

Facile synthesis of some new pyrazolo[3,4-b]pyrazines and their antifungal activity

T.I. El-Emary, A.M. Kamal El-Dean, H.S. El-Kashef *

Chemistry Department, Faculty of Science, Assiut University, 71516 Assiut, Egypt

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Abstract

The synthesis of new pyrazolo[3,4-b]pyrazines and related heterocycles has been reported. The key intermediate 1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-b]pyrazine-5-carbonitrile **2** was obtained in one pot synthesis from the reaction of 5-amino-3-methyl-4-nitroso-1-phenylpyrazole **1** with benzoylacetone nitrile. Modest antifungal activity was shown for some of the prepared compounds. © 1998 Elsevier Science S.A. All rights reserved.

Keywords: Pyrazolopyrazine; Pyrazine; Pyrazole; Antifungal activity

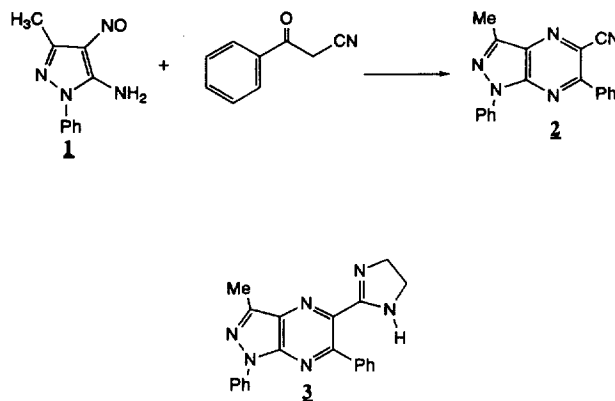
1. Introduction

The pyrazolo[3,4-b]pyrazine ring system is an interesting class of heterocycles. It has been reported that some of its derivatives are used as bone metabolism improvers [1], antiinflammatories [2], blood platelet aggregation inhibitors [2] and anticancer agents with low toxicity [3,4]. Pyrazolo[3,4-b]pyrazines are also used as fluorescent [5] and disperse dyes in dye chemistry [6].

With this in mind and in continuation to our program directed towards the synthesis of new fused pyrazine heterocycles [7–10], we wish to report herein a facile synthesis of the hitherto unknown pyrazolo[3,4-b]pyrazines along with their antifungal activity.

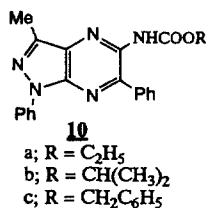
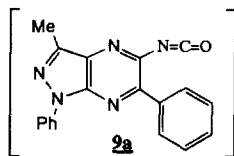
2. Chemistry

This synthesis involves a cyclodehydration reaction of the readily available 5-amino-3-methyl-4-nitroso-1-phenylpyrazole **1** with benzoylacetone nitrile in refluxing pyridine to afford 1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-b]pyrazine-5-carbonitrile **2**. When the cyano function of **2** was interacted with ethylene diamine in the presence of carbon disulphide [11], 1,6-diphenyl-5-(2-imidazolin-2-yl)-3-methyl-1*H*-pyrazolo[3,4-b]pyrazine **3** resulted.

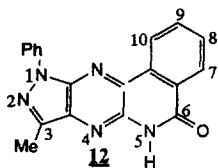


On the other hand, the alkaline hydrolysis of **2** afforded the carboxylic acid derivative **4**. The treatment of the acid **4** with absolute methanol in the presence of concentrated sulfuric acid gave the corresponding methyl ester **5**, while its treatment with thionyl chloride led to the formation of the acid chloride derivative **6**. The ester function of **5** was readily converted into an acid hydrazide group by heating under reflux with ethanolic solution of hydrazine hydrate giving **8**. The treatment of an acetic acid solution of **8** with nitrous acid afforded the corresponding pyrazolo pyrazinecarboxy acid azide **9**. It is worth noting that this acid azide **9** is sensitive to moisture on standing for more than 24 h in the open air. Thus, it is preferable to engage it directly after drying in the next reaction.

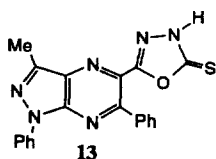
* Corresponding author. Tel.: +20 88 333 837; fax: +20 88 312 564; e-mail: assiut@frcu.eun.eg



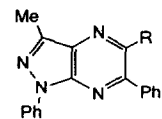
The acid azide **9** proved to be a useful key intermediate in the synthesis of the pyrazolo[3,4-*b*]pyrazine derivatives. Thus, when **9** was heated in a boiling alcohol the product was the corresponding carbamate **10**. Obviously the reaction underwent via the intermediacy of the isocyanate **9a**, formed through Curtius rearrangement of **9**, which reacted in situ with the alcohol used to give the carbamate **10**. Similarly, the *N,N'*-disubstituted urea **11** was obtained when the azide **9** was subjected to Curtius rearrangement by heating it in an inert solvent (benzene) and in the presence of isopropylamine. However, when **9** was heated in the inert high boiling solvent xylene and in the absence of any reactive entity, Curtius rearrangement occurred with concomitant ring closure of the isocyanate intermediate **9a**. This ring closure led to the formation of the tetracyclic structure, namely 3-methyl-1-phenyl-1*H*-pyrazolo[3',4':6,5]pyrazino[2,3-*c*]isoquinolin-9-(10*H*)one **12**. This cyclization occurred via intramolecular electrophilic attack of the isocyanate carbonyl carbon of **9a** on position 2 of the phenyl substituent.



The synthesis of pyrazolo[3,4-*b*]pyrazines substituted in position 5 by 5-membered heterocycles was also achieved. The interaction of the acid hydrazide **8** with carbon disulphide in refluxing pyridine gave 1,6-diphenyl-3-methyl-5-(2,3-dihydro-2-thioxo-1,3,4-oxadiazol-5-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine **13**.

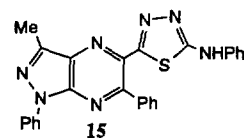


However, the synthon leading to the thiadiazolyl **15** and triazolyl **16** derivatives is 1-phenyl-4-(1,6-diphenyl-3-methyl-1*H*-pyrazolo[3,4-*b*]pyrazin-5-oyl)thiosemicarbazide **14**.

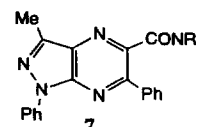


- 4**; R = COOH
5; R = COOCH₃
6; R = COCl
8; R = CONHNH₂
9; R = CON₃
11; R = NHCONHCH(CH₃)₂
14; R = CONHNHCSNHPh

This latter compound was obtained by the interaction of the hydrazide **8** with phenylisothiocyanate in refluxing ethanol. When **14** was dehydrated with concentrated sulfuric acid the product was 1,6-diphenyl-3-methyl-5-(2-phenylamino-1,3,4-thiadiazol-5-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine **15**.



Finally, the treatment of **14** with boiling 2*N* sodium hydroxide solution gave 1,6-diphenyl-3-methyl-5-(3-mercapto-4-phenyl-1,2,4-triazol-5-yl)-1*H*-pyrazolo[3,4-*b*]pyrazine **16**.



- a; R = piperidino.
 b; R = morphitino.
 c; R = dimethylamino.

Although the presence of the -SH group of **16** was quite clear in its IR spectrum (2750 cm⁻¹), no signal corresponding to the -SH proton was observed in its NMR spectrum (run in deuterated chloroform). For this reason the *S*-ethyl derivative **17** was prepared, where its NMR spectrum showed clearly the presence of the ethylthio substituent.

3. Experimental

All melting points are uncorrected and were determined on a Mel-Temp II melting point apparatus. IR spectra were

recorded on a Pye-Unicam SP3-100 spectrophotometer using the KBr wafer technique. ^1H NMR spectra were recorded on a 90 MHz Varian EM-390 NMR spectrometer in the suitable deuterated solvents using tetramethylsilane (TMS) as the internal standard. Elemental analyses were determined on a Perkin-Elmer 240 C microanalyser and the results for the indicated elements were within $\pm 0.4\%$ of the calculated values, unless otherwise indicated.

3.1. 5-Amino-3-methyl-4-nitroso-1-phenylpyrazole 1

This compound was prepared according to the literature [12].

3.2. 1,6-Diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxamide 2

A mixture of compound 1 (20.2 g, 0.1 mol) and ω -cyanoacetophenone (14.5 g, 0.1 mol) in pyridine (50 ml) was heated under reflux for 3 h. After cooling, the precipitate formed was collected, washed with a little cold ethanol and dried. Recrystallization from an ethanol/dioxane mixture (1:1) gave yellow crystals, m.p. 230°C, yield 23.9 g (77%). IR: ν (cm^{-1}) 2220 (CN). ^1H NMR (CDCl_3): δ 2.77 (s, 3H, CH_3), 7.20–7.53 (m, 6H, Ar-H), 7.93–8.07 (m, 2H, Ar-H), 8.17–8.27 (m, 2H, Ar-H). Anal. (C, H, N) for $\text{C}_{19}\text{H}_{13}\text{N}_5$.

3.3. 1,6-Diphenyl-5-(2-imidazolin-2-yl)-3-methyl-1H-pyrazolo[3,4-b]pyrazine 3

Carbon disulphide (5 drops) was added to a cold mixture of the cyano compound 2 (0.311 g, 0.001 mol) and excess ethylene diamine (5 ml), then the reaction mixture was heated on a water bath overnight. The cold reaction mixture was then poured into ice-cold water with stirring. The solid product formed was filtered, washed with water, dried and recrystallized from diluted ethanol to give shiny fluffy needles, m.p. 240°C, yield 0.27 g (76%). IR: ν (cm^{-1}) 3150 (NH), 1610 (δ -NH). ^1H NMR (CDCl_3): δ 2.73 (s, 3H, CH_3), 3.67 (s, 4H, 2 CH_2), 7.33–7.43 (m, 6H, Ar-H), 7.67–7.85 (m, 2H, Ar-H), 8.28–8.30 (m, 2H, Ar-H). Anal. (C, H, N) for $\text{C}_{21}\text{H}_{18}\text{N}_6$.

3.4. 1,6-Diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxylic acid 4

A mixture of 2 (15.5 g, 0.05 mol), aqueous NaOH solution (40 ml, 20%) and ethanol (100 ml) was heated under reflux for 6 h. The reaction mixture was filtered while hot and the filtrate was cooled and acidified with 6N HCl. The solid precipitate was filtered, washed with water and air dried. Recrystallization from diluted acetone gave yellow crystals, m.p. 190°C, yield 9.24 g (56%). IR: ν (cm^{-1}) 3500–3250 (COOH), 1680 (C=O). ^1H NMR (CDCl_3): δ 2.77 (s, 3H, CH_3), 5.27 (s, 1H, OH), 7.23–7.57 (m, 6H, Ar-H), 7.77–7.80 (m, 2H, Ar-H), 8.20–8.33 (m, 2H, Ar-H). Anal. Calc.

for $\text{C}_{19}\text{H}_{14}\text{N}_4\text{O}_2$: C, 69.08; H, 4.27; N, 16.96. Found: C, 69.74; H, 4.50; N, 16.72%.

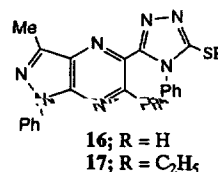
3.5. Methyl 1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxylate 5

A mixture of 4 (16.5 g, 0.05 mol), absolute methanol (100 ml) and concentrated sulfuric acid (3 ml) was refluxed for 5 h. The reaction mixture was concentrated under reduced pressure, cooled and then poured onto a concentrated solution of NaHCO_3 . The solid product was filtered, washed with water, dried and recrystallized from methanol as yellow needles, m.p. 133°C, yield 12.9 g (75%). IR: ν (cm^{-1}) 1710 (C=O). ^1H NMR (CDCl_3): δ 2.80 (s, 3H, CH_3), 3.82 (s, 3H, CH_3 ester), 7.17–7.57 (m, 6H, Ar-H), 7.67–7.80 (m, 2H, Ar-H), 8.20–8.34 (m, 2H, Ar-H). Anal. Calc. for $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_2$: C, 69.76; H, 4.68; N, 16.27. Found: C, 69.20; H, 4.35; N, 15.83%.

3.6. 1,6-Diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazin-5-oyl chloride 6

A mixture of the acid 4 (8.25 g, 0.025 mol) and thionyl chloride (30 ml) was heated under reflux for 2 h. The excess thionyl chloride was evaporated under reduced pressure and the solid residue was recrystallized from petroleum ether (b.p. 60–80°C) to give yellow crystals, m.p. 150°C, yield 6.8 g (78%). IR: ν (cm^{-1}) 1760 (C=O). ^1H NMR (CDCl_3): δ 2.73 (s, 3H, CH_3), 7.17–7.77 (m, 8H, Ar-H), 8.17–8.27 (m, 2H, Ar-H). Anal. Calc. for $\text{C}_{19}\text{H}_{13}\text{N}_4\text{OCl}$: C, 65.43; H, 3.76; N, 16.06; Cl, 10.16. Found: C, 64.90; H, 3.78; N, 15.47; Cl, 10.33%.

3.7. 1,6-Diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazine-5-carboxylic acid amides 7a–c



3.7.1. General procedure

A solution of the acid chloride 6 (0.348 g, 0.001 mol) in dioxane (10 ml) was added dropwise with stirring to an ice-cold solution of 0.001 mol of the appropriate secondary amine (piperidine, morpholine, dimethylamine) in dioxane (5 ml). After completion of the addition, the reaction mixture was further stirred at room temperature overnight. It was then poured into water and the precipitate formed was filtered and washed with water, dried, and recrystallized from the appropriate solvent.

3.7.2. 7a

Yellow crystals from benzene, m.p. 130°C, yield 0.27 g (68%). IR: ν (cm⁻¹) 1620 (C=O). ¹H NMR (CDCl₃): δ 0.93 (m, 2H, piperidine), 1.47 (m, 4H, piperidine), 2.73 (s, 3H, CH₃), 2.90 (m, 2H, piperidine), 3.77 (m, 2H, piperidine), 7.20–7.57 (m, 6H, Ar-H), 7.83–7.93 (m, 2H, Ar-H), 8.23–8.33 (m, 2H, Ar-H). *Anal.* (C, H, N) for C₂₄H₂₃N₅O.

3.7.3. 7b

Yellow crystals from ethanol, m.p. 211°C, yield 0.28 g (70%). IR: ν (cm⁻¹) 1630 (C=O). ¹H NMR (CDCl₃): δ 2.77 (s, 3H, CH₃), 3.00 (m, 4H, morpholine), 3.60 (m, 4H, morpholine), 7.23–7.57 (m, 6H, Ar-H), 7.87–7.95 (m, 2H, Ar-H), 8.23–8.33 (m, 2H, Ar-H). *Anal.* (C, H, N) for C₂₃H₂₁N₅O₂.

3.7.4. 7c

Pale yellow crystals from ethanol, m.p. 186°C, yield 0.26 g (73%). IR: ν (cm⁻¹) 1625 (C=O). ¹H NMR (CDCl₃): δ 2.60 (s, 3H, CH₃), 2.77 (s, 3H, CH₃), 3.03 (s, 3H, CH₃), 7.23–7.57 (m, 6H, Ar-H), 7.83–7.93 (m, 2H, Ar-H), 8.27–8.33 (m, 2H, Ar-H). *Anal.* (C, H, N) for C₂₁H₁₉N₅O.

3.8. 1,6-Diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazin-5-carbohydrazide 8

A mixture of the ester **5** (8.6 g, 0.025 mol) and excess hydrazine hydrate (6 ml) was heated under reflux for 1 h. The solid product was filtered, washed with water and dried. Recrystallization from ethanol gave shiny yellow needles, m.p. 231°C, yield 6.5 g (76%). IR: ν (cm⁻¹) 3250 and 3150 (NHNH₂), 1645 (C=O), 1620 (δ -NH₂). ¹H NMR (DMSO-d₆): δ 2.73 (s, 3H, CH₃), 4.17 (s, 2H, NH₂), 7.23–7.57 (m, 6H, Ar-H), 7.67–7.80 (m, 2H, Ar-H), 8.20–8.27 (m, 2H, Ar-H), 9.27 (s, 1H, NH). *Anal.* (C, H, N) for C₁₉H₁₆N₆O.

3.9. 1,6-Diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazin-5-carboxylic acid azide 9

A cold solution of sodium nitrite (0.69 g, 0.01 mol/5 ml water) was added gradually to a well-stirred solution of the hydrazide **8** (1.72 g, 0.005 mol) in acetic acid (15 ml), and the mixture was stirred for 10 minutes then poured into cold water. The solid precipitate formed was filtered, washed with water and air dried, m.p. 110°C (decomp.), yield quantitative. IR: ν (cm⁻¹) 3050 (CH arom.), 2900 (CH aliph.), 2250, 2150 (N₃), 1680 (C=O).

This azide should be kept very dry since it is sensitive to moisture, thus it is better to use it directly in the next reaction. *Anal.* (C, H, N) for C₁₉H₁₃N₇O.

3.10. Alkyl N-(1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazin-5-yl) carbamates 10a–c

3.10.1. General procedure

A solution of the azide **9** (0.355 g, 0.001 mol) in 5 ml of the appropriate alcohol (ethanol, 2-propanol or phenylme-

thanol) was heated under reflux for 1 h. After cooling, the precipitate of the ethyl derivative was filtered, while in the remaining cases the reaction mixture was evaporated to near dryness, then triturated with an ethanol/water mixture. The precipitate was collected, dried and crystallized from the appropriate solvent.

3.10.2. Ethyl derivative 10a

Pale-yellow needles from diluted ethanol, m.p. 191°C, yield 0.2 g (53.6%). IR: ν (cm⁻¹) 3200 (NH), 1680 (C=O). ¹H NMR (CDCl₃): δ 1.13 (t, 3H, CH₃ ester), 2.73 (s, 3H, CH₃), 4.03 (q, 2H, CH₂), 4.83 (s, 1H, NH), 7.13–7.53 (m, 6H, Ar-H), 7.73–7.83 (m, 2H, Ar-H), 8.17–8.27 (m, 2H, Ar-H). *Anal.* (C, H, N) for C₂₁H₁₉N₅O₂.

3.10.3. 2-Propyl derivative 10b

Small pale-yellow needles from ethanol, m.p. 148°C, yield 0.16 g (41%). IR: ν (cm⁻¹) 3150 (NH), 1710 (C=O). ¹H NMR (CDCl₃): δ 1.13 (d, 6H, 2CH₃), 2.67 (s, 1H, NH), 2.77 (s, 3H, CH₃), 4.90 (m, 1H, CH-isoprop.), 7.17–7.67 (m, 6H, Ar-H), 7.87–8.00 (m, 2H, Ar-H), 8.27–8.40 (m, 2H, Ar-H). *Anal.* (C, H, N) for C₂₂H₂₁N₅O₂.

3.10.4. Benzyl derivative 10c

Yellow needles from ethanol, m.p. 179°C, yield 0.12 g (35%). IR: ν (cm⁻¹) 3190 (NH), 1690 (C=O). ¹H NMR (CDCl₃): δ 2.73 (s, 3H, CH₃), 4.8 (s, 1H, NH), 5.03 (s, 2H, CH₂), 7.23–7.53 (m, 11H, Ar-H), 7.67–7.87 (m, 2H, Ar-H), 8.20–8.27 (m, 2H, Ar-H). *Anal.* Calc. for C₂₆H₂₁N₅O₂: C, 71.71; H, 4.86; N, 16.08. Found: C, 71.24; H, 4.45; N, 17.24%.

3.11. N-1,6-Diphenyl-3-methyl-1H-pyrazolo[3,4-b]pyrazin-5-yl-N'-isopropylurea 11

A mixture of the azide **9** (0.355 g, 0.001 mol) and excess isopropylamine in benzene (10 ml) was heated under reflux for 2 h. The reaction mixture was left to cool and then diluted with petroleum ether (b.p. 60–80°C, 20 ml) the solid precipitate was collected, dried and recrystallized from dioxane/ethanol (3:2) to give yellow needles, m.p. 235°C, yield 0.24 g (62%). IR: ν (cm⁻¹) 3250 (NH), 1640 (C=O). ¹H NMR (DMSO-d₆): δ 1.07 (d, 6H, 2CH₃), 2.70 (s, 3H, CH₃), 4.00 (sept, 1H, CH(CH₃)₂), 7.50–7.60 (m, 6H, Ar-H), 7.73–7.83 (m, 2H, Ar-H), 8.20–8.27 (m, 2H, Ar-H). *Anal.* Calc. for C₂₂H₂₂N₆O: C, 68.38; H, 5.74; N, 21.75. Found: C, 68.58; H, 5.33; N, 20.92%.

3.12. 3-Methyl-1-phenyl-1H-pyrazolo[3',4':6,5]pyrazino-[2,3-c]isoquinolin-9-(10H)one 12

The azide **9** (0.355 g, 0.001 mol) was heated in dry xylene (15 ml) under reflux for 2 h. The reaction mixture was then cooled and diluted with petroleum ether (b.p. 60–80°C) where a solid product was precipitated. This was filtered, dried and crystallized from a mixture of dioxane/ethanol

Table 2
Antifungal activity of some pyrazolo[3,4-*b*]pyrazines^a

Compound	<i>Aspergillus terreus</i>	<i>Penicillium chrysogenum</i>	<i>Rhizopus stolonifer</i>	<i>Fusarium moniliforme</i>
3	10	12		14
4	10			15
6	11	10		10
7b			10	12
8	12	8		

^a The activity is expressed as the diameter of the inhibition zone (mm). Only the active compounds are indicated.

(2:1) to give pale-yellow crystals, m.p. 275°C, yield 0.1 g (31%). IR: ν (cm⁻¹) 3400 (NH), 1680 (C=O). ¹H NMR (CDCl₃): δ 2.50 (s, 3H, CH₃), 7.23–7.57 (m, 5H, Ar-H), 7.77–7.83 (m, 2H, Ar-H), 8.23–8.30 (m, 2H, Ar-H), 9.73 (s, 1H, NH). Anal. (C, H, N) for C₁₉H₁₃N₅O.

3.15. 1,6-Diphenyl-3-methyl-5-(2,5-dihydro-2-thioxo-1,3,4-thiadiazol-5-yl)-1H-pyrazolo[3,4-*b*]pyrazine 13

A mixture of the hydrazide **8** (0.344 g, 0.001 mol) and carbon disulfide (10 ml) in pyridine (5 ml) was heated under reflux over a water bath for 5 h. After cooling, the reaction mixture was poured with stirring into cold water then acidified with HCl. The solid precipitate was filtered, washed with water, dried and crystallized from ethanol to give fluorescent greenish-yellow short needles, m.p. 250°C, yield 0.19 g (49.2%). IR: ν (cm⁻¹) 3350 (NH), 1170 (C=S). ¹H NMR (CDCl₃): δ 2.80 (s, 3H, CH₃), 7.23–7.57 (m, 9H, Ar-H and NH), 8.23–8.33 (m, 2H, Ar-H). Anal. (C, H, N, S) for C₂₀H₁₄N₆OS.

3.14. 1-Phenyl-4-(1,6-diphenyl-3-methyl-1H-pyrazolo[3,4-*b*]pyrazin-5-yl) thiosemicarbazide 14

A mixture of the hydrazide **8** (0.688 g, 0.002 mol) and phenylisothiocyanate (0.27 g, 0.002 mol) in absolute ethanol (40 ml) was heated under reflux for half an hour. The solid precipitate was filtered and crystallized from a mixture of dioxane/water (2:1) to give shiny small pale-yellow needles, m.p. 210°C, yield 0.9 g (94%). IR: ν (cm⁻¹) 3300, 3150 (NH), 1680 (C=O), 1150 (C=S). ¹H NMR (DMSO-d₆): δ 2.73 (s, 3H, CH₃), 7.17–7.70 (m, 11H, Ar-H), 7.91–8.00 (m, 2H, Ar-H), 8.23–8.33 (m, 2H, Ar-H), 9.73 (s, 1H, NH), 10.2 (s, 1H, NH), 11.00 (s, 1H, NH). Anal. (C, H, N) for C₂₆H₂₁N₇OS.

3.15. 1,6-Diphenyl-3-methyl-5-(2-phenylamino-1,3,4-thiadiazol-5-yl)-1H-pyrazolo[3,4-*b*]pyrazine 15

The thiosemicarbazide **14** (0.479 g, 0.001 mol) was dissolved in concentrated sulfuric acid (4 ml, 96%) and stirred at room temperature for 0.5 h. The dark-red solution formed was then poured into crushed ice and neutralized with sodium

carbonate solution. The solid precipitate was filtered, washed with water and recrystallized from dioxane to give yellow platelets, m.p. 325°C, yield 0.35 g (76%). IR: ν (cm⁻¹) 3200 (NH). ¹H NMR (trifluoroacetic acid): δ 3.00 (s, 3H, CH₃), 7.20 (m, 2H, Ar-H), 7.43–7.67 (m, 11H, Ar-H), 7.74 (m, 2H, Ar-H). Anal. (C, H, N, S) for C₂₆H₁₉N₇S.

3.16. 1,6-Diphenyl-3-methyl-5-(3-mercapto-4-phenyl-1,2,4-triazol-5-yl)-1H-pyrazolo[3,4-*b*]pyrazine 16

A suspension of the thiosemicarbazide **14** (0.479 g, 0.001 mol) in an aqueous solution of NaOH (20 ml, 2N) was heated under reflux for 3 h. The mixture was cooled, acidified with diluted HCl and the separated solid was filtered off and crystallized from diluted ethanol to give yellow crystals, m.p. 290°C, yield 0.3 g (65%). IR: ν (cm⁻¹) 2750 (SH). ¹H NMR (CDCl₃): δ 2.77 (s, 3H, CH₃), 6.63 (m, 2H, Ar-H), 7.03–7.53 (m, 11H, Ar-H), 8.23 (m, 2H, Ar-H). Anal. (C, H, N, S) for C₂₆H₁₉N₇S.

3.17. 1,6-Diphenyl-3-methyl-5-(3-ethylthio-4-phenyl-1,2,4-triazol-5-yl)-1H-pyrazolo[3,4-*b*]pyrazine 17

A mixture of **16** (0.46 g, 0.001 mol), ethyl iodide (1 ml) and fused sodium acetate (0.5 g) in ethanol (20 ml) was heated over a boiling water bath for 30 minutes. After cooling the reaction mixture was poured onto cold water and the precipitate formed was collected and recrystallized from diluted ethanol as light-yellow needles, m.p. 210°C, yield 0.42 g (86%). IR: ν (cm⁻¹) 3050 (CH arom.), 2920 (CH aliph.), 1580, 1540, 1495, 1420, 1480, 1190, 745, 690. ¹H NMR (CDCl₃): δ 1.40 (t, 3H, CH₂CH₃), 2.80 (s, 3H, CH₃), 3.27 (q, 2H, CH₂CH₃), 6.42 (m, 2H, Ar-H), 7.07–7.53 (m, 11H, Ar-H), 8.27 (m, 2H, Ar-H). Anal. (C, H, N, S) for C₂₈H₂₃N₇S.

4. Antifungal activities

Eight of the newly synthesized compounds were screened for their antifungal activity against four species of fungi, namely, *Aspergillus terreus*, *Fusarium moniliforme*, *Penicillium chrysogenum* and *Rhizopus stolonifer* using the disc-diffusion method [13,14].

The tested compounds were dissolved in *N,N*-dimethylformamide (DMF) to get a solution of 1% concentration. Filter paper discs (Whatman No. 3 filter paper, 5 mm diameter) were saturated with this former solution. The saturated filter paper discs were placed on the surface of solidified Czapek's Dox agar dishes seeded by the test fungi. The inhibition zones were measured in mm at the end of an incubation period of 48 h at 28°C. The results are illustrated in Table 1; five compounds exhibited a moderate activity, with compound **3** being the best.

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