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RESEARCH ARTICLE

Syntheses and antimicrobial activity of new benzofuran, pyrrole, imidazole and thioxoimidazolidin incorporating antipyrine moiety

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Abstract

Pyrazolo[3,4:3',2']pyrrolo[2,3-*d*]pyrimidin**,** thioxoimidazolidine and 3-chloro pyrazole derivatives were reported to act as potent antimicrobial activity. A series of novel derivatives of 4-substituted-pyrazole, pyrrolo[3,2-*c*]pyrazole**,** acetamide, pyrrole, ethoxy, thiourea, urea and pyrazolo[4,3-*d*]imidazole, bearing antipyrine were synthesized. The structures of these compounds were confirmed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the new synthesized compounds were tested against antimicrobial activities by the disc diffusion method, which exhibited higher promising biological activities.

Introduction

Antipyrine derivatives have attracted the attention of several research groups due their potential activities (Abdel Latif 2006, Jain et al. 2003, El Maati 2002; Turan-Zitouni et al. 2001, Gürsoy et al. 2000). A broad spectrum of bioactive antipyrine derivatives has been investigated and diversities of bioactivities such as analgesic (Cechinel et al., 1999; Sondhi et al. 1999) anti-inflammatory (Ismail et al., 2007), antimicrobial (Hala et al. 2011; Raman et al., 2004, Raman et al. 2002, Mishra 1999) and anticancer activity (Hala et al., 2012, Sondhi et al., 2001) have been reported. Synthesis of a variety of heterocyclic systems from the readily obtainable inexpensive starting materials was performed to evaluate the biological screening program. Some new heterocyclic moieties incorporating the antipyrine moiety starting from 4-amino antipyrine were synthesized. Also, the behavior of the unreported 4-amino antipyrine **1** towards some active methylene and electrophilic reagent was investigated.

Results and discussion

Chemistry

In this investigation, a series of new compounds incorporating benzofuran, pyrrole, imidazole and thioxoimidazolidin moieties that attached to phenazone moiety (**2–23**), was designed and synthesized (Scheme**s 1–4**). The biological evaluation for these compounds in vitro antimicrobial activity was studied. Thus, interaction of 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one **1** with different active methylene (malononitrile, ethyl cyanoacetate, acetyl acetone, 1-(4,6-dimethoxybenzofuran-5-yl)butane-1,3-dione) **5,** and triethyl orthoformate in the presence of acetic acid yielded the corresponding antipyrine derivatives **2-6**, respectively (Mostafa et al., 2010) (Scheme **1**). Assignments of compounds **2-6** were based on the basis of elemental and spectral data. The infrared spectrum of compound **2** exhibited the absence of NH_2 group absorption band while it showed the presence of characteristic CN bands. Also, its mass spectrum showed a molecular ion peak at m/z 279 [M^+] (10.98) together with the base peak at m/z 55 (100). IR spectrum of compound **3** showed the presence of CN and ester bands, while its ^{1}HNMR spectrum showed the presence of triplet and quartet signals characteristic of ester group. The IR spectra of compounds **4** and **6** exhibited the presence of a C=O bands that characteristic to the acetyl group.

The interaction of 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydropyrazol-3-one **1** with malononitrile in dioxane as solvent in the presence of piperidine that furnished the pyrrolo[3,2-*c*]pyrazole derivative **8** (Mostafa et al., 2008) was studied (Scheme **2**). Structure of the latter product was based on analytical and spectral data, IR spectrum exhibited the disappearance of carbonyl group of 4-aminoantipyrine and presence of bands that characteristic for cyano and amino groups. Its mass spectrum exhibited a molecular ion peak at m/z 251 (2.82 %) together with a base peak at m/z 207. The formation of pyrrolo[3,2-*c*]pyrazole derivative **8** was assumed to proceed through initial condensation of malononitrile with (C=O) group in compound **1** to form intermediate compound **7**. Intramolecular cyclization was followed to one of the cyano group and tautomerization to furnish pyrrolo[3,2-*c*]pyrazole derivative **8** (Scheme **2**). Also, the reactivity of pyrrolo[3,2-*c*] pyrazole derivative **8** against nucleophilic reagent was studied in order to obtain the biologically active pyrrolo[2,3-*d*]pyrimidin derivative **10** bearing antipyrine moiety.

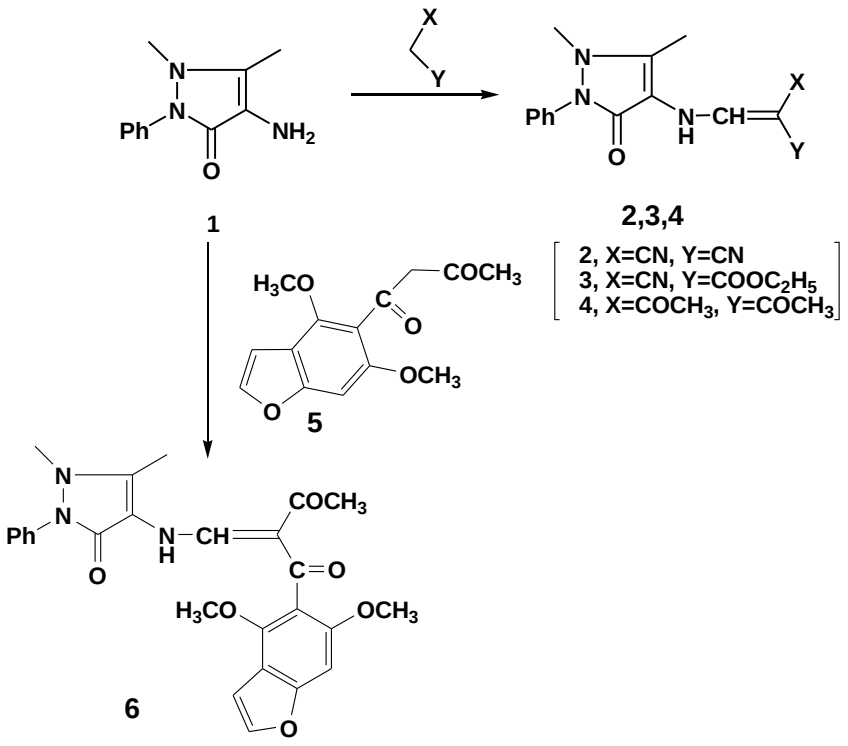
Thus, interaction of compound **8** with urea in sodium ethoxide yielded the corresponding pyrrolo[2,3-*d*]pyrimidin derivative **10** and the other expected pyrrolo[3,2-*c*]pyrazoluredo derivative **9** was ruled out on the basis of analytical and spectral data. The IR spectrum of **10** showed the absence of (C≡N) absorption band and presence of (NH_2) at 3390, 3299, (CH arom.) at 3112, (CH aliph.) at 2958, (C=O) at 1665cm^{-1}. The mass spectrum of **10** exhibited a molecular ion peak m/z at 296 (M+2, 17.9%), with a base peak at 77. Its ^{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), 6.4 (s, 2H, NH_2), 7.5-7.9 (m, 6H, Ar-H+NH) and ^{13}C NMR (DMSO-d_6) δ: 165.9, 163.6, 160, 157 , 137.2, 134.2, 129.3, 129.2, 119.4, 112.2, 114.2, 87, 39.1, 11.0. Treatment of antipyrine derivative **1** with chloroacetyl chloride at room temperature afforded 2-chloro-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide **11**. Structure of the latter product was confirmed on the basis of its correct elemental analysis and spectral data. The ^{1}H NMR spectrum of compound **11** was characterized by the complete disappearance of the signals attributed to assign for the amino group on antipyrine moiety and presence of the signals attributed to $COCH_2$ group at δ 4.2. The behavior of compound **11** towards active methylene was investigated. On the other hand, ethyl 2-amino-1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-5-oxo-4,5-dihydro-1*H*-pyrrole-3-carboxylate **12** was prepared by reacting of 2-chloro-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide **11** (Raiziss and Clemence,1930) with ethyl cyano acetate. Both elemental and spectral data supported the proposed structure **12** and formation of **13** and **14** that were ruled out, (Scheme **3**). The infrared spectrum of compound **12** indicated the presence of characteristic absorption bands for NH_2 and ester functional groups. In addition, the molecular structure of compound **12** was established by ^{1}H NMR spectrum which exhibited the lack of characteristic signal of ($COCH_2$) group and the presence of triplet at 1.2 ppm, quartet at 4.2 ppm assigned for ethyl ester moiety in addition to amino protons at 6.4 ppm. The molecular ion peak of compound **12** was found in the mass spectrum at m/z 356 (M^++1, 6.9 %) and 111 (100% base peak).

In addition, ethyl *N*-1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl- formimidate **15** was obtained in 87 % yield by the alkylation reaction of starting material **1** with excess of triethyl orthoformate at 200 ºC under solvent free condition and attempts to cyclize the ethoxy derivative **15** by refluxing in ethanol containing piperidine and/or pyridine to afford 2,3 -dimethyl-1-phenyl-2,6a-dihydro-1*H*-pyrazolo[4,3-*d*]oxazole **16** which was unsuccessful on the basis of analytical and spectral data.

When compound **1** was allowed to reflux with thiourea in ethanol in the presence of sodium ethoxide as a base yield thiourea derivative **17** via elimination of ammonia and the expected 2,3-dimethyl-1-phenyl-1,2-dihydropyrazolo[4,3-*d*]- imidazole-5 (4*H*) thione **18** was obtained by fusion of 4-aminoantipyrine **1** with thiourea on oil bath. Both of compounds **17** and **18** were elucidates by analytical and spectral data. This was proved by IR spectrum of compound **17** that showed bands at 3352, 3226, 3195 (NH, NH_2), 1669 (C=O) and 1259 cm^{-1} (C=S). The mass spectrum of compound **17** exhibited a molecular ion peak m/z at 262 (M-2, 3.59%), with a base peak at 64 corresponding to the molecular formula $C_{12}H_{14}N_4OS$. In a similar, 4-aminoantipyrine **1** underwent reacted with equimolar amount of urea afforded the 1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)urea **19**. Both elemental and spectral data supported the proposed structure **19** and the formation of 2,3-dimethyl-1-phenyl-1,2-dihydropyrazolo[4,3-*d*]imidazol-5(4*H*)-one **20** was ruled out, (Scheme **4**). 1,5-dimethyl-4-(4-oxo-2-thioxoimidazolidin-1-yl)-2-phenyl-1,2-dihydro pyrazol-3-one **21** was occurred in excellent yield by treatment of thiourea derivative **17** with ethyl chloroacetate under reflux in ethanol in the presence of sodium acetate. A Knoevenagel condensation of the active methylene group in thioxoimidazolidin derivative **21** with aromatic aldehyde in ethanol under reflux in the presence of piperidine yielded the 1,5-dimethyl-4-(4-oxo-5-(pyridin-4-ylmethylene)-2-thioxo imidazolidin-1-yl)-2-phenyl-1,2-dihydropyrazol-3-one **22**. Assignment of **22** was based on the basis of elemental and spectral data. Finally, chlorination of 4-amino antipyrine **1** with thionyl chloride afforded the 3-chloro-1,5-dimethyl-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-amine **23**. Structure of the latter product was confirmed on the basis of its correct elemental analysis and spectral data, (Scheme **4**). The investigation of antibacterial and antifungal screening data revealed that all the tested compounds **2, 3, 4, 6, 8, 10, 11, 12, 15, 17, 18, 19, 21, 22** and **23** showed comparatively good activity against all the bacterial strains. The organisms were tested against the activity of solutions with concentration; 1.0 mg/mL of

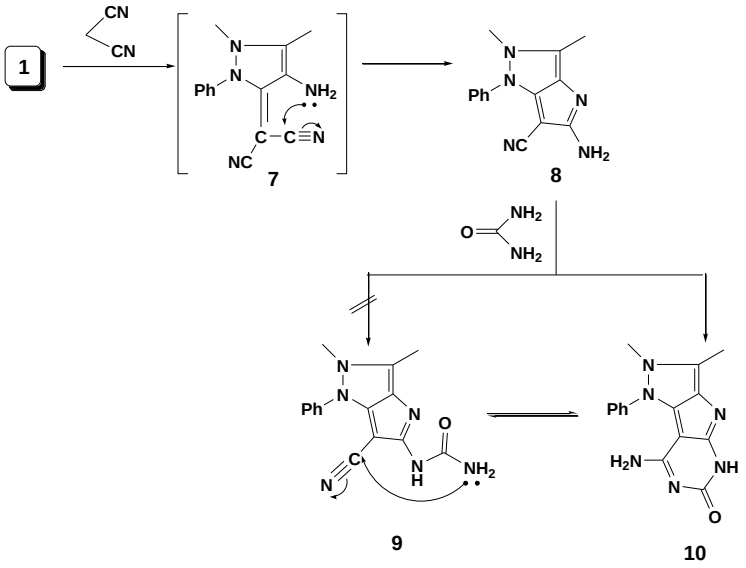
each compound; and using inhibition zone diameter (IZD) in centimeter as the criterion for antimicrobial activity. Itraconazole and Clotrimazole as an antifungal agents & Penicillin G and Streptomycin as antibacterial agents were used as references to evaluate the potency of the tested compounds under the same conditions. The minimum inhibitory concentration (MIC) of the biologically active compounds was measured by a two-fold serial dilution method. The results are depicted in Tables **1-3**. Most of the compounds were effective against Aspergillus fumigatus, Geotrichum candidum, and Candida albicans. In case of Itraconazole and Clotrimazole, compounds **2, 4, 15, 17 and 19** are mostly effective, while **3, 6, 8, 11, 12, 18, 21** have no activity. The pyrazolo[3,4:3',2']pyrrolo[2,3-d]pyrimidin **10,** thioxoimidazolidine **22** and 3-chloro pyrazole derivatives **23** were nearly as active as the standard fungicides Itraconazole and Clotrimazole. Among these fifteen active compounds, the most moderate effective compounds against Syncephalastrum racemosum than the standard fungicides Itraconazole and Clotrimazole were **4, 10, 22** and **23** while other effective compounds have no activity; the results were depicted in Table **1**. In addition, most of the compounds were effective against Staphylococcus aureus, Bacillus subtilis (as gram positive bacteria) and Escherichia coli (as gram negative bacteria). In case of Penicillin G and Streptomycin; compounds **2, 4, 8, 10, 12, 15, 17, 19, 22 and 23** are mostly effective, while **3, 6, 11, 18, 21** have no activity. The pyrazolo[3, 4: 3', 2'] pyrrolo[2,3-d]pyrimidin **10,** thioxoimidazolidine **22** and 3-chloro pyrazole derivatives **23** nearly as active as the standard bactericides Penicillin G and Streptomycin.

Scheme 1. Synthetic pathways for compounds 2, 3, 4 and 6.



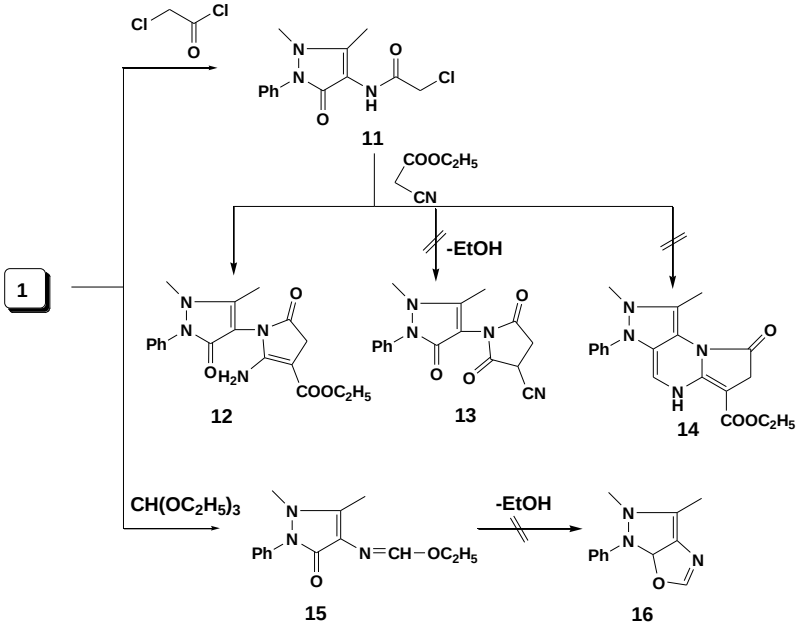
Scheme 1.

Scheme 2. Synthetic pathways for compounds 8, 10



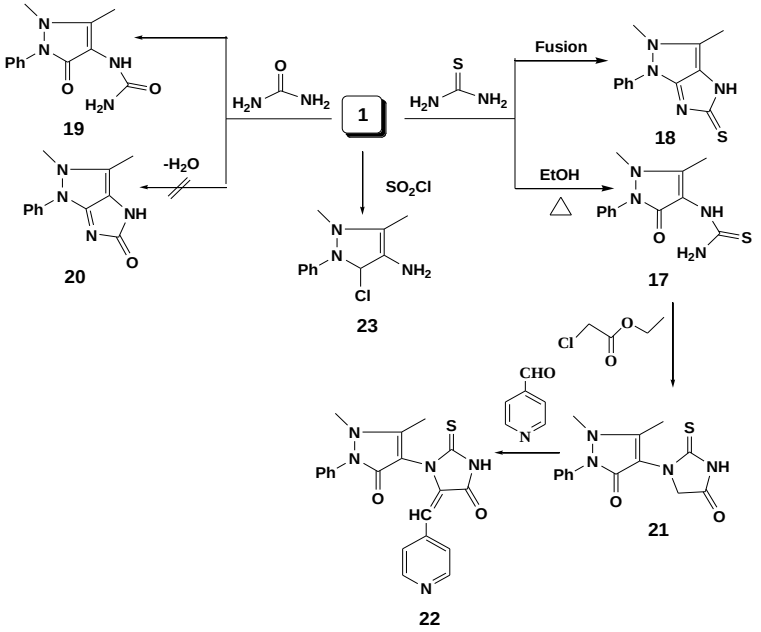
Scheme 2.

Scheme 3. Synthetic pathways for compounds 11-15.



Scheme 3.

Scheme 4. Synthetic pathways for compounds 17-23.



Scheme 4.

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)**	**Syncephalastrumr acemosum (RCMB 005003)**
2	15.3 ± 0.08	16.2 ± 0.5	14.7 ± 0.1	NA
3	NA	NA	NA	NA
4	11.3 ± 0.05	10.5± 0.07	NA	9.4± 0.04
6	NA	NA	NA	NA
8	NA	NA	NA	NA
10	19.3± 0.02	18.2 ± 0.06	16.4 ± 0.09	10.3 ± 0.05
11	NA	NA	NA	NA
12	NA	NA	NA	NA
15	10.5 ± 0.2	12.2 ± 0.05	11.8 ± 0.08	NA
17	15.2 ± 0.08	18.4 ± 0.5	15.4 ± 0.1	NA
18	NA	NA	NA	NA
19	11.7± 0.02	10.8± 0.08	10.0± 0.07	NA
21	NA	NA	NA	NA
22	20.6 ± 0.07	19.7 ± 0.05	16.8 ± 0.08	11.3 ± 0.03
23	19.2± 0.08	17.4± 0.09	14.2± 0.1	8.5± 0.05
Itraconazole	**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 (10mg/ml) concentration of tested samples and standard using (10mg/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 the tested chemical compounds.

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	19.8 ± 0.01	20.08 ± 0.03	NA	18.6 ± 0.5
3	NA	NA	NA	NA
4	11.0± 0.05	11.2± 0.03	NA	NA
6	NA	NA	NA	NA
8	12.4 ± 0.5	15.4 ± 0.1	NA	NA
10	21.3 ± 0.04	24.2 ± 0.03	19.4 ± 0.02	22.7 ± 0.06
11	NA	NA	NA	NA
12	10.4± 0.03	12.4 ± 0.2	NA	8.2± 0.08
15	11.5± 0.03	12.5± 0.1	NA	NA
17	12.4± 0.03	13.5± 0.04	NA	11.5± 0.03
18	NA	NA	NA	NA
19	12.2± 0.03	11.8 ± 0.1	NA	NA
21	NA	NA	NA	NA
22	22.9 ± 0.02	26.1 ± 0.03	20.2 ± 0.08	23.9 ± 0.04
23	18.4 ± 0.5	19.2± 0.1	16.2± 0.2	13.8 ± 0.1
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 (10mg/ml) concentration of tested samples and standard using (10mg/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 3: Antimicrobial activity as MICS (µg/ml) of the tested compounds against some microorganisms

	Minimum inhibitory concentration (µg/ml)							
	Antifungal				Antibacterial			
					Gram positive bacteria		Gram negative bacteria	
Compd No.	Aspergillus fumigatus (RCMB 002003)	Geotrichum candidum (RCMB 052006)	Candida albicans (RCMB 005002)	Syncephalastrum racemosum (RCMB 005003)	Staphylococcus aureus (RCMB 000106)	Bacillis subtilis (RCMB 000107)	Pseudomonas aeruginosa (RCMB 000102)	Escherichia coli (RCMB 000103)
10	39	78	78	313	39	9.5	39	19
22	19	39	39	156	19	9.5	19	9.5
23	68	58	136	635	78	29	78	156

Microbiological studies

Antifungal

Materials and methods

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 (Rathore et al, 2000), 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 Petri dish 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.

Procedure

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 (4mm×2mm) 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) were loaded into the wells of the plates. All compounds were prepared in dimethyl sulfoxide (DMSO), which 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. This 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 compounds **2-23** and the results were summarized in Table **1**

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 pellets 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 (typetone 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 DMSO, which 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 (Rahman et al.,

2001). Inhibition zones were determined for **2, 3, 4, 6, 8, 10, 11, 12, 15, 17, 18, 19, 21, 22** and **23**; the results were summarized in Table **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 (Damyanova et al., 2000). 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 appropriate 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.

Conclusion

This study reports the successful synthesis and antimicrobial activity of new benzofuran, pyrrole, imidazole and thioxoimidazolidin moieties bearing antipyrine moiety. The antimicrobial activity study revealed that all the tested compounds showed moderate to good growth inhibitory activity against both bacterial and fungal strains. Among these fifteen active compounds, the most moderate effective compound against Pseudomonas aeruginoca than the standard bactericides Penicillin G and Streptomycin were **10, 22** and **23** while other effective compounds do not active; the results were depicted in Tables **1, 2**. The newly synthesized compounds especially **10**, **22** and **23** have good activity against antimicrobial activity, so the MIC showed significant growth inhibition zones for these compounds; the results were depicted in Table **3**. 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.

Experimental

Measurements

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, UK). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5: 0.5 ml) mixture was used as a developing solvent system at room temperature and the spots were visualized by ultraviolet light and/or iodine. Infrared spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra (in DMSO-d_6) were recorded on Bruker Ac-300 ultra shield NMR spectrometer (Bruker, Flawil, Switzerland, ppm) at 300 MHz, using TMS as 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. Electron impact Mass Spectra were recorded on, Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. (Shimadzu, Tokyo, Japan). Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). Elemental analyses were carried out by the Micro analytical Research Centre, Faculty of Science, Cairo University. Analytical results for C, H, N and S were within ±0.4 of the calculated values.

Synthesis

General procedure for the reaction of 4-aminoantipyrine with active methylene derivatives and triethyl orthoformate A mixture of 4-aminoantipyrine derivative **1** (2.03 g, 10 mmol), active methylene compounds (such as malononitrile, ethyl cyanoacetate, acetyl acetone, 1-(4,6-dimethoxybenzofuran-5-yl)butane-1,3-dione) **5** and/ or triethyl orthoformate in the presence of acetic acid (1 mL) in methanol (50 mL) were refluxed for 5h. The solid which separated was filtered, dried and crystallized from a suitable solvent ethanol to give compounds **2-6** respectively**.**

2-((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino) methylene) malononitrile **(2)**

Yellow crystals: (72% yield), m.p. 240-242 °C; IR(KBr, cm^{-1}): 3294 (NH), 2967(CH aliph.), 3105 (CH arom.), 2100 (2C≡N), 1662 (C=O, antipyrine), MS(m/z): 279(M^+,2.24%), 55(100% base peak), ^{1}HNMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 3.1 (s, 3H, N-CH_3), 6.2 (d, 1H, CH), 6.6(s, 1H, NH), 7.5-7.8(m, 5H, Ar-H),. Anal. Found: C, 64.49; H, 4.39; N, 24.67; $C_{15}H_{13}N_5O$ Calcld C, 64.51; H, 4.69; N, 25.07.

Ethyl 2-cyano-3-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino) acrylate (3)

Off white crystals: (69% yield), m.p. 190-192 °C; IR (KBr, cm^{-1}): 3218 (NH), 2923, 2890 (CH aliph.), 3099 (CH arom.), 2210 (C≡N), 1668 (C=O, antipyrine), ^{1}HNMR (DMSO-d_6) δ: 1.3 (t, 3H, CH_3), 2.3 (s, 3H, CH_3), 3.3 (s, 3H,

N-CH_3), 4.1 (q, 2H, CH_2), 6.1 (d, 1H, CH), 6.8(s, 1H, NH), 7.8-8.0 (m, 5H, Ar-H), Anal. Found: C, 62.27; H, 5.36; N, 17.07; $C_{17}H_{18}N_4O_3$ Calcld C, 62.57; H, 5.56; N, 17.17.

3-((1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino) methylene) pentane-2,4-dione **(4)**

Black crystals: (77% yield), m.p. 250-252 °C; IR (KBr, cm^{-1}): 3230 (NH), 3019 (CH arom.), 2953, 2855 (CH aliph.), 1655, 1668 (2C=O), ^{1}HNMR (DMSO-d_6) δ: 1.7 (s, 3H, CH_3), 2.3, 2.4 (2s, 6H, 2$COCH_3$), 3.2 (s, 3H, N-CH_3), 5.9 (d, 1H, CH), 6.5(s, 1H, NH), 7.3-7.7 (m, 5H, Ar-H), Anal. Found: C, 64.86; H, 6.01; N, 13.21; $C_{17}H_{19}N_3O_3$ Calcld C, 65.16; H, 6.11; N, 13.41.

1-(4,6-Dimethoxybenzofuran-5-yl)-2-((1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-ylamino)methylene)butane-1,3-dione **(6)**

White crystals: (86% yield), m.p. 220-222 °C; IR (KBr, cm^{-1}): 3225 (NH), 2933, 3109 (CH arom.), 2835 (CH aliph.), 1665, 1668 (2C=O), MS(m/z): 475(M^++2, 3.04%), 55 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 2.3, (1s, 3H, $COCH_3$), 3.3 (s, 3H, N-CH_3), 3.7 (2s, 6H, OCH_3), 6.2 (d, 1H, CH), 6.5(s, 1H, NH), 6.9-7.5 (m, 8H, Ar-H),. Anal. Found: C, 65.37; H, 5.10; N, 8.44; $C_{26}H_{25}N_3O_6$ Calcld C, 65.67; H, 5.30; N, 8.84.

5-Amino-2,3-dimethyl-1-phenyl-1,2-dihydropyrrolo[3,2-c]pyrazole-6-carbonitrile **(8)**

A mixture of compound **1** (2.03 g, 0.01 mol) and malononitrile (0.7 g, 0.01 mol) was refluxed in dioxane (30 mL) containing piperidine (0.5 g) for 4 h. The reaction mixture was acidified with diluted HCl. The solid obtained was recrystallized from methanol to give brown crystals **8**: (90% yield), m.p. 110-112 °C; IR (KBr, cm^{-1}): 3318, 3300 (NH_2), 3059 (CH arom.), 2913 (CH aliph.), 2202 (C≡N), MS(m/z): 251(M^++2, 5.24%), 57 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 1.8 (s, 3H, CH_3), 3.1 (s, 3H, N-CH_3), 6.2(s, 2H, NH_2), 7.1-7.6 (m, 5H, Ar-H), Anal. Found: C, 66.52; H, 5.01; N, 27.57; $C_{14}H_{13}N_5$ Calcld C, 66.92; H, 5.21; N, 27.87.

3-Amino-1,8-dimethyl-2-phenyl-1,2-dihydro1H-pyrazolo[3,4:3',2'] pyrrolo[2, 3-d]pyrimidin-5-one **(10)**

A mixture of compound **8** (4.2 g, 0.01 mol) and urea (0.6 g, 0.01 mol) in ethanol (30 mL) containing sodium ethoxide (0.5 g, 0.01 mol) was refluxed for 3 h. The reaction mixture was cooled and acidified with diluted HCl. The separated crystals were recrystallized from ethanol to give pale brown crystals **10**: (95% yield), m.p. 157-159 °C; IR (KBr, cm^{-1}): 3390, 3299 (NH_2), 3112 (CH arom.), 2958 (CH aliph.), 1665 (C=O), MS (m/z): 296 (M^++2, 17.9%), 77 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 1.9 (s, 3H, CH_3), 2.9 (s, 3H, N-CH_3), 6.4 (s, 2H, NH_2), 7.5-7.9 (m, 6H, Ar-H+NH). ^{13}C NMR(DMSO-d_6) δ : 165.9, 163.6, 160,157 ,137.2, 134.2, 129.3, 129.2, 119.4, 112.2, 114.2, 87, 39.1, 11.0. Anal. Found: C, 61.41; H, 4.99; N, 28.65; $C_{14}H_{13}N_5$ Calcld C, 61.21; H, 4.79; N, 28.55.

2-Chloro-N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl) acetamide **(11)**

A suspension of compound **1** (2.03 g, 0.01 mole), and chloroacetyl chloride (1.2 g, 0.01 mole) was stirred in dimethylformamide (20 ml) at room temperature, for 30min. The reaction mixture was cooled, diluted with water and the solid obtained was collected and recrystallized from dioxane to give pale browen crystals **11**: (66% yield), m.p. 200-202 °C; IR (KBr, cm^{-1}): 3200 (NH), 3122 (CH arom.), 2950 (CH aliph.), 1660 (C=O), MS (m/z): 261(M^++2, 17.9%), 56 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 3.3 (s, 3H, N-CH_3), 4.2 (s,2H, $COCH_2$), 9.4 (s, 2H, NH), 7.5-7.9 (m, 6H, Ar-H+NH),. Anal. Found: C, 55.62; H, 4.74; N, 14.82; $C_{13}H_{14}ClN_3O_2$ Calcld C, 55.82; H, 5.04; N, 15.02.

Ethyl 2-amino-1-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-5-oxo-4,5-dihydro-1H-pyrrole-3-carboxylate **(12)**

A suspension of compound **11** (2.03g, 0.01 mole)and ethyl cyanoacetate (1.2 g, 0.01 mole) in dimethyl formamide (50 ml) containing anhydrous potassium carbonate (0.5 gm.) was refluxed for 4 h., then left to cool to room temperature. The solid obtained was recrystallized from ethanol to give yellow crystals **12**: (74% yield), m.p. 224-226 °C; IR (KBr, cm^{-1}): : 3318 (NH_2), 2903, 2870 (CH aliph.), 3109 (CH arom.), 1759, 1668 (C=O), MS(m/z): 356(M^++1, 6.9%), 111 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 1.2 (t, 3H, CH_3), 2.1 (s, 3H, CH_3), 2.8 (s,2H,CH_2), 3.0 (s, 3H, N-CH_3), 4.2 (q, 2H, CH_2), 6.4 (s, 2H, NH_2), 7.0-7.4 (m, 5H, Ar-H). ^{13}C NMR(DMSO-d_6) δ = 166.9, 160.8, 160.6, 154.2, 136.3, 129.8, 129, 119.3, 112.8, 106, 80.8, 61.3, 38.8, 38, 14.6, 12.6. Anal. Found: C, 60.96; H, 5.76; N, 15.92; $C_{18}H_{20}N_4O_4$ Calcld C, 60.66; H, 5.66; N, 15.72.

Ethyl-N-1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl formimidate **(15)**

A suspension of **1** (2.03g, 0.01 mol), triethyl orthoformate (20 mL) was refluxed for 3 h. and the solid separated was recrystallized from ethanol solvent to afford yellow crystals **15**: (87 % yield), m.p. 200-202 °C; IR (KBr, cm^{-1}): 3154 (CH arom.), 2980 (CH aliph.), 1668 (C=O), MS (m/z): 259(M^+-1, 10.0%), 55 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 1.4 (t, 3H, CH_3), 1.7 (s, 3H, CH_3), 2.8 (s,2H,CH_2), 2.9 (s, 3H, N-CH_3), 3.8 (q, 2H, CH_2), 7.4 (s, 1H, N=CH), 7.7-8.1 (m, 5H, Ar-H). ^{13}C NMR (DMSO-d_6) δ =160.4,154.5, 152.2,129.3,112.6, 136.6, 119.2,110.7,61.7,39.3, 15.0, 12.8. Anal. Found: C, 64.45; H, 6.31; N, 16.00; $C_{14}H_{17}N_3O_2$ Calcld C, 64.85; H, 6.61; N, 16.20.

General procedure for the reaction of 4-aminoantipyrine with urea or thiourea

A mixture of **1** (2.03 g, 0.01 mol) and thiourea or urea (0.01 mol) in ethanol (50 mL) containing sodium ethoxide (0.53 g, 0.01 mol) was refluxed for 3 h. The reaction mixture was cooled and acidified with diluted HCl. The separated crystals were recrystallized from methanol solvent to afford the corresponding pyrazole derivatives **17** and **19** respectively.

1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)thiourea **(17)**

Yellow crystals: (77% yield), m.p. 170-172 °C; IR (KBr, cm^{-1}): 3360, 3349 (NH_2/ NH), 3008 (CH arom.), 2947 (CH aliph.), 1669 (C=O), MS(m/z): 262(M^+-2, 29.0%), 64 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 1.9 (s, 3H, CH_3), 3.1 (s, 3H, N-CH_3), 7.5-8.3 (m, 5H, Ar-H), 9.4 (s, 2H, NH_2),11.2 (s, 1H, NH). Anal. Found: C, 54.64; H, 5.18; N, 21.16; S, 12.02; $C_{12}H_{14}N_4OS$ Calcld C, 54.94; H, 5.38; N, 21.36; S, 12.22.

1-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)urea **(19)**

White crystals: (79% yield), m.p. 137-139 °C; IR (KBr, cm^{-1}): 3390, 3249 (br,NH_2/ NH), 3138 (CH arom.), 2900 (CH aliph.), 1656 (C=O), MS(m/z): 246(M^+, 21.3%), 51 (100% base peak), ^{1}HNMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 3.2 (s, 3H, N-CH_3), 7.2-7.9 (m, 5H, Ar-H), 8.3 (s, 2H, NH_2), 9.0 (s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ = 160.4, 149.3, 136.7, 131.7, 129.2, 119.2, 112.5, 103.1, 39, 12.5. Anal. Found: C, 58.33; H, 5.53; N, 22.35; $C_{12}H_{14}N_4O_2$ Calcld C, 58.53; H, 5.73; N, 22.75.

2,3-Dimethyl-1-phenyl-1,2-dihydroimidazo[4,5-c]pyrazole-5(4H)-thione **(18)**

A mixture of compound **1** (2.27 g, 0.01 mol) and thiourea (0.76 g, 0.01 mol) was fused on an oil bath at 220 °C for 30 min., the solid obtained was crystallized from ethanol solvent to afford brown crystals **18**: (88% yield), m.p. < 250 °C; IR (KBr, cm^{-1}): 3300 (NH), 3199 (CH arom.), 2900 (CH aliph.), 1499 (C=S), MS(m/z): 244(M^++1, 20.29%), 56 (100% base peak). ^{1}H NMR (DMSO-d_6) δ: 1.8 (s, 3H, CH_3), 2.8 (s, 1H, NH), 3.2 (s, 3H, N-CH_3), 7.5–8.1 (m, 5H, Ar-H). Anal. Found: C, 58.99; H, 4.95; N, 22.93; S, 13.12; $C_{12}H_{12}N_4S$ Calcld C, 58.99; H, 4.95; N, 22.93; S, 13.12.

1,5-Dimethyl-4-(4-oxo-2-thioxoimidazolidin-1-yl)-2-phenyl-1,2-dihydro pyrazol-3-one **(21)**

A mixture of compound **17** (2.6 g, 0.01 mol) and ethyl chloroacetate (0.2 g, 0.01 mol) in ethanol (50 mL) containing fused sodium acetate (0.53 g, 0.01 mol) The reaction mixture was cooled and the separated crystals were recrystallized from ethanol to afford white crystals **21**: (83% yield), m.p. 196-198 °C; IR (KBr, cm^{-1}): 3320 (NH), 3169 (CH arom.), 2890 (CH aliph.), 1435 (C=S), 1666, 1654 (C=O), MS(m/z): 302 (M^+, 3.29%), 77 (100% base peak). ^{1}H NMR (DMSO-d_6) δ: 2.1 (s, 3H, CH_3), 3.1 (s, 3H, N-CH_3), 4.3 (s, 2H, CH_2 thioxoimidazolidin), 7.3–7.6 (m, 5H, Ar-H), 8.5 (s, 1H, NH), Anal. Found: C, 55.91; H, 4.87; N, 18.93; S, 10.81; $C_{14}H_{14}N_4O_2S$ Calcld C, 55.61; H, 4.67; N, 18.53; S, 10.61.

1,5-Dimethyl-4-(4-oxo-5-(pyridin-4-ylmethylene)-2-thioxoimidazolidin-1-yl)-2-phenyl-1,2-dihydropyrazol-3-one **(22)**

A mixture of compound **21** (3.2 g, 10 mmol) with isonicotinaldehyde (10 mmol) in dioxane (50 mL) containing drops of triethylamine was reflux for 4 h. The reaction mixture was allowed to cool to room temperature and was poured into water (100 mL), whereby a solid was filtered off and crystallized from methanol to produce **22**: (83% yield), m.p. > 250 °C; IR (KBr, cm^{-1}): 3320 (NH), 3109 (CH arom.), 2936, 2890 (CH aliph.), 1465 (C=S), 1662, 1659 (C=O), MS (m/z): 391(M^+, 3.29%), 77 (100% base peak), ^{1}H NMR (DMSO-d_6) δ: 2.3 (s, 3H, CH_3), 3.0 (s, 3H, N-CH_3),6.2 (s, H, CH=CH), 7.0–7.8 (m, 9H, Ar-H), 11.0 (s, 1H, NH). ^{13}C NMR(DMSO-d_6) δ : 165.1, 161.4, 160.5, 149.1, 143.5, 136.3, 136.7, 129.5, 129.1, 120.7, 117, 119.6, 112.6, 105.8, 39.6, 13.1. Anal. Found: C, 61.67; H, 4.58; N, 17.99; S, 8.39; $C_{20}H_{17}N_5O_2S$ Calcld C, 61.37; H, 4.38; N, 17.89; S, 8.19.

3-Chloro-1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine **(23)**

A mixture of compound **1** (2.03 g, 0.01 mol) in dioxane (20mL) 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 ethanol to afford compound **23:** IR (KBr, cm^{-1}): 3320 (NH), 3169 (CH arom.), 2890 (CH aliph.), 1435 (C=S), 1666, 1654 (C=O), MS (m/z): 223 (M^+, 1.29%), 56 (100% base peak), Anal. Found: C, 59.36; H, 6.41; N, 18.88; $C_{11}H_{14}ClN_3$ Calcld C, 59.06; H, 6.31; N, 18.78.

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