ion peak *m*/*z* at 343 [M + 1] (11.07) with a base peak at 63. In addition, treatment of carboxamide derivative **16** with thionyl chloride afforded the thienothiadiazine derivative **17** which elucidate by elemental analysis and spectral data.

The acetohydrazide derivative **18** was obtained from the hydrazinolysis of the ester group in 4-oxaloacetylantipyrine **3** (Scheme 5). The chemical structure of **18** was elucidated on the basis of elemental analysis and spectral data. Whereas, the ^{1}H NMR spectrum were characterized by the complete disappearance of the signals attributed to a triplet and a quartet assigned for the ester moiety and presence the amino hydrazinyl moiety. Interaction of hydrazide **18** with phenyl isothiocyanate in presence of (1N) sodium hydroxide afforded the corresponding 1,2,4-triazol-3-yl-propanamide derivative **19**. Finally, condensation of the acetohydrazide derivative **18** with isonicotinaldehyde in butanole yielded the hydrazone derivative **20**. The chemical structure of **19** and **20** were elucidated on the basis of elemental analysis and spectral data in which IR measurements showed the absence of NH_2 and presence of NH band.

3. Biological screening

3.1. Preliminary screening for antimicrobial activity

3.1.1. Antifungal

Newly prepared compounds were screened separately *in vitro* for their antifungal activity against four fungal species, namely *Aspergillus fumigatus* (RCMB 002003), *Geotrichum candidum* (RCMB 052006), *Candida albicans* (RCMB 005002) and *Syncephalastrum racemosum* (RCMB 005003) on *Sabouraud dextrose agar plates*. The

culture of fungi was purified by single spore isolation technique. The antifungal activity was determined by agar well diffusion method [18], by the following procedure:

Sabouraud dextrose agar plates: A homogeneous mixture of glucose–peptone–agar (40:10:15) was sterilized by autoclaving at 121 °C for 20 min. The sterilized solution (25 mL) was poured in each sterilized petridish in laminar flow and left for 20 min to form the solidified sabouraud dextrose agar plate. These plates were inverted and kept at 30 °C in incubator to remove the moisture and to check for any contamination.

Antifungal assay: Fungal strain was grown in 5 mL Sabouraud dextrose broth (glucose: peptone; 40:10) for 3–4 days to achieve 10^5 CFU mL^{-1} cells. The fungal culture (0.1 mL) was spread out uniformly on the sabouraud dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5–10 min so that culture is properly adsorbed on the surface of Sabouraud dextrose agar plates. Now small wells of size (4 mm × 2 mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100 μl of the tested samples (10 mg mL^{-1}) were loaded into the wells of the plates. All compounds were prepared in dimethyl sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 30 °C for 3–4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. Clotrimazole and Itraconzole were used as references to evaluate the potency of the tested compounds under the same conditions. Zones of inhibition were determined for **2**, **3**, **6**, **7**, **9**, **10**, **11**, **12**, **13**, **15**,**16**, **17**, **18**,**19** and **20** and the results were summarized in Table 1.

3.1.2. Antibacterial

Antibacterial activities were investigated using agar well diffusion method. The activity of tested samples was studied against the *Staphylococcus aureus* (RCMB 000106) and *Bacillus subtilis* (RCMB 000107) (as gram positive bacteria) while *Pseudomonas aeruginoca* (RCMB 000102) and *Escherichia coli* (RCMB 000103) (as gram negative bacteria). Centrifuged pelletes of bacteria from a 24 h old culture containing approximately 10^4–10^6 CFU (colony forming unit) per ml were spread on the surface of Nutrient agar (tryptone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1%, 1000 ml of distilled water, PH 7.0) which was autoclaved under 121 °C for at least 20 min. Wells were created in medium with the help of a sterile metallic bores and then cooled down to 45 °C. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100 μl of the tested samples (10 mg mL^{-1}) were loaded into the wells of the plates. All compounds were prepared in Dimethyl Sulfoxide (DMSO), DMSO was loaded as control. The plates were kept for incubation at 37 °C for 24 h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacterium culture. *Penicillin G* and *Streptomycin* were used as antibacterial standard drugs [19]. Inhibition zones were determined for **2, 3, 6, 7, 9, 10, 11, 12, 13, 15**, **16, 17, 18, 19** and **20**, the results were summarized in Table 2.

3.2. Minimum inhibition concentration

The agar plate method was used to determine the minimum inhibition concentration (MIC) of tested samples, two-fold serial dilutions of each sample were added to nutrient broth for bacteria (beef extract 5 g, peptone 10 g added to 1000 mL distilled water, pH 7.0) and to sabouraud dextrose broth for fungi, (DMSO) was used as the control [20]. Then they were heated in autoclave at 121 °C for 25 min. The culture of each organism was diluted by sterile distilled water to 10^5–10^6 CFU mL^{-1}. A loop of each suspension was inoculated on apropiate medium with the sample or the control added. After inoculation, the plates were incubated at 37 °C for 24 h for bacteria, and at 30 °C for 3–4 days for fungi. The colonies were counted and the MIC values were obtained. The MIC was considered to be the lowest concentration that completely inhibits against inoculums comparing with the control, disregarding a single colony or a faint haze caused by the inoculums. Whereas, the good activity of the newly synthesized compounds especially **6**, **7**, **11** and **13** against antimicrobial activity, so the minimum inhibition concentration (MIC) were determined for these compounds, the results were depicted in Table 3.

4. Conclusion

The researches study the successful synthesis and antimicrobial activity of new acetamide, oxaloacetyl, acetohydrazide, thiophene and thiophenacrylamide derivatives bearing antipyrine moiety. The investigation of antifungal and antibacterial screening data revealed that all the tested compounds, **2**–**20** showed moderate to good inhibition in DMSO. The compounds **2, 3, 6, 7, 10, 11, 13, 15, 17** and **18** showed comparatively good activity against all the fungal strains. The good activity is attributed to the presence of pharmacologically active OCH_2CH_3, $-COCH_2$, $-CH_3$, chlorine groups and thiophene moiety attached to nitrogen atom incorporation of the

Table 1
Antifungal activity data of chemical substances tested.

Compound No.	Inhibition zone diameter (mm)			
	Aspergillus fumigatus (RCMB 002003)	*Geotrichum candidum*(RCMB 052006)	*Candida albicans* (RCMB 005002)	*Syncephalastrumracemosum* (RCMB 005003)
2	14.3 ± 0.09	13.4 ± 0.1	12.2 ± 0.05	NA
3	11.1 ± 0.1	13.4 ± 0.08	10.6 ± 0.2	NA
6	24.3 ± 0.09	22.4 ± 0.1	17.2 ± 0.05	18.9 ± 0.03
7	22.1 ± 0.2	22.0 ± 0.05	16.4 ± 0.08	18.2 ± 0.03
10	14.2 ± 0.1	NA	13.4 ± 0.4	NA
11	19.2 ± 0.08	17.4 ± 0.09	14.2 ± 0.1	8.5 ± 0.05
12	NA	NA	NA	NA
13	20.1 ± 0.2	18.3 ± 0.05	15.4 ± 0.08	11.2 ± 0.03
15	12.5 ± 0.09	12.8 ± 0.1	11.0 ± 0.05	13.4 ± 0.08
16	NA	NA	NA	NA
17	15.1 ± 0.01	14.4 ± 0.1	13.4 ± 0.4	10.6 ± 0.2
18	11.2 ± 0.03	13.4 ± 0.04	NA	13.4 ± 0.04
Itraconzole	28 ± 0.05	27 ± 0.1	26 ± 0.02	22 ± 0.09
Clotrimazole	26 ± 0.1	23 ± 0.03	18 ± 0.1	20 ± 0.2

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/ml) concentration of tested samples and standard using (30 μg/ml). The test was done using the diffusion agar technique, Well diameter: 6.0 mm (100 μl was tested). NA: No activity, data are expressed in the form of mean ± SD.

Table 2
Antibacterial activity of chemical substances tested.

Compound No.	Inhibition zone diameter (mm)			
	Gram positive bacteria		Gram negative bacteria	
	Staphylococcus aureus (RCMB 000106)	*Bacillis subtilis* (RCMB 000107)	*Pseudomonas aeruginosa* (RCMB 000102)	*Escherichia coli* (RCMB 000103)
2	15.9 ± 0.03	13.2 ± 0.04	NA	11.4 ± 0.04
3	16.3 ± 0.1	16.2 ± 0.08	NA	NA
6	23.9 ± 0.03	27.2 ± 0.04	22.8 ± 0.1	24.4 ± 0.04
7	20.2 ± 0.04	25.3 ± 0.07	20.8 ± 0.09	23.8 ± 0.08
10	11.0 ± 0.05	11.2 ± 0.03	NA	NA
11	18.4 ± 0.5	19.2 ± 0.1	16.2 ± 0.2	13.8 ± 0.1
12	NA	NA	NA	NA
13	19.4 ± 0.03	24.1 ± 0.04	18.9 ± 0.09	16.2 ± 0.2
15	13.9 ± 0.03	15.2 ± 0.04	NA	10.4 ± 0.09
16	NA	NA	NA	NA
17	18.3 ± 0.08	19.2 ± 0.1	NA	NA
18	17.2 ± 0.05	14.4 ± 0.5	NA	NA
Penicillin G	29.4 ± 0.08	32.5 ± 0.05	28.3 ± 0.10	33.5 ± 0.07
Streptomycin	25 ± 0.2	29 ± 0.04	24 ± 0.1	25 ± 0.03

Mean zone of inhibition in mm ± Standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (10 mg/ml) concentration of tested samples and standard using (30 μg/ml). The test was done using the diffusion agar technique, Well diameter: 6.0 mm (100 μl was tested). NA: No activity, data are expressed in the form of mean ± SD.

aminoantipyrine ring. It is worth mentioning that incorporation of thiophene ring to the acetamide of antipyrine nucleus at position 4 via aryilidine derivatives to give compounds **6** and **7** that produce strong antifungal activity. Also, ethoxymethylene derivative **11** and chloroacetylthiophene **13** gave another strong antifungal activity. Conversion of acetylantipyrine **2** to thiophenacrylamide derivative **10** enhanced the antifungal activity. Unfortunately, incorporation of the pyrimidine nucleus to thiophene at position 2 and 3 in compound **15** produced moderate antimicrobial activity; compounds **12** and **16** have no activity. The compounds **2**–**20** showed comparatively good activity against all the bacterial strains. In conclusion, we reported herein a simple and convenient route for the synthesis of some new heterocyclic based on acetylantipyrine for antimicrobial evaluation. Most of the compounds were effective against *S. aureus* and *Bacillus subtilis* (as gram positive bacteria), *P. aeruginoca*, *E. coli* (as gram negative bacteria) when compared with *Penicillin G* and *Streptomycin* especially **2, 3, 6, 7, 10, 11, 13, 15, 17** and **18** showed strong, compounds **12** and **16** no activity. Structure and biological activity relationship of title compounds showed that presence pyrazole moiety and biologically active groups like $-COCH_3$, $-CH_3$, chlorine groups and thiophene moiety attached to nitrogen atom incorporation of the 4-aminoantipyrine ring for good antimicrobial activity. As we consider all results obtained from antifungal and antibacterial tests together we can say that entire compounds tested are more active towards fungi and some bacteria. These preliminary results of biological screening of the tested compounds could offer an encouraging framework in this field that may lead to the discovery of novel antimicrobial agent.

5. Experimental

5.1. Chemistry

All melting points are uncorrected and were determined on a Stuart melting point apparatus. IR spectra were recorded on a Shimadzu-440 IR spectrophotometer using the KBr technique (Shimadzu, Japan). ^{1}H NMR spectra were measured on a Varian Mercury VX-300 NMR spectrometer in DMSO-d_6 as a solvent and were run at 300 MHz, using tetramethylsilane (TMS) as an internal standard. The ^{13}C NMR (500 MHz) spectra were run in dimethylsulfoxide (DMSO-d_6). Chemical shifts were related to that of the solvent. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. The purity of the synthesised compounds was monitored by TLC. Elemental analyses were carried out by the Microanalytical Research Centre, Faculty of Science, Cairo University. Analytical results for C, H, N and S were within ±0.4 of the calculated values.

5.1.1. *N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)acetamide (**2**)*

A solution of **1** (2.03 g, 10 mmol) in acetic anhydride (20 mL) was refluxed for 1 h, the reaction mixture was then concentrated, the solid separated was recrystallized from ethanol to give brown powder **2**: Yield, 95%; m.p. 165–167 °C; IR, cm^{-1}: 3220(NH), 3032 (CH-arom.), 2935, 2812 (2CH-aliph.), 1660 (C=O, antipyrine), 1721 (C=O, acetyl). ^{1}H NMR (DMSO-d_6) δ: 2.1 (s, 3H, $COCH_3$), 1.9 (s, 3H, CH_3), 3.4 (s, 3H, N-CH_3), 7.2–7.5 (m, 5H, Ar-H), 9.0 (s, H, NH). MS, *m/z*

Table 3
Antimicrobial Activity as MICS (μg/ml) of tested samples against tested microorganismis.

Compound No.	Minimum inhibitory concentration (μg/ml)							
	Antifungal				Antibacterial			
					Gram Positive Bacteria		Gram negative Bacteria	
	Aspergillus fumigatus (RCMB 002003)	*Geotrichum candidum* (RCMB 052006)	*Candida albicans* (RCMB 005002)	*Syncephalastrumracemosum* (RCMB 005003)	*Staphylococcus aureus* (RCMB 000106)	*Bacillis subtilis* (RCMB 000107)	*Pseudomonas aeruginosa* (RCMB 000102)	*Escherichia coli* (RCMB 000103)
6	19	19	39	39	19	9.5	39	19
7	19	39	78	39	39	19	39	19
11	78	78	156	625	78	39	78	156
13	39	78	156	313	39	19	39	78

(%):247[M+2] (1.44), 84 (100). Anal. Calcd. For $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13. Found: C, 63.36; H, 6.06; N, 17.03.

*5.1.2. Ethyl 4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino)-2,4-dioxobutanoate (**3**)*

To a stirred solution of **2** (2.3 g, 10 mmol) and diethyl oxalate (5.4 g, 40 mmol) was added drop wise a solution of sodium ethoxide (1.36 g, 20 mmol). The reaction mixture was refluxed for 5 h, cooled and then acidified with acetic acid (3%). The solid separated was collected and crystallized from dioxane to give yellow crystals **3**: Yield, 76%; m.p. 100–102 °C; IR, cm^{-1}: 3259 (NH), 3050 (CH-arom.), 2968 (CH-aliph.), 1750 (C=O, α- ketoester), 1700 (β-diketone), 1669(C=O, antipyrine). ^1H NMR (DMSO-d_6) δ: 1.6 (t, 3H, CH_3 ester), 2.7(s, 3H, CH_3), 3.1 (s, 3H, N–CH_3), 3.4(s, 2H, –$COCH_2CO$-), 4.1 (q, 2H, CH_2 ester), 7.2–7.5 (m, 5H, Ar–H), 9.1 (s, H, NH). MS, *m*/*z* (%): 345[M + 2](7.11), 132(100). Anal. Calcd. For $C_{17}H_{19}N_3O_5$: C, 59.12; H, 5.75; N, 12.17. Found: C, 59.42; H, 6.06; N, 12.37.

5.1.3. General procedure for the reaction of 4-acetyl antipyrine with sulfur and activated nitrile derivatives

A mixture of acetylantipyrine **2** (2.3 g, 10 mmol), activated nitrile such as malononitrile and/or ethyl cyanoacetate (10 mmol), sulfur (10 mmol), ethanol (50 mL) and piperidine were refluxed for 3h. The solid which separated was filtered, washed with ethanol, dried and crystallized from ethanol to give **6** and **7.**

*5.1.3.1. Spectral data of 2-amino-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl amino) thiophene-3-carbonitrile (**6**).* Yield, 85%; m.p. 115–117 °C; IR, cm^{-1}: 3390, 3226 (br, NH_2/NH), 3188 (CH-arom.), 2922 (CH-aliph.), 2206 (C≡N), 1662 (C=O). ^1H NMR (DMSO-d_6) δ: 1.9 (s, 3H, CH_3), 3.1 (s, 3H, N–CH_3), 4.3 (s, 2H, NH_2), 5.5 (s, H, CH thiophene), 7.5–7.9 (m, 5H, Ar–H), 8.3 (s, 1H, NH). MS, *m*/*z* (%): 325 [M^+](10.17), 63(100%). Anal. Calcd. For $C_{16}H_{15}N_5OS$: C, 59.06; H, 4.65; N, 21.52; S, 9.85. Found: C, 59.46; H, 4.95; N, 21.72; S, 9.95.

*5.1.3.2. Spectral data of ethyl 2-amino-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl amino)thiophene-3-carboxylate (**7**).* Yield, 70%; m.p. 240–242 °C; IR, cm^{-1}: 3309(NH_2), 3247 (NH), 3065 (CH-arom.), 2996 (CH-aliph.), 1705, 1685 (2C=O). ^1H NMR (DMSO-d_6) δ: 1.2 (t, 3H, CH_2CH_3), 1.6 (s, 3H, CH_3), 3.3 (s, 3H, N–CH_3), 4.1 (q, 2H, CH_2CH_3), 5.9 (s, H, CH thiophene), 7.4–7.6 (m, 5H, Ar–H), 8.3 (s, 1H, NH), 9.6 (s, H, NH_2). MS, *m*/*z* (%):372(M^+) (100%). Anal. Calcd. For $C_{18}H_{20}N_4O_3S$: C, 58.05; H, 5.41; N, 15.04; S, 8.61. Found: C, 58.45; H, 5.61; N, 15.24; S, 8.91.

*5.1.4. Ethyl-2-amino-6-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino)-4-(furan-2-yl)-4H-pyran-3-carboxylate (**9**).* To a mixture of 4-acetylantipyrine **2** (2.3 g, 10 mmol) and in ethanol (50 mL) containing a few drops of piperidine of ethyl 2-cyano-3-(furan-2-yl)acrylate were added. The reaction mixture was refluxed for 2 h; and then left to cool at room temperature. The solid separated was collected and crystallized from dioxane to give brown crystals **9**: Yield, 87%; m.p. 100–102 °C; IR, cm^{-1}: 3325, 3250 (NH_2, NH), 3009 (CH-arom.), 2905(CH-aliph.), 1780, 1668 (2C=O). ^1H NMR (DMSO-d_6) δ: 1.3(t, 3H, CH_3 ethyl), 2.4(s, 3H, CH_3), 3.2 (s, 3H, N–CH_3), 4.2(q, 2H, CH_2 ethyl), 4.6(s, 1H, NH), 6.3–7.5(m, 12H, Ar–H+ NH_2). ^{13}C NMR (DMSO-d_6): 12.5 (**C**H_3, aliphatic), 13.9 (**C**H_3, ester), 38.9 (N–**C**H_3), 60.9 (**C**H_2, ester), 30.3, 75.5, 75.9, 105.2, 106.0, 109.4, 112.1, 119.4, 129.1, 131.2, 136.5, 140.0, 15104, 152.2 (14**C**, aromatic carbons), 160.2, 166.5(2**C**=O),167.0(pyrane–**C**–NH_2). MS, *m*/*z* (%): 339[M + 1] (1.94), 50 (100%). Anal. Calcd. For $C_{23}H_{24}N_4O_5$: C, 63.29; H, 5.54; N, 12.84. Found: C, 63.49; H, 5.74; N, 12.94.

*5.1.5. N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-(thiophen-2-yl)acrylamide (**10**).* To a mixture of 4-acetyl -antipyrine **2** (2.3 g, 10 mmol) and thiophene-2-carbaldehyde (1.12 g, 10 mmol) in ethanol (50 mL) cooled at 5–10 °C was added aqueous sodium hydroxide (70%, 5 mL) drop wise with constant stirring. The reaction mixture was further stirred for 2 h and left over night. The reaction mixture was neutralized with concentrated hydrochloric acid, and then the solid separated was collected and crystallized from benzene to give brown crystals **10**: Yield, 75%; m.p. 240–242 °C; IR, cm^{-1}: 3218 (NH), 3079 (CH-arom.), 2993 (CH-aliph.), 1711, 1665 (2C=O). ^1H NMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 3.3 (s, 3H, N–CH_3), 6.8 (s, 2H, CH=CH), 7.1–7.8 (m, 8H, Ar–H+ NH), 9.0 (s, H, NH). ^{13}C NMR (DMSO-d_6): 12.4 (**C**H_3, aliphatic), 39.6 (N–**C**H_3), 103.5, 113.0, 119.1, 127.6, 128.6, 129.8, 130.9, 132.5, 136.2, 137.6 (10**C**, aromatic carbons), 160.8, 164.9(2**C**=O). MS, *m*/*z* (%): 339 [M + 2] (2.04), 121 (100%). Anal. Calcd. For $C_{18}H_{17}N_3O_2S$: C, 63.70; H, 5.05; N, 12.38; S, 9.45. Found: C, 63.40; H, 4.85; N, 12.08; S, 9.35.

*5.1.6. Ethyl N-3-cyano-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino)thiophen-2-ylformimidate (**11**).* A mixture of compound **6** (1.6 g, 5 mmol) and triethyl orthoformate (20 mL) was heated under reflux for 2 h. The solid product was collected and crystallized from benzene to give orange crystals **11**: Yield, 83%; m.p. 240–242 °C; IR, cm^{-1}: 3228 (NH), 3097 (CH-arom.), 2935(CH-aliph.), 2218 (C≡N), 1661 (C=O), 1593 (CH=N). ^1H NMR (DMSO-d_6) δ: 1.1(t, 3H, CH_3 ethyl), 2.2(s, 3H, CH_3), 3.4 (s, 3H, N-CH_3), 4.1(q, 2H, CH_2 ethyl), 4.4(s, 1H, NH), 7.4–8.4(m, 6H, Ar–H+ N=CH). Anal. Calcd. For $C_{19}H_{19}N_5O_2S$: C, 59.82; H, 5.02; N, 18.36; S, 8.41. Found: C, 59.92; H, 5.32; N, 18.56; S, 8.71.

*5.1.7. 4-(3-Amino-4-imino-3,4-dihydrothieno[2,3-d]pyrimidin-5-ylamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one(**12**).* A mixture of **11** (1.9 g, 5 mmol) and hydrazine hydrate (0.25 g, 5 mmol) in ethanol (60 mL) was heated under reflux for 3 h. The solid product was filtered on hot, dried, and crystallized from ethanol to give yellow crystals **12**: Yield, 80%; m.p. 220–222 °C; IR, cm^{-1}: 3310, 3215 (NH_2/NH), 3007 (CH-arom.), 2919 (CH-aliph.), 1664 (C=O). ^1H NMR (DMSO-d_6) δ: 2.0(s, 3H, CH_3), 3.1 (s, 3H, N–CH_3), 6.0 (s, 2H, NH_2), 7.5–7.9 (m, 8H, Ar-H+C=NH). ^{13}C NMR (DMSO-d_6): 12.3 (**C**H_3, aliphatic), 39.5 (N–**C**H_3), 102.6, 113.5, 115.9, 119.3, 127.0, 128.9, 131.2, 136.8, 145.3, 163.1, 164.2 (11**C**, aromatic carbons), 160.7 (pyrazole–**C**=O). MS, *m*/*z* (%):367 [M + 2] (100%). Anal. Calcd. For $C_{17}H_{17}N_7OS$: C, 55.57; H, 4.66; N, 26.68; S, 8.73. Found: C, 55.87; H, 4.96; N, 26.88; S, 8.93.

*5.1.8. 2-Chloro-N-(3-cyano-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino)thiophen-2-yl)acetamide (**13**).* To stirred suspension of **6** (1.6 g, 0.005 mol), and chloroacetyl chloride (0.5 g, 5 mmol) in dimethylformamide (20 mL) at room temperature, for 30 min was added. The reaction mixture was cooled, diluted with water and the solid obtained was collected and recrystallized from dioxane to give orange crystals **13**: Yield, 60%; m.p. 120–122 °C; IR, cm^{-1}: 3248 (2NH), 3091 (CH-arom.), 2960 (CH-aliph.), 2168 (C≡N), 1724, 1669 (2C=O). ^1H NMR (DMSO-d_6) δ: 4.2 (s, H, CH_2), 5.8 (s, H, CH thiophene), 7.0–7.9 (m, 6H, Ar–H+ NH), 9.4(s, 1H, NH). ^{13}C NMR (DMSO-d_6): 12.4 (**C**H_3, aliphatic), 39.3 (N–**C**H_3), 43.5 (**C**H_2, aliphatic), 116.09 (**C**≡N), 64.6, 93.2, 113.0, 116.0, 119.0, 128.6, 129.4, 130.8, 136.5, 145 (10**C**, aromatic carbons), 160.8, 164.9 (2**C**=O). MS, *m*/*z* (%): 401 [M + 2] (22.07), 311 (100%). Anal. Calcd. For $C_{18}H_{16}ClN_5O_2S$: C, 53.80; H, 4.01; N, 17.43; S, 7.98. Found: C, 53.90; H, 4.21; N, 17.73; S, 8.28.

*5.1.9. 4-(4-Aminothieno[2,3-d]pyrimidin-5-ylamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (**15**).* A solution of compound **6** (1.6 g, 5 mmol), in formamide (20 mL) was refluxed for 5 h; the reaction mixture was cooled, and then poured onto ice cooled water. The formed solid was recrystallized from dioxane to yellow

crystals **15**: Yield, 83%; m.p. 240–242 °C; IR, cm^{-1}: 3340, 3230 (br, NH_2/NH), 3104 (CH arom.), 2893 (CH aliph.), 1669 (C=O). ^1H NMR (DMSO-d_6) δ: 1.6 (s, 3H, CH_3), 2.6 (s, 3H, N–CH_3), 4.8(s, 1H, NH), 6.5 (s, 2H, NH_2), 7.5–8.4 (m, 7H, Ar–H). ^{13}C NMR (DMSO-d_6): 12.5 (**C**H_3, aliphatic), 39.5 (N–**C**H_3), 102.9, 113.3, 116.9, 119.5, 127.0, 129.9, 131.1, 136.5, 143.3,157.0,158.0 (11**C**, aromatic carbons), 160.4(pyrazole–**C**=O). MS, *m*/*z* (%):352 (M + 2, 3.14%), 52 (100%). Anal. Calcd. For $C_{17}H_{16}N_6OS$: C, 57.94; H, 4.58; N, 23.85; S, 9.10. Found: C, 57.64; H, 4.78; N, 23.95; S, 9.40.

*5.1.10. 2-Amino-4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino) thiophene-3-carboxamide (**16**).* A sample of compound **6** (1.6 g, 5 mmol), was dissolved in conc. Sulfuric acid (10 mL) and stirred at r.t. for 2 h. The reaction mixture was diluted with ice-cold water and neutralized with ammonium hydroxide. The resulting precipitate was collect by filtration and recrystallized from ethanol to give yellow crystals **16**: Yield, 74%; m.p. 180–182 °C; IR, cm^{-1}: 3390, 3159 (br, 2NH_2/NH), 3031 (CH-arom.), 2896 (CH-aliph.), 1728, 1660 (2C=O). ^1H NMR (DMSO-d_6) δ: 1.9 (s, 3H, CH_3), 2.9 (s, 3H, N–CH_3), 4.5(s, 1H, NH), 4.9 (s, 2H, NH_2), 7.2–7.7 (m, 8H, Ar–H+ NH_2). MS, *m*/*z* (%): 343 [M + 1] (11.07), 63 (100%). Anal. Calcd. For $C_{16}H_{17}N_5O_2S$: C, 55.96; H, 4.99; N, 20.39; S, 9.34. Found: C, 55.66; H, 4.59; N, 20.19; S, 9.14.

*5.1.11. 4-(4-Oxo-2-sulphoxido-1,3,4-trihydrothieno[2,3-d]thiadiazine-5-ylamino)-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one (**17**).* A mixture of compound **16** (3.7 g, 10 mmol) in dioxane (20 mL) and DMF (5 mL), thionyl chloride (10 mL) was added. The reaction mixture was refluxed for 4 h and the solid separated was recrystallized from benzene to afford compound **17**. Yield, 88%; m.p. 98–100 °C; IR, cm^{-1}: 3275 (NH), 3057 (CH-arom.), 2969 (CH-aliph.), 1707, 1634 (2C=O). ^{13}C NMR (DMSO-d_6): 12.6 (**C**H_3, aliphatic), 39.4 (N–**C**H_3), 102.1, 104.5, 113.3, 116.09, 119.5, 124.0, 129.7, 131.3, 136.3, 138 (10**C**, aromatic carbons), 160.6, 169.0(2**C**=O). MS, *m*/*z* (%): 389 [M + 1] (7.03), 256 (100%). Anal. Calcd. For $C_{16}H_{15}N_5O_3S_2$: C, 49.34; H, 3.88; N, 17.98; S, 16.47. Found: C, 49.14; H, 3.48; N, 17.98; S, 16.27.

*5.1.12. N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-hydrazinyl-3,4-dioxobutanamide (**18**).* To a suspension of the compound **3** (1.7 g, 5 mmol), in absolute ethanol (25 mL), was added hydrazine hydrate (5 mmol, 98%). The mixture was reflux for 3 h, and then the solid precipitate so formed was filtered and crystallized from and crystallized from dioxane to afford the hydrazide as orange crystals **18**: Yield, 83%; m.p. 236–238 °C; IR, cm^{-1}: 3342,3355, 3210 (NH_2/NH), 3144 (CH arom.), 2863 (CH aliph.), 1769,1720,1699, 1669 (4C=O). ^1H NMR (DMSO-d_6) δ: 2.0 (s, 3H, CH_3), 2.7 (s, 3H, N–CH_3), 3.4(s, H, CH_2), 4.4(s, 1H, NH_2), 7.5–7.9 (m, 7H, Ar–H+ 2NH). MS, *m*/*z* (%):331 [M–NCH_3] (11.07), 63 (100%). Anal. Calcd. For $C_{15}H_{17}N_5O_4$: C, 54.38; H, 5.17; N, 21.14. Found: C, 54.48; H, 5.37; N, 21.34.

*5.1.13. N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-3-oxo-3-(4-phenyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl) propanamide (**19**).* A mixture of compound **18** (3.7 g, 10 mmol) and phenyl isothiocyanate (1.35 g, 10 mmol) in the presence of (1*N*) sodium hydroxide (20 mL) was refluxed for 3 h. The solid separated was crystallized from ethanol to afford compound **19**. Yield, 75%; m.p. 136–138 °C; IR, cm^{-1}: 3388 (NH), 3105 (CH arom.), 1745, 1685, 1669(3C=O), 1306(C=S). ^1H NMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 3.2 (s, 3H, N–CH_3), 3.4(s, H, CH_2CO), 7.4–8.3 (m, 10H, Ar–H), 9.5(s, 1H., NH), 12.5(s, 1H.,NH triazol). ^{13}C NMR (DMSO-d_6): 12.5 (**C**H_3, aliphatic), 38.6(**C**H_2, aliphatic), 39.3 (N–**C**H_3), 103.1, 113.3, 119.5, 124.0, 126.7, 129.0, 129.6, 133.3, 133.9, 136.5 (10**C**, aromatic carbons), 167.4 (**C**=S), 160.5, 167.9, 201.0 (3**C**=O). MS, *m*/*z* (%): 448 [M^+] (15.48), 77 (100%). Anal. Calcd. For $C_{22}H_{20}N_6O_3S$: C, 58.92; H, 4.49; N, 18.74; S, 7.15. Found: C, 58.72; H, 4.29; N, 18.74; S, 6.85.

*5.1.14. N-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-4-(isonicotinacetylhydrazone)-3,4-dioxobutanamide (**20**).* A mixture of compound **18** (3.7 g, 10 mmol) and isonicotinaldehyde(1.07 g, 10 mmol) in butanole (20 mL) was refluxed for 8 h. The solvent was evaporated and the solid was crystallized from dioxane to yellow crystals **20**: Yield, 80%; m.p. 106–108 °C; IR, cm^{-1}: 3325 (NH), 3108 (CH arom.), 1735, 1703, 1689, 1665 (4C=O). ^1H NMR (DMSO-d_6) δ: 2.3 (s, 3H, CH_3), 3.1 (s, 3H, N–CH_3), 3.3(s, H, CH_2CO), 7.4–8.9 (m, 11H, Ar–H+ 2NH), 12.1(s, 1H, N=CH). ^{13}C NMR (DMSO-d_6): 12.3 (**C**H_3, aliphatic), 37.2(**C**H_2, aliphatic), 39.5 (N–**C**H_3), 103.3, 113.5, 119.3, 124.5, 129.0, 133.5, 136.3, 144.6, 198.2 (9**C**, aromatic carbons), 143.0(N=**C**H), 160.6, 164.9, 168.0, 197.2 (4**C**=O). MS, *m*/*z* (%): 420 [M^+] (28.3), 77 (100%). Anal. Calcd. For $C_{21}H_{20}N_6O_4$: C, 59.99; H, 4.79; N, 19.99. Found: C, 59.69; H, 4.59; N, 19.59.

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