European Journal of Medicinal Chemistry 62 (2013) 478–485

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Laboratory note

First synthesis and biological evaluation of indeno[2,1-*e*]pyrazolo[3,4-*b*]pyrazin-5-one and related derivatives

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ARTICLE INFO

Article history:
Received 18 October 2012
Received in revised form
17 January 2013
Accepted 19 January 2013
Available online 26 January 2013

Keywords:
Pyrazolo[3,4-*b*]pyrazine
Indeno[2,1-*e*]pyrazolo[3,4-*b*]pyrazin-5-one
Biological evaluation

ABSTRACT

The synthesis of indeno[2,1-*e*]pyrazolo[3,4-*b*]pyrazin-5-one was achieved by intramolecular Friedel–Crafts reaction of the acid chloride 3-methyl-1,6-diphenyl-1*H*-pyrazolo[3,4-*b*] pyrazine-5-carboxylic acid chloride (**4**) using $AlCl_3$ in boiling CS_2. Compound **4** proved to be a versatile compound for the synthesis of several Indenopyrazolopyrazinone derivatives. The antibacterial and antifungal activities of selected derivatives were evaluated.

1. Introduction

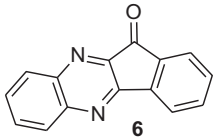
Pyrazolopyrazines constitute an important class of heterocyclic compounds. They are used in the prevention and/or treatment of a wide variety of conditions known to be related to adenosine receptors such as depression, dementia, Parkinson's disease, anxiety, pain, cerebrovascular disease, and heart failure [1–3].

A perusal of the literature revealed that there are rather limited reports on the synthesis of pyrazolo[3,4-*b*]pyrazines in spite of their importance as therapeutic agents. The latter compounds were reported to play a role as bone metabolism improvers useful for the treatment of osteoporosis, periodontosis, hypercalcemia, Paget's disease and rheumatoid arthritis [4a]. Some reports indicated their use as blood platelets aggregation inhibitors, antiinflammatories [4b] and anticancer agents with low toxicity [5a,b]. As well as, it showed antiparasitic, antifungal and antibacterial activities [6a–c]. Other derivatives are also used as fluorescent dyes [7a] or dispersed dyes for polyester fibers [7b]. Very recently, a microwave-assisted synthesis of fused pyrazolo[3,4-*b*]pyrazines by the reaction of ortho-aminonitrosopyrazoles and cyclic β-diketones was reported [8].

2. Results and discussions

2.1. Chemistry

In continuation to our interest in the synthesis of pyrazole-based heterocycles [9–13], and in particular the synthesis pyrazolo[3,4-*b*]pyrazines of antiparasitic, antifungal and antibacterial activities [6a–c] we wish to report herein the first synthesis of 3-methyl-1-phenyl-1*H*-indeno[2,1-*e*]pyrazolo[3,4-*b*]pyrazin-5-one (**5**), the pyrazole isostere of indeno[1,2-*b*] quinoxalin-*11*-one (**6**), and to evaluate the biological activities of this new tetrafused heterocycle and its derivatives.



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Indeno[1,2-*b*]quinoxalin-11-one

The synthesis of **5** was achieved by intramolecular Friedel–Crafts reaction of the acid chloride **4** using $AlCl_3$ in boiling CS_2 (Scheme 1). The synthesis of **4** was described earlier by us [6a] starting from the easily accessible 5-amino-3-methyl-4-nitroso-1-phenyl-1*H*-pyrazole (**1**) which was interacted with benzoyl acetonitrile to give the pyrazolopyrazine carbonitrile **2**. Alkaline hydrolysis of **2** gave the acid **3** followed by treatment of **3** with thionyl chloride at reflux to give the acid chloride **4** as shown in Scheme 1.

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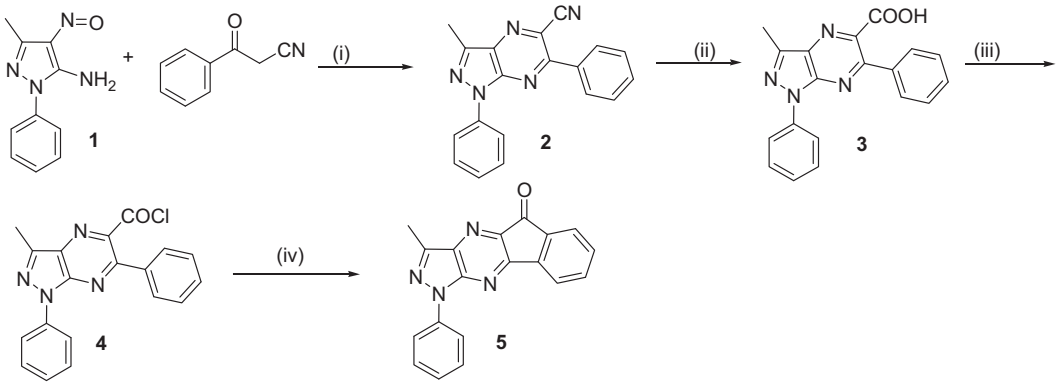
http://dx.doi.org/10.1016/j.ejmech.2013.01.025



Scheme 1. Synthesis of compounds **1**–**5**. Reagent and conditions: i) pyridine, reflux 3 h.; ii) aq. NaOH (20%), reflux 6 h.; iii) $SOCl_2$, reflux, 2 h.; iv) $AlCl_3/CS_2$, reflux, 6 h.

Several derivatives were obtained from the versatile compound **5**. Thus, **5** was condensed with some active methylene compounds such as malononitrile, acetylacetone, ethyl acetoacetate, ethyl cyanoacetate, benzoyl acetonitrile, and diethyl malonate to give the corresponding indenopyrazolopyrazin-5-ylidene derivatives **7**–**12** respectively (Scheme 2).

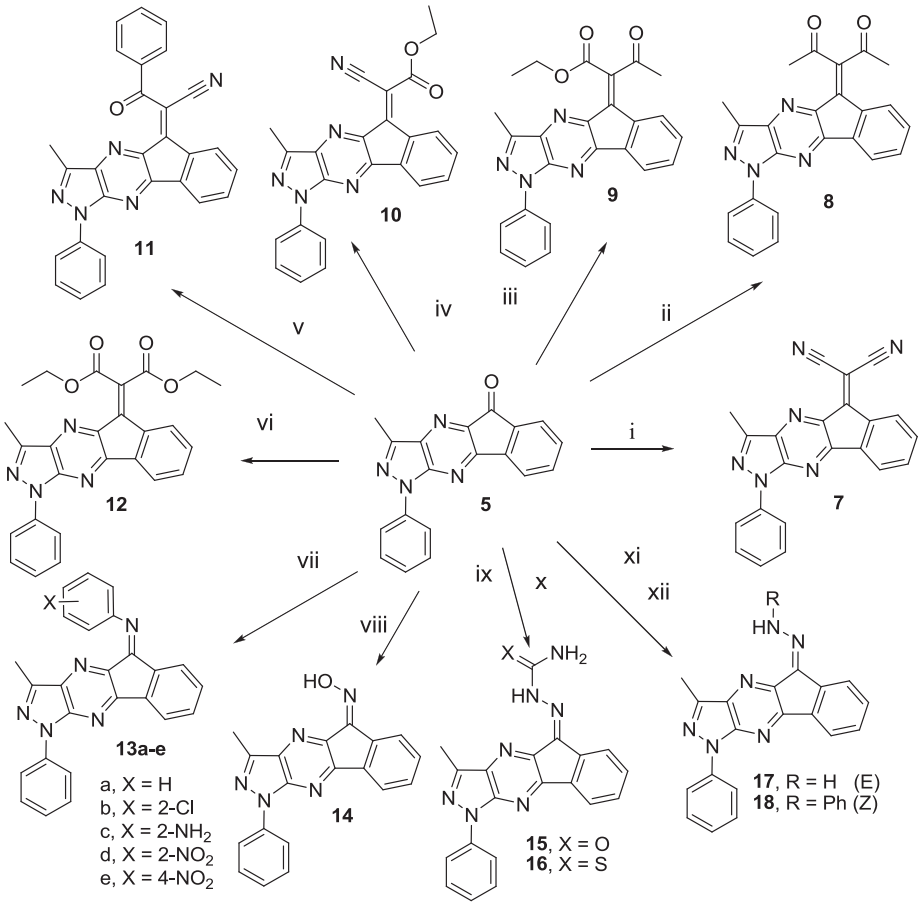
A series of anils **13a**–**e** were obtained when **5** was interacted with the corresponding aromatic amines. The reaction of **5**, in boiling pyridine, with hydroxylamine hydrochloride, semicarbazide hydrochloride and thiosemicarbazide gave the corresponding oxime **14**, semicarbazone **15** and thiosemicarbazone **16** respectively.

The hydrazones **17** and **18** were obtained via the reaction of **5** with hydrazine hydrate and phenyl hydrazine respectively (Scheme 2).

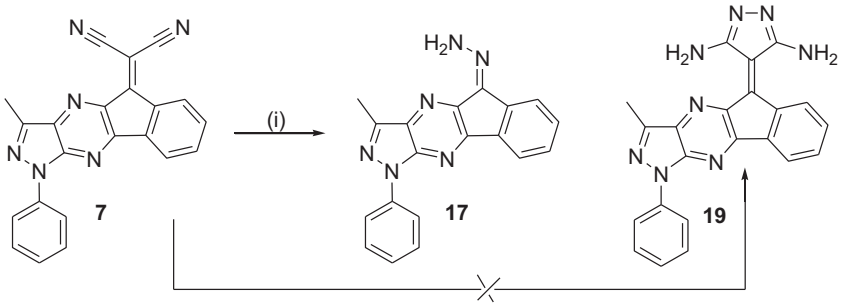
It is of interest to note that when **7** was reacted with hydrazine hydrate the product was identified as the hydrazone **17** and not the expected diamino product **19** (Scheme 3).

When the hydrazone **17** was reacted with some aromatic aldehydes the corresponding arylidene derivatives **20a**–**d** were obtained and when it was reacted with triethyl orthoformate it gave the ethoxymethylene derivative **21** (Scheme 4).

The thiosemicarbazone **16** was allowed to reacted with some α-halo compounds such as diethyl bromomalonate, ethyl chloroacetate



Scheme 2. Synthesis of compounds **7**–**18**. Reagent and conditions: i) $CH_2(CN)_2$, dry pyridine, rt.; ii) $CH_2(COCH_3)_2$, dry pyridine, reflux 10 h.; iii) $CH_3COCH_2COOC_2H_5$, dry pyridine, reflux 10 h.; iv) $NCCH_2COOC_2H_5$, dry pyridine, reflux 10 h. (v) $C_6H_5COCH_2CN$, dry pyridine, reflux 6 h.(vi) $CH_2(COOC_2H_5)_2$ dry pyridine, reflux 10 h.; vii) *x*-$C_6H_5NH_2$, dry pyridine, reflux 8 h.; viii) $NH_2OH{\cdot}HCl$, dry pyridine, reflux 3 h.



Scheme 3. Synthesis of compound **17**. Reagent and conditions: i) $NH_2NH_2 \cdot H_2O$, reflux 15 min.

and/or phenacyl bromide in boiling ethanol in the presence of anhydrous sodium acetate, to give the corresponding thiazolidinylidene derivatives **22**–**24** (Scheme 5).

On the other hand, the carbonyl group of **5** could be reduced under Wolff–Kishner reduction conditions yielding the 3-methyl-1-phenyl-1*H*,5*H*-indeno[2,1-*e*]pyrazolo[3,4-*b*]pyrazine (**25**). The latter compound when reacted with some aromatic aldehydes in refluxing ethanol in the presence of sodium ethoxide the corresponding arylidene derivatives **26a**–**c** were formed, and the anils **27a**,**b** were obtained when **25** was allowed to react with 4-nitroso-*N*,*N*-diemethyl(or diethyl)aniline in refluxing ethanol in the presence of KOH (Scheme 6).

2.2. Biological results

Ten selected indenopyrazolopyrazine derivatives (**5**, **10**, **14**, **16**, **17**, **22**, **23**, **24**, **25**, and **26b**) were evaluated for their antibacterial and antifungal activities.

Thus, these compounds were screened against *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus* as a Gram positive bacteria and *Escherichia coil*, *Pseudomonas aeruginosa*, *Serratia marcescens* as Gram negative bacteria. None of the tested compounds showed significant activity against these bacterial strains (Table 1).

On the other hand, the compounds under testing (in a concentration of 20 mg/ml) were screened for their antifungal activities against fungal strains; (*Candida albicans* AUMC No. 418, *Trichophyton rubrum* AUMC No. 1804, *Aspergillus flavus* AUMC No. 1276, *Fusarium oxysporum* AUMC No. 5119, *Scopulariopsis brevicaulis* AUMC No. 729, *Geotrichum candidum* AUMC No. 226 (Table 2). The results indicated that compounds 16, 22, 23, 24 and 26b were active against both *Trichophyton rubrum* AUMC No. 1804, *A. flavus* AUMC No. 1276) [14]. The MIC of these compounds against the latter two fungal strains was evaluated and the results were depicted in Table 3. None of the results obtained was promising.

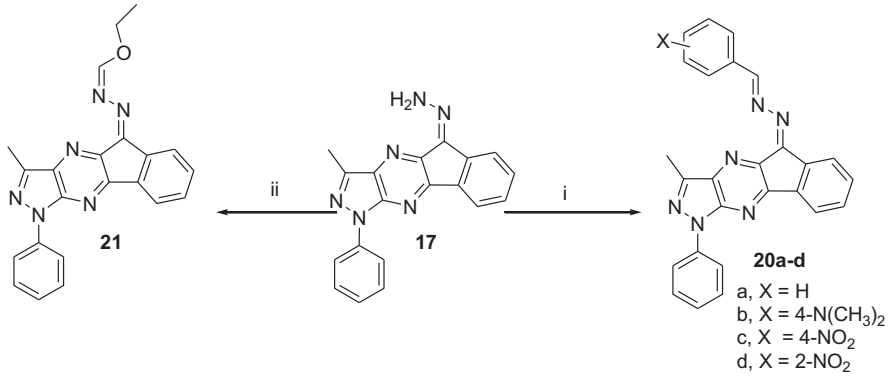
3. Conclusion

In conclusion, we have reported the synthesis novel heterocyclic system; indeno[2,1-*e*]pyrazolo[3,4-*b*]pyrazine and its related derivatives. Ten synthesized compounds have been screened for their antibacterial and antifungal activities. The results obtained showed that the compounds under investigation did not show significant activity against the used bacterial strains. Meanwhile, 5 tested compounds (**16**, **22**, **23**, **24**, and **26b**) showed weak inhibitory activity against two fungal strains *Trichophyton rubrum* AUMC No. 1804 and *A. flavus* AUMC No. 3214.

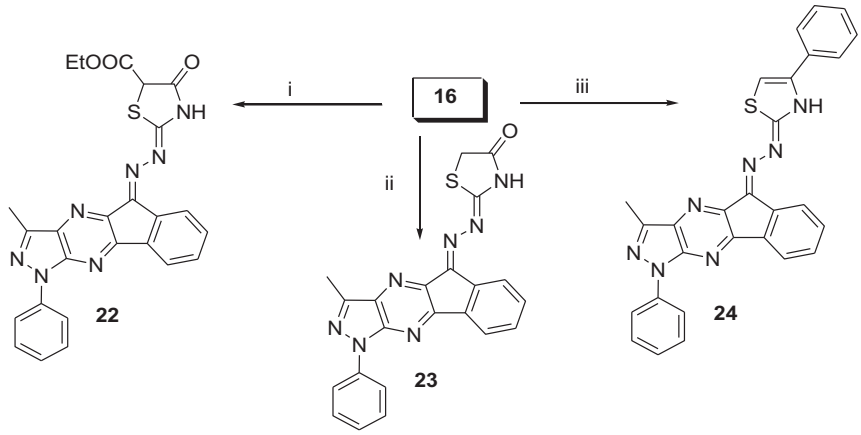
4. Experimental

4.1. Chemistry

All melting points were determined on a Kofler melting point apparatus. IR spectra were recorded on a Pye Unicam SP3-100 spectrophotometer using KBr wafer technique. The ^{1}H NMR spectra were recorded on a Bruker ARX 200 spectrometer (200 MHz for ^{1}H and 50 MHz for ^{13}C) at the Faculty of Pharmacy, University of Aix Marseille, France. ^{1}H and ^{13}C NMR chemical shifts (δ) were reported in parts per million (ppm) and were referenced to the solvent peak; $CDCl_3$ (7.26 ppm for ^{1}H and 76.90 ppm for ^{13}C) and DMSO-d_6 (2.50 ppm for ^{1}H and 39.70 ppm for ^{13}C). Multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were taken on a Jeol JMS600 instrument spectrometer at ionizing potential of 70 eV (EI) at Assiut University. Elemental analyses were carried out using a Perkin–Elmer 240 C Micro analyzer at Assiut University and they were found to be within ±0.4% of the theoretical values. Their results were found in good agreement with the calculated values. The geometry around



Scheme 4. Synthesis of compounds **20**–**21a**–**d**. Reagent and conditions: i) ArCHO, aq. NaOH, EtOH, reflux 3 h.; ii) $CH(OEt)_3$ (5 ml), reflux 5 h.



Scheme 5. Synthesis of compounds **22**–**24**. Reagent and conditions: i) $BrCH(COOC_2H_5)_2$,fused CH_3COONa, EtOH, reflux 10 h., i) $C_6H_5COCH_2Br$, fused CH_3COONa, EtOH, reflux 10 h.

the new double bond (*E* and/or *Z*) and the minimum energy of each isomer was calculated and assigned according to ChemBio 3D11.0 (2008) program. Thus we depend on the minimum energy calculation for the assignment of *E*-/and or in *Z*- isomers of the compounds prepared.

*4.1.1. 3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-one (**5**)*

A mixture of the acid chloride **4** [14] (1.74 g, 0.005 mol) and anhydrous $AlCl_3$ (2 g, 0.015 mol) in CS_2 (10 ml) was heated under reflux on a water bath for 6 h. After removal of the solvent under reduced pressure diluted HCl was added to the residue and the resulting mixture was stirred at 40 °C for 1 h. The solid product obtained was then collected, washed with water, dried and recrystallized from ethanol–dioxane (1:2) to give yellow crystals, m.p. 227–229 °C, yield 0.98 g (63%). IR (KBr, cm^{-1}): $\nu = 3050$ (CH arom.), 2910, 2850 (CH aliph.), 1715 (CO). ^1H NMR ($CDCl_3$): $\delta = 2.55$ (S, 3H, CH_3), 7.15–8.18 (m, 9H, Ar–H). ^{13}C NMR ($CDCl_3$) δ ppm: 11.44, 120.63 (2C), 122.15, 124.35, 126.66, 129.17 (2C), 132.32, 134.80, 136.00, 136.20, 138.45, 140.34, 143.73, 144.32, 146.40, 158.41, 189.10.

Anal. Calcd. for $C_{19}H_{12}N_4O$ (312. 32) C, 73.07; H, 3.87; N, 17.94. Found: C, 73.23; H, 3.89; N, 17.73.

*4.1.2. General procedure for the reaction of **5** with active methylene compounds. Formation of compounds **7**–**12***

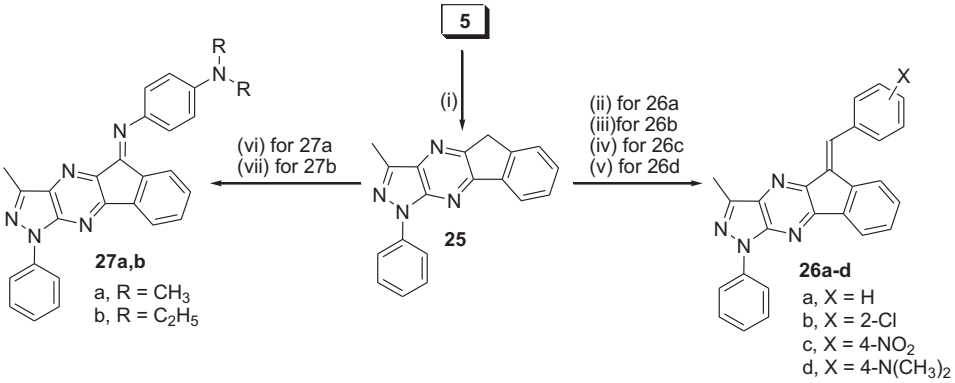
An equimolar mixture (0.002 mol) of compound **5** and the active methylene compound in dry pyridine (20 ml) was heated under reflux for 6 h. The reaction mixture was allowed to cool and then it was poured onto ice-water mixture. The precipitate thus formed was collected by filtration, washed with water, dried and crystallized from the proper solvent to afford **7**–**12**.

*4.1.2.1. 2-(3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene) malononitrile (**7**).* A mixture **5** (1.56 g, 0.005 mol) and malononitrile (0.33 g, 0.005 mol) in pyridine (25 ml) was stirred at room temperature for 15 min. The red precipitate formed was filtered, dried and recrystallized fro DMF to give red needled, m.p. 314–316 °C, yield quantitative. IR (KBr, cm^{-1}): $\nu = 3050$ (CH arom.), 2910 (CH aliph.), 2210 (CN). ^1H NMR, the product was insoluble in most deuterated solvents. MS: 360.0 (100%), 361.1 (22.7%), 362.2 (3.97%), 344.8 (27.47.%), 318.9 (17.28%).

Anal. Calcd for $C_{22}H_{12}N_6$ (360.37): C, 73.32; H, 3.36; N, 23.32. Found: C, 73.23; H, 3.69; N, 23.63.

*4.1.2.2. 3-(Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene)pentane-2,4-dione (**8**).* A mixture of the oxo compound 2 (0.624 g, 0.002 mol) and acetylacetone (0.2 g, 0.002 mol) in dry pyridine (20 ml) was heated under reflux for 10 h. The cold reaction mixture was poured onto ice–water and the solid product formed was filtered, dried and crystallized from dioxane–water (3:2) to give fine yellowish-brown crystals, m.p. 281–283 °C yield (38%). IR (KBr, cm^{-1}): $\nu = 3050$ (CH arom.) 2910 (CH aliph.), 1720 (CO). ^1H NMR ($CDCl_3$): δ ppm = 2.65 (s, 6H, 2 $COCH_3$), 2.85(s, 3H, CH_3), 7.22–8.53 (m, 9H, Ar–H).

Anal. Calcd. for $C_{24}H_{18}N_4O_2$ (394.43): C, 73.08; H, 4.60; N, 14.20. Found: C, 73.23; H, 4.89; N, 14.33.



Scheme 6. Synthesis of compounds **25**–**27a**,**b**. Reagent and conditions: i) KOH, $NH_2NH_2 \cdot H_2O$, $HO(CH_2)_2OH$, 150 °C, 14 h.; ii) $4\text{-}(R)_2NC_6H_4NO$, C_2H_5ONa, EtOH, reflux 2 h.

Table 1
Antibacterial activity of tested compounds at 20 mg concentration, (inhibition zone in mm).

Compound no.	**5**	**10**	**14**	**16**	**17**	**22**	**23**	**24**	**25**	**26b**	Control
Bacterial strains											
Staphylococcus aureus (+ve) AUMC No. B-54	8	8	8	8	8	8	10	10	8	10	18
Bacillus cereus (+ve) AUMC NoB-52	0	0	0	0	0	0	0	0	0	0	22
Escherichia coli (−ve) AUMC No. B-53	10	8	0	0	8	8	8	8	0	8	18
Pseudomonas aeruginosa (−ve) AUMC No. B-73*g*	0	8	8	0	0	8	8	0	0	8	18
Serratia marcescens (−ve) AUMC No. B-55	0	0	0	0	0	0	0	0	0	0	20
Micrococcus luteus (+ve) AUMC NoB-112	0	0	0	0	0	0	0	0	0	0	20

The amount added in each pore is 50 μL.
p.i. = Partial Inhibition.
AUMC = Assiut University Mycological Center.
Control = Chloramphenicol as antibacterial standard.

*4.1.2.3. (Z) Ethyl 2-(3-methyl-1-phenyl-1H,5H-indeno[2,1-e]pyrazolo [3,4-b]pyrazin-5(1H)-ylidene)oxobutanoate (**9**).* A mixture of the oxo compound 2 (0.624 g, 0.001 mol) and ethyl acetoacetate (0.26 g, 0.001 mol) in dry pyridine (20 ml) was heated under reflux for 10 h. The cold reaction mixture was poured onto ice–water and the solid product formed was filtered, dried and crystallized from dioxane–water (5:2) to give fine yellowish-brown crystals, m.p. 234–236 °C, yield 0.4 g (49%). IR (KBr, cm^{-1}): ν = 3050 (CH arom.), 2950, 2920 (CH. aliph.), 1728(CO). ^{1}H NMR ($CDCl_3$): δ ppm = 1.32–1.52 (t, 3H, $CH_2\underline{CH_3}$), 2.62 (s, 3H, $COCH_3$), 2.92 (s, 3H, CH_3), 4.25–4.50 (q, 2H, $\underline{CH_2}CH_3$), 7.35–8.52 (m, 9H, Ar).

Anal. Calcd. for $C_{25}H_{20}N_4O_3$ (424. 45): C, 70.74; H, 4.75; N, 13.20. Found: C, 70.53; H, 4.89; N, 13.33.

*4.1.2.4. (E) Ethyl 2-cyano-2(3-methyl-1-phenylindeno[2,1-e]pyrazolo [3,4-b]pyrazin-5(1H)-ylidene)acetate (**10**).* A mixture of **5** (0.624 g, 0.002 mol) and ethyl cyanoacetate (0.226 g, 0.001 mol) in dry pyridine (10 ml) was heated under reflux for 10 h. The cold reaction mixture was poured onto ice–water and the solid product formed was filtered, dried and crystallized from dioxane–water (5:2) to give brownish-yellow crystals, m.p. 286–288 °C, yield 0.47 g (58%). IR (KBr, cm^{-1}): ν = 3500 (CH arom.) 2910 (CH aliph.) 2200 (CN) 1700 (CO). MS, 407 (58%). ^{1}H NMR ($CDCl_3$): δ ppm = 1.43–1.55 (t, 3H, $CH_2\underline{CH_3}$), 2.68 (s, 3H, CH_3), 4.52–4.63 (q, 2H, $\underline{CH_2}CH_3$), 7.36–8.55 (m, 9H, Ar–H). ^{13}C NMR ($CDCl_3$) δ ppm: 11.24, 13.91, 63.34, 101.20, 115.03, 120.61, 120.70(2C), 122.68, 125.46, 126.56, 129.24(2C), 132.00, 133.28, 136.18, 137.49, 138.71, 143.81, 144.88, 145.27, 153.51, 161.64.

Anal. Calcd. for $C_{24}H_{17}N_5O_2$ (407.42): C, 70.75; H, 4.21; N, 17.19. Found: C, 70.43; H, 4.11; N, 17.13.

*4.1.2.5. (Z) 2-(3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene)-3-oxo-3-phenylpropanenitrile (**11**).* A mixture of the oxo compound 2 (0.62 g, 0.002 mol) and benzoyl acetonitrile (0.29 g, 0.002 mol) in dry pyridine (20 ml) was heated under reflux for 6 h. The cold reaction mixture was then poured onto ice–water. The precipitate that formed was collected by filtration washed with water, dried and crystallized from DMF-H_2O (1:1) to give small brownish-yellow needles, m.p. 294–296 °C, yield 0.51 g (58%). IR (KBr, cm^{-1}): ν = 3050 (CH arom.) 2900 (CH aliph.), 2200 (CN), 1681 (CO). ^{1}H NMR ($CDCl_3$): δ ppm = 2.73 (s, 3H, CH_3), 7.32–8.28 (m, 14H, Ar–H). ^{13}C NMR ($CDCl_3$) δ ppm: 10.86, 107.59, 115.50, 120.62(2C), 122.73(2C), 125.44(2C), 126.46(2C), 128.90 (2C), 129.20(2C), 129.45(2C), 131.99, 133.11, 134.28, 136.08, 136.29, 138.69, 145.04, 145.21, 145.82, 153.37, 188.06. MS: 439.19 (0.6%), 439.97 (0.4%), 440.99 (0.2%), 411.86 (30.1%), 400.20 (38.9%).

Anal. Calcd. for $C_{28}H_{17}N_5O$ (439.47): C, 76.52; H, 3.90; N, 15.94. Found: C, 76.23; H, 3.89; N, 15.73.

*4.1.2.6. Diethyl 2-(3-methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b] pyrazin-5(1H)-ylidene) malonate (**12**).* A mixture of the oxo compound 2 (0.624 g, 0.002 mol) and diethyl malonate (0.32 g, 0.002 mol) in dry pyridine (20 ml) was heated under reflux for 10 h. The cold reaction mixture was poured onto ice–water and the solid product formed was filtered, dried and crystallized from dioxane–water (5:2) to give fine yellowish-brown crystals, m.p. 231–233 °C yield (38%). IR (KBr, cm^{-1}): ν = 3500 (CH arom.) 2910 (CH aliph.), 1720 (CO). ^{1}H NMR ($CDCl_3$): δ ppm = 1.11–1.28 (t, 6H,$CH_2\underline{CH_3}$), 2.53 (s, 3H, CH_3), 4.15–4.35 (q, 4H, $\underline{CH_2}CH_3$), 6.56–8.32 (m, 9H, Ar-H).

Anal. Calcd. for $C_{26}H_{22}N_4O_4$ (454.48): C, 68.71; H, 4.88; N, 12.33. Found: C, 68.63; H, 4.89; N, 13.23.

*4.1.3. General procedure for the reaction of compound 5 with aromatic amines. Formation of **13a–e***

An equimolar mixture of **5** (0.002 mol) and appropriate aromatic amine namely (aniline, 2-chloroaniline, 2-phenelenediamine, 2-nitroaniline and 4-nitroaniline) in dry pyridine (20 ml) was heated under reflux for 8 h. After cooling to room temperature the reaction mixture was then poured onto ice–water mixture. The precipitate thus formed was filtered, washed with water, dried and crystallized from the appropriate solvent.

*4.1.3.1. (E) N-(3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene)aniline (**13a**).* Yellowish-orange needles from dioxane–water (1:1), m.p. 229–231 °C, yield 0.35 g (46%). IR (KBr, cm^{-1}): ν = 3050 (CH arom.) 2910 (CH aliph.). ^{1}H NMR ($CDCl_3$): δ ppm = 2.82 (s, 3H, CH_3), 6.70–8.37 (m, 14H, Ar–H). ^{13}C NMR ($CDCl_3$) δ ppm: 11.75, 118.09 (2C), 120.66(2C), 122.43, 124.62, 126.32, 126.92, 129.20(2C), 129.36(2C), 131.15, 132.86, 133.32, 134.42, 138.95, 139.51, 144.39, 145.55, 147.11, 151.35, 154.87, 158.53.

Anal. Calcd. for $C_{25}H_{17}N_5$ (387.44): C, 77.50; H, 4.42; N, 18.08. Found: C, 77.23; H, 4.23; N, 18.23.

*4.1.3.2. (E) 2-Chloro-N-(3-methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene)aniline (**13b**).* Crystallised from dioxane–water (1:1) to give brownish-yellow crystals, m.p. 164–166 °C, yield 0.55 g (41%). IR (KBr, cm^{-1}): ν = 3050 (CH arom.) 2910 (CH aliph.). ^{1}H NMR ($CDCl_3$): δ ppm = 2.65 (s, 3H, CH_3), 7.35–8.35 (m, 13H, Ar–H).

Table 2
Antifungal sensitivity (inhibition zone in mm) of the tested compounds at 20 mg concentration.

Compound no.	**5**	**10**	**14**	**16**	**17**	**22**	**23**	**24**	**25**	**26b**	Clotrimazole
Fungal strains											
Candida albicans AUMC No. 418	0	10	0	0	0	0	0	0	0	0	24
Trichophyton rubrum AUMC No. 1804	0	0	0	30	0	30	26	30	0	26	36
Aspergillus flavus AUMC No. 1276	0	0	0	16	0	13	12	16	0	9	44
Fusarium oxysporum AUMC No. 5119	0	0	0	0	0	0	0	0	0	0	20
Scopulariopsis brevicaulis AUMC No. 729	0	0	0	0	0	0	0	0	0	0	28
Geotrichum candidum AUMC No. 226	0	0	0	17 pi.	0	10	10	10	0	0	26

The amount added in each pore is 50 μL.
p.i. = Partial Inhibition.
AUMC = Assiut University Mycological Center.
Clotrimazole (as antifungal reference drug).

Table 3
Antifungal activity of **16**, **22**, **23**, **24** and **26b**.

Sample no.	*Trichophyton rubrum* AUMC no. 1804		*Aspergillus flavus* AUMC no. 3214	
	Inhibition zone (mm)	MIC (mg/mL)	Inhibition zone (mm)	MIC (mg/mL)
16	14	0.3	14	2.5
22	16	0.3	10	2.5
24	14	0.3	12	2.5
23	12	0.6	10	5
26b	10	0.6	9	20
Clotrimazole[a]	35	0.08	15	0.15

[a] Clotrimazole was used as standard reference drug.

Anal. Calcd. for $C_{25}H_{16}ClN_5$(421.88): C, 71.17; H, 3.82; N, 16.60. Found: C, 71.23; H, 3.89; N, 16.73.

*4.1.3.3. (E) N^1-(3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene) benzene-1,2- diamine (**13c**).* Crystallised from dioxane–water (1:1) to give brownish-red needles, m.p. 246–248 °C, yield 0.42 g (53%). IR (KBr, cm^{-1}): ν = 3400–3300 (NH_2) 3050 (CH arom.) 2910 (CH aliph.).

^{1}H NMR ($CDCl_3$): δ ppm = 2.73 (s, 3H, CH_3), 5.88 (s, 2H, NH_2), 7.26–8.37 (m, 13H, Ar–H). MS: 401.62 (85.5%), 402.08 (27.6%), 403.72 (19.3), 312.3 (100%).

Anal. Calcd. for $C_{25}H_{18}N_6$ (402.45): C, 74.61; H, 4.51; N, 20.88. Found: C, 74.53; H, 4.89; N, 20.73.

*4.1.3.4. (E) N-(3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene)-2-nitroaniline (**13d**).* Crystallised from dioxane–water (3:1) to give brownish-red needles, m.p. 261–263 °C, yield 0.42 g (53%). IR (KBr, cm^{-1}): ν = 3040 (CH arom.) 2920 (CH aliph.).^{1}H NMR ($CDCl_3$): δ ppm = 2.73 (s, 3H, CH_3), 7.35–8.35 (m, 13H, Ar–H).

Anal. Calcd. for $C_{25}H_{16}N_6O_2$ (432.43): C, 69.44; H, 3.73; N,19.43. Found: C, 69.23; H, 3.82; N, 19.73.

*4.1.3.5. (E) N-(3-methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene)-4-nitroaniline. (**13e**).* Reddish-brown crystals from DMF–H_2O (2:1), m.p. 258–260 °C, yield 0.46 g (53%). IR (KBr, cm^{-1}): ν = 3050 (CH arom.), 2970 (CH aliph.). ^{1}H NMR (DMSO-$_{d6}$): δ ppm = 2.70 (s, 3H, CH3), 7.32–8.35 (m, 13H, Ar–H).

Anal. Calcd. for $C_{26}H_{19}N_6O_2$ (447.47): C, 69.79; H, 4.28; N, 18.78. Found C, 69.58; H, 4.32; N, 18.89.

*4.1.4. Reaction of compound **5** with hydroxylamine hydrochloride, semicarbazide hydrochloride and thiosemicarbazide. Formation of compounds **14**–**16***

An equimolar mixture of compound **5**(0.002 mol) and hydroxylamine hydrochloride, semicarbazide hydrochloride or thiosemicarbazide in dry pyridine (15 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was then poured onto ice–water mixture. The solid precipitate thus formed was then filtered, washed with water, dried and crystallized the proper solvent to give compounds **14**–**16**.

*4.1.4.1. (Z) 3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-one-oxime (**14**).* Yellow needles from ethanol–dioxane (1:1), m.p. 304–306 °C, yield 0.95 g (58%). IR (KBr, cm^{-1}): ν = 3200 (OH), 3050 (CH arom.) 2900 (CH aliph.). ^{1}H NMR (DMSO-d_6): δ ppm = 2.93 (s, 3H, CH_3), 7.73 (m, 9H, Ar–H), 9.9 (s, 1H, OH). ^{13}C NMR ($CDCl_3$) δ ppm: 11.33, 120.12(2C), 121.92, 126.20, 128.63, 129.48(4C), 132.21, 132.35, 133.07, 135.54, 138.83, 143.39, 144.06, 146.87, 152.11.

Anal. Calcd. for $C_{19}H_{13}N_5O$ (327.34) C, 69.71; H, 4.00; N, 21.39. Found: C, 69.83; H, 3.89; N, 21.53.

*4.1.4.2. (Z) 2-(3-Methyl-1-phenyindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene) hydrazinecarboxamide (**15**).* Yellow crystals from dioxane, m.p. 267–269 °C, yield 0.45 g (61%). IR (KBr, cm^{-1}): ν = 3400–3200 (NH_2 + NH), 3050 (CH arom.), 2900 (CH aliph), 1650 (CO), ^{1}H NMR ($CDCl_3$): δ ppm = 2.72 (s, 3H, CH_3), 3.73 (s, 1H, NH), 6.45 (s, 2H, NH_2) 7.25–8.35 (m, 9H, Ar–H) Anal. Calcd. for $C_{20}H_{15}N_7O$ (369.38): C, 65.03; H, 4.09; N, 26.54. Found: C, 65.32; H, 3.94; N, 26.23.

*4.1.4.3. (Z) 2-(3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene) hydrazinecarbothioamide (**16**).* Yellow crystals from DMF–H_2O (3:1), m.p. 264–266 °C, yield 0.45 g (61%). IR (KBr, cm^{-1}): ν = 3400–3200 (NH_2 + NH), 3050 CH (arom.), 2900 CH. aliph). ^{1}H NMR ($CDCl_3$): δ ppm = 2.73 (s, 3H, CH_3), 3.70 (s, 1H, NH), 6.49 (s, 2H, NH_2) 7.26–8.30 (m, 9H, Ar–H). ^{13}C NMR ($CDCl_3$) δ ppm: 11.27, 120.60, 120.80, 122.43, 122.45, 126.51, 129.20, 129.23, 131.06, 131.68, 132.39, 134.88, 135.67, 137.69, 144.62, 144.49, 153.83, 158.54, 179.88.

Anal. Calcd. for $C_{20}H_{15}N_7S$ (385.44): C, 62.32; H, 3.92; N, 25.44. Found: C, 62.23; H, 3.89; N, 25.73.

*4.1.5. (E) 5-Hydrazono-3-methyl-1-phenyl-1,5-dihydroindeno[2,1-e]pyrazolo[3,4-b]pyrazine (**17**)*

4.1.5.1. Method A. A mixture of compound **5** (1.56 g, 0.005 mol) and excess hydrazine hydrate (80%, 5 ml) in ethanol (20 ml) was heated under reflux for half an hour. The solid product formed was filtered, washed with water, dried and recrystallized from dioxane–ethanol (1:1) to give small yellow needles, m.p. 236–288 °C, yield 1.1 g (66%). IR (KBr, cm^{-1}): ν = 3328–3175 (NH_2), 3050 (CH arom.) 2900 (CH aliph.), ^{1}H NMR ($CDCl_3$): δ ppm = 2.65 (s, 3H, CH_3), 7.25–8.7 (m, 9H, Ar–H), 9.85 (NH_2). ^{13}C NMR ($CDCl_3$) δ ppm: 11.40, 119.90, 120.59(4C), 122.01, 126.07, 128.16, 129.16(4C), 131.31, 133.26, 134.56, 139.17, 139.83, 142.66, 143.63, 143.98, 152.50. MS: 326.1 (100%), 327.1 (27.9%), 328.1 (3.69%), 297.9 (38.3%), 282.8 (17.6%).

Anal. Calcd. for $C_{19}H_{14}N_6$ (326.35): C, 69.92; H, 4.32; N, 25.75. Found: C, 69.73; H, 4.59; N, 25.73.

4.1.5.2. Method B. A mixture of **7** (0.36 g, 0.001 mol) and excess hydrazine hydrate (2 ml, 80%) was heated under reflux for 15 min. The heavy yellowish precipitate filtered, washed with water, dried and recrystallized from dioxane–ethanol (1:1) to give small yellow needles, m.p., mixed m.p. (238 °C) and IR spectrum were found to be identical with those of an authentic sample (method A), yield 0.24 g (68%).

*4.1.6. (Z) 3-Methyl-1-phenyl-5-(2-phenylhydrazono)1,5-dihydroindeno[2,1-e]pyrazolo[3,4-b] pyrazine (**18**)*

A mixture of **5** (0.312 g, 0.001 mol) and excess phenyl hydrazine (2 ml) was heated for 15 min (no solvent at reflux). The orange precipitate was filtered washed with water, dried and crystallized from dioxane to give orange needles, m.p. 294–296 °C, yield 0.27 g (68%). IR (KBr, cm^{-1}): ν = 3220 (NH) 3050 (CH arom.) 2910 (CH aliph.). ^{1}H NMR (CF_3COOD): δ ppm = 2.95 (s, 3H, CH3), 7.75 (m, 14H, Ar–H).

Anal. Calcd. for $C_{25}H_{18}N_6$ (402.45): C, 74.61; H, 4.51; N, 20.88. Found: C, 74.23; H, 4.82; N, 20.73.

*4.1.7. General procedure for the reaction of the hydrazono derivative **17** with some aromatic aldehydes. Formation of compounds **20a**–**d**25 ml*

To an equimolar mixture of **17** (0.02 mol) and the appropriate aldehyde in ethanol (), aqueous sodium hydroxide solution (5 ml, 20%) was added and the mixture was allowed to reflux for 3hr. After cooling, the obtained precipitate was filtered, washed with water, dried and crystallized from the suitable solvent.

*4.1.7.1. (Z) 5-(E)(Benzylidenehydrazono)-3-methyl-1-phenyl-1,5-dihydroindeno[2,1-e] pyrazolo[3,4-b]pyrazine (**20a**).* As reddish-brown fine needles, from dioxane–water (3:1), m.p. 241–243 °C, yield 0.46 g (56%). IR (KBr, cm^{-1}): ν = 3050, 3020 (CH, arom.), 2960, 2920(CH, aliph.). 1H NMR ($CDCl_3$): 2.72 (s, 3H, CH_3), 7.25–8.35 (m, 14H, Ar–H), 8.99 (s, 1H, CH).

Anal. Calcd. for $C_{26}H_{18}N_6$ (414.46): C, 75.35; H, 4.38; N, 20.28. Found: C, 75.23; H, 4.59; N, 20.53.

*4.1.7.2. N,N-Dimethyl-4-(E)-(Z)((3-methyl-1-phenylindeno[2,1-e] pyrazolo[3,4-b]pyrazin-5 (1H)-ylidene)hydrazinyl)aniline (**20b**).* Golden needles from DMF–H_2O (2:1), m.p. 173–175 °C, yield 0.48 g (53%). IR (KBr, cm^{-1}): ν = 3070, 3020 (CH. arom). 2970, 2930 (CH. aliph.) ^1H NMR ($CDCl_3$) 2.65 (s, 3H, CH_3), 2.95 (s, 6H, $2CH_3$), 6.65–8.50 (m, 13H, Ar–H), 8.9 (s, 1H, CH).

Anal. Calcd. for $C_{28}H_{23}N_7$ (457.53): C, 73.50; H, 5.07; N, 21.43. Found: C, 73.23; H, 5.21; N, 21.73.

*4.1.7.3. (Z) 3-(E)-Methyl-5-((4-nitrobenzylidene)hydrazono)-1-phenyl-1,5-dihydroindeno [2,1-e] pyrazolo[3,4-b]pyrazine (**20c**).* Brownish-red crystals from DMF-H_2O (4:1), yield 0.53 g (58%), m.p. 244–246 °C. IR (KBr, cm^{-1}): ν = 3020 (CH, arom.), 2950, 2920(CH, aliph.). ^1H NMR (DMSO-d_6) 2.75 (s, 3H, CH3), 7.45–8.30 (m, 13H, Ar–H), 8.85 (s, 1H, CH).

Anal. Calcd. for $C_{26}H_{17}N_7O_2$ (459.46): C, 67.97; H, 3.73; N, 21.34. Found: C, 67.73; H, 3.81; N, 21.53.

*4.1.7.4. (Z)-3-(E)(Methyl-5-(2-nitrobenzylidene)hydrazono)-1-phenyl-1,5-dihydroindeno[2,1-e]pyrazolo[3,4-b]pyrazine (**20d**).* Brownish-red crystals from DMF–H_2O (2:1), mp 236–238 °C, yield 0.47 g (51%) IR (KBr, cm^{-1}): ν = 3050 (CH arom.), 2920 (CH aliph.), ^1H NMR (DMSO-d_6): 2.75 (s, 3H, CH3), 7.40–8.50 (m, 13H, Ar–H), 8.65 (s, 1H, CH).

Anal. Calcd. for $C_{26}H_{17}N_7O_2$ (459.46): C, 67.97; H, 3.73; N, 21.34. Found: C, 67.73; H, 3.89; N, 21.73.

*4.1.7.5. (^{1}Z,N'Z) Ethyl 2-((3-methyl-1-phenylindeno[2,1-e]pyrazolo [3,4-b]pyrazin-5(1H)-ylidene) hydrazono)acetate (**21**).* A mixture of the hydrazono derivative **14** (0.02 mol) and triethyl orthoformate (5 ml) was heated under reflux for 4 h. The reaction mixture was evaporated under reduced pressure and the residue was triturated with cold ethanol, filtered, dried and crystallized from ethanol to give yellow crystals, yield 0.4 g (52%) m.p. 161–163 °C. IR (KBr, cm^{-1}): ν = 3020 (CH, arom.), 2960, 2920 (CH, aliph.). ^1H NMR ($CDCl_3$): 1.13–1.35 (t, 3H, $CH_2\underline{CH_3}$), 2.75(s, 3H, CH_3), 4.15–4.40 (q, 4H, $\underline{CH_2}CH_3$), 7.35–8.75 (m, 10H, Ar–H + CH).

Anal. Calcd. for $C_{22}H_{18}N_6O$ (382.42): C, 69.10; H, 4.74; N, 21.98. Found: C, 69.23; H, 4.89; N, 21.73.

*4.1.8. General procedure for the reaction of the thiosemicarbazone 16 with some α-halocarbonyl compounds. Formation of **22–24***

An equimolar (0.002 mol) mixture of the thiosemicarbazone **16** the respective α-halocarbonyl compound (diethyl bromomalonate, ethyl chloroacetate and/or phenacyl bromide), and fused sodium acetate (0.005 mol) in absolute ethanol (25 ml) was refluxed for 10 h. After cooling the reaction mixture was poured onto cold water, and the precipitate formed was filtered off, washed with water, dried and crystallized from the proper solvent.

*4.1.8.1. (Z) Ethyl-2-(E)((3-methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene) hydrazono)-4-oxothiazolidine-5-carboxylate (**22**).* As reddish-brown crystals, from dioxane–water (3:1), m.p. 244–246 °C, yield 0.42 (42%). IR (KBr, cm^{-1}): ν = 3270 (NH), 3050 (CH, arom.), 2950, 2920, 2890 (CH, aliph.). ^1H NMR ($CDCl_3$), 1.10–1.25 (t, 3H, $CH_2\underline{CH_3}$), 2.75 (s, 3H, CH_3), 3.75 (s, 1H, CH), 4.15–4.30 (q, 2H, $\underline{CH_2}CH_3$), 7.35–8.35 (m, 9H, Ar–H).

Anal. Calcd. for $C_{25}H_{19}N_7O_3S$ (497.53): C, 60.35; H, 3.85; N, 19.71. Found: C, 60.23; H, 3.89; N, 19.73.

*4.1.8.2. (Z) 2-(E)((3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b] pyrazin-5(1H)-ylidene) hydrazono)thiazolidine-4-one (**23**).* Brownish-orange crystals from DMF-water (3:1), m.p. 238–240 °C, yield 0.54 g (47%). IR (KBr, cm^{-1}): ν = 3335 (NH), 3050 (CH, arom.), 2950, 2920, 2890 (CH, aliph.). ^1H NMR ($CDCl_3$): 2.76 (s, 3H, CH_3), 4.15 (s, 2H, CH_2), 7.35–8.35 (m, 9H, Ar–H).

Anal. Calcd. for $C_{22}H_{15}N_7OS$ (425.47): C, 62.10; H, 3.55; N, 23.04. Found: C, 62.43; H, 3.65; N, 23.13.

*4.1.8.3. (Z) 2-(E)((3-Methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b] pyrazin-5(1H)-ylidene) hydrazono)-4-phenyl-2,3-dihydrothiazole (**24**).* Yellowish-brown crystals, from DMF–water (3:1), yield 0.46 (47%), m.p. 282–284 °C, IR (KBr, cm^{-1}): ν = 3300 (NH), 3050 (CH, arom.), 2920 (CH, aliph.). ^1H NMR: ($CDCl_3$), 2.84 (s, 3H, CH_3), 7.03 (s, 1H, CH), 7.33–8.35 (m, 14H, Ar–H). ^{13}C NMR ($CDCl_3$) δ ppm: 11.66, 105.02, 120.69(3C), 121.03, 122.23, 125.99(3C), 127.92, 128.67(3C), 129.18(3C), 129.66, 131.56, 134.56, 138.34, 138.93, 142.98, 143.30, 144.12, 152.16, 153.38, 167.39.

Anal. Calcd. for $C_{28}H_{19}N_7S$ (485.56): C, 69.26; H, 3.94; N, 20.19. Found: C, 69.23; H, 3.89; N, 20.73.

*4.1.8.4. 3-Methyl-1-phenyl-1,5-dihydroindeno[2,1-e]pyrazolo[3,4-b] pyrazine (**25**).* A mixture of compound **5** (1.56 g, 0.005 mol), KOH (0.75 g, 0.014 mol) and hydrazine hydrate (2 ml) in ethylene glycol (30 ml) was heated under reflux in an oil bath at 150 °C for 14 h. After cooling, the reaction mixture was poured onto water and acidified with HCl. The solid product was filtered off, washed with water, dried and crystallized from dioxane–water (1:1) as buff crystals, m.p. 175–177 °C, yield 0.6 g (52%). IR (KBr, cm^{-1}): ν = 3010 (CH arom) 2910 (CH aliph.). ^1H NMR: ($CDCl_3$): 2.76 (s, 3H, CH_3), 4.07 (s, 2H, CH_2), 7.32–8.39 (m, 9H, Ar–H). ^{13}C NMR ($CDCl_3$) δ ppm: 11.40, 35.41, 120.35(2C), 122.46, 125.56, 125.71, 127.66, 127.80, 129.10(2C), 130.61, 132.41, 138.07, 139.44, 143.03, 144.93, 153.18, 154.82. MS: 298.01 (100%), 298.36 (0.4%), 298.76 (0.2%).

Anal. Calcd. For $C_{19}H_{14}N_4$ (298.34): C, 76.49; H, 4.73; N, 18.78. Found: C, 76.23; H, 4.82; N, 18.73.

*4.1.9. General procedure for the reaction of the indeno derivative **25** with some aromatic aldehydes. Formation of **26a–c***

An equimolar mixture of the indeno derivative **25** (0.002 mol), the appropriate aromatic aldehyde and sodium ethoxide (0.002 mol) in absolute ethanol (25 ml) was heated under reflux for 2 h. The reaction mixture was poured onto water, neutralized with diluted. HCl. The solid precipitate formed was filtered off, washed with water, dried and recrystallized from proper solvent.

*4.1.9.1. (E) 5-Benzyledine-3-methyl-1-phenyl-1,5-dihydrindeno[2,1-e]pyrazolo[3,4-b] pyrazine (**26a**).* Small Orange-red flakes from dioxane–H_2O (2:1), m.p. 231–233 °C, yield 0.42 g, (54%). IR (KBr, cm^{-1}): ν = 3010 (CH arom.) 2950 (CH aliph.). ^1H NMR ($CDCl_3$): 2.85 (s, 3H, CH_3), 7.35–8.30 (m, 14H, Ar–H), 8.9 (s, 1H, CH), MS: 385 (100), 386 (75%), 387 (18%), 344.1 (7.1%), 308.9 (0.4%).

Anal. Calcd. for $C_{26}H_{18}N_4$ (386.45): C, 80.81; H, 4.69; N, 14.50. Found: C, 80.68; H, 4.89; N, 14.73.

*4.1.9.2. (E) 5-(2-Chlorobenzyledine)-3-methyl-1-phenyl-1,5-dihydroindeno[2,1-e]pyrazolo[3,4-b]pyrazine (**26b**).* Reddish brown needles, m.p. 247–249 °C from dioxane–water (3:1), yield 0.46 g (53%), IR (KBr, cm^{-1}): ν = 3020 (CH arom) 2950C (CH aliph.). ^1H

NMR ($CDCl_3$): 2.70 (s, 3H, CH_3), 7.25–8.35 (m, 13H, Ar–H), 8.95 (s, 1H, CH).

Anal. Calcd. for $C_{26}H_{17}ClN_4$ (420.89): C, 74.19; H, 4.07; N, 13.31. Found: C, 74.23; H, 4.19; N, 13.23.

*4.1.9.3. (E) 3-Methyl-5-(4-nitrobenzylidene)-1-phenyl-1,5-dihydroindeno[2,1-e]pyrazolo[3,4-b]pyrazine (**26c**).* Reddish-brown needles, m.p. 287–289 °C from DMF–water (3:1), yield 0.46 g (53%), IR (KBr, cm^{-1}): ν = 3020 (CH arom.) 2950C (CH aliph.). ^1H NMR ($CDCl_3$): 2.65 (s, 3H, CH_3), 7.35–8.45 (m, 13H, Ar–H).

Anal. Calcd. for $C_{26}H_{17}N_5O_2$ (431.45): C, 72.38; H, 3.97; N, 16.23. Found: C, 72.23; H, 3.89; N, 16.43.

*4.1.9.4. (E) N,N-Dimethyl-4-((3smethyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene)methyl)aniline (**26d**).* Orange needles, m.p. 241–243 °C from Dioxane–H_2O 3:1, yield 0.44 g (51%). IR (KBr, cm^{-1}): ν = 3020 (CH arom.) 2900 (CH aliph.). ^1H NMR ($CDCl_3$): 2.75 (s, 3H, CH_3), 2.97 (s, 6H, $2CH_3$), 6.60–8.50 (m, 13 H, Ar–H), 9.0 (s, 1H, CH).

Anal. Calcd. for $C_{28}H_{23}N_5$ (429.52): C, 78.30; H, 5.40; N, 16.31. Found: C, 78.23; H, 5.69; N, 16.13.

*4.1.10. General procedure for the reaction of **25** with 4-nitroso-N,N-dimethyl(and/or diethyl)aniline. Formation of compounds **27a**,**b***

To an equimolar mixture of **25** (0.002 mol) and 4-nitroso-*N*,*N*-dimethyl (and/or *N*,*N*-diethyl) aniline in ethanol (25 ml), 5 ml of an aqueous KOH (20%) was added and the mixture was heated under reflux for 2 h. The solid product formed after cooling was filtered off, washed with water, dried and crystallized from ethanol.

*4.1.10.1. (E) N^1,N^1-Dimethyl-N^4-(3-methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene) benzene-1,4-diamine (**27a**).* Violet needles, crystallized from dioxane–water (1:1), m.p. 241–243 °C, yield 0.56 (54%). IR (KBr, cm^{-1}): ν = 3050 (CH arom.) 2950, 2910 (CH aliph.). ^1H NMR $CDCl_3$: 2.25 (s, 3H, CH_3), 2.95 (s, 6H, $2CH_3$), 6.55–8.35 (m, 13H, Ar–H).

Anal. Calcd. for $C_{27}H_{22}N_6$ (430.50): C, 75.33; H, 5.15; N, 19.52. Found: C, 75.23; H, 5.25; N, 19.73.

*4.1.10.2. (E) N^1,N^1-Diethyl-N^4-(3-methyl-1-phenylindeno[2,1-e]pyrazolo[3,4-b]pyrazin-5(1H)-ylidene) benzene-1,4-diamine (**27b**).* Brownish-violet needles, crystallized from dioxane–water (1:1), m.p. 197–199 °C, yield 0.47 g (52%). IR (KBr, cm^{-1}): ν = 3050 (CH arom.) 2950 (CH aliph.). ^1H NMR ($CDCl_3$): 1.15–1.28 (t, 6H, $2CH_2\underline{CH_3}$), 2.75 (s, 3H, CH_3), 3.35–3.50 (q, 4H, $2\underline{CH_2}CH_3$), 7.52 (m, 13H, Ar–H).

Anal. Calcd. for $C_{29}H_{26}N_6$ (458.56): C, 75.69; H, 5.71; N, 18.33. Found: C, 75.73; H, 5.89; N, 18.23.

4.2. Biology

The antimicrobial activity of 10 selected compounds was evaluated against 6 bacterial and 6 fungal strains. All microbial strains were kindly provided by the Assiut University Mycological Centre (AUMC). These strains are common contaminants of the environment in Egypt and some of which are involved in human and animal diseases (Trichophyton rubrum, Candida albicans, Geotrichum candidum, *S. brevicaulis*, *A. flavus*), plant diseases (*F. oxysporum*) or frequently reported from contaminated soil, water and food substances (*Escherichia coli*, *B. cereus*, *Pseudomonas aeruginosa*, *S. marcescens*, *S. aureus*, *M. luteus*).

To prepare inocula for bioassay, bacterial strains were individually cultured for 48 h in 100 ml conical flasks containing 30 ml nutrient broth medium. Fungi were grown for 7 days in 100 ml conicals containing 30 ml Sabouraud's dextrose broth.

Bioassay was done in 10 cm sterile plastic Petri plates in which microbial suspension (1 ml/plate) and 15 ml of appropriate agar medium (15 ml/plate) were poured. Nutrient agar and Sabouraud's dextrose agar were respectively used for bacteria and fungi. After solidification of the media, 5 mm diameter cavities were cut in the solidified agar (4 cavities/plate) using sterile cork borer. The tested compounds were dissolved in dimethyl sulfoxide (DMSO) at 2%w/v (=20 mg/ml), pipetted and poured in the cavities (20 μL/cavity). Cultures were then incubated at 28 °C for 48 h in case of bacteria and up to 7 days in case of fungi. Results were read as the diameter (in mm) of inhibition zone around cavities [14].

To determine the minimum inhibitory concentrations (MICs), several concentrations in DMSO, of the compounds under testing that gave positive results, have been prepared in descending manner down to a concentration of 0.02 mg/ml. The solutions of different compounds were similarly assayed as mentioned before and the least concentration (below which no activity was observed) was recorded as the MIC.

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