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STUDIES ON SYNTHESIS AND ANTIFUNGAL ACTIVITY OF PYRAZINE CONNECTED 1,2,4-TRIAZOL DERIVATIVES

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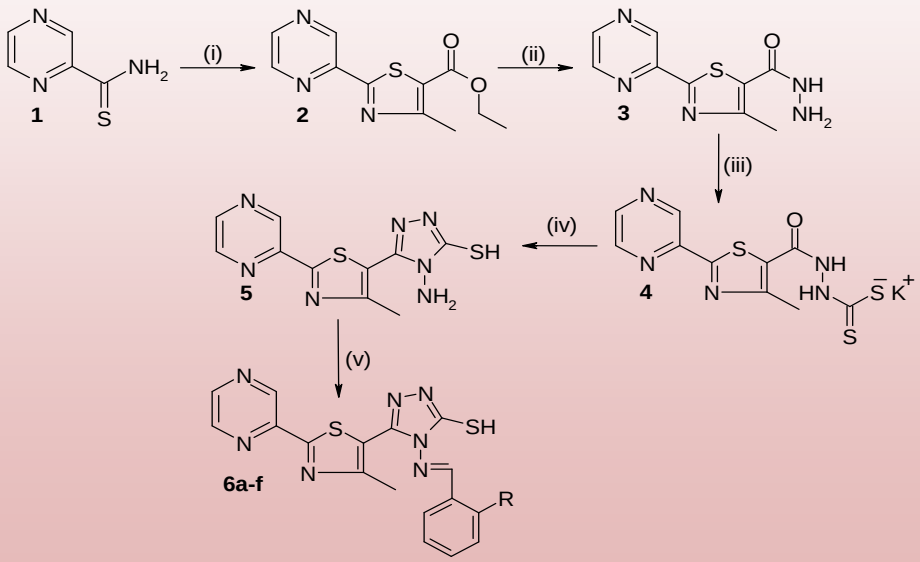
ABSTRACT
Encouraged by the broad spectrum of biological activities of pyrazine and 1,2,4-triazoles, we have decided to synthesis a novel series of 4-(benzylidene-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol and its derivatives (6a-f) by involving various intermediates like 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid ethyl ester (2), 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid hydrazide (3), potassium *N*-(4-methyl-2-pyrazine-2-yl-thiazole-5-carbonyl)-hydrazine carbodithioate (4) and 3-methyl-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-[1,2,4]-triazol-4-ylamine (5) and from pyrazine-2-carbothioic amide (1) as raw material. Structures of the synthesized compounds were elucidated by IR, ^{1}H-NMR and mass spectral data. All of the compounds were tested for antifungal activity and found that all the compounds exhibited marginal to good antifungal activity.



KEYWORDS: Pyrazine, 1,2,4-Triazole, antifungal activity.

1. INTRODUCTION
1,2,4-Triazole and its derivatives represent an important class of heterocyclic compounds. The synthesis of compounds containing 1,2,4-triazole rings in their structure has attracted widespread attention, mainly in connection with their wide range of pharmacological properties such as anti-inflammatory[1], analgesic[2], antibacterial[3], antifungal[4], antitubercular[5], antiviral[6], antitumoral[7] and antidepressant.[8] Pyrazine and its derivatives play an important role in the drug discovery realm. In particular the structural analogue of pyrazine derivatives presents various pharmacological activities such as antimicrobial[9], anti-inflammatory[10], antidepressant[11] and antiproliferative activities.[12]

2. RESULTS AND DISCUSSION

In the present study, as a continuation of our studies on obtaining bioactive molecules, we have performed the synthesis of some new pyrazine associated 1,2,4-triazole derivatives and investigation of antifungal activity of newly synthesized compounds.

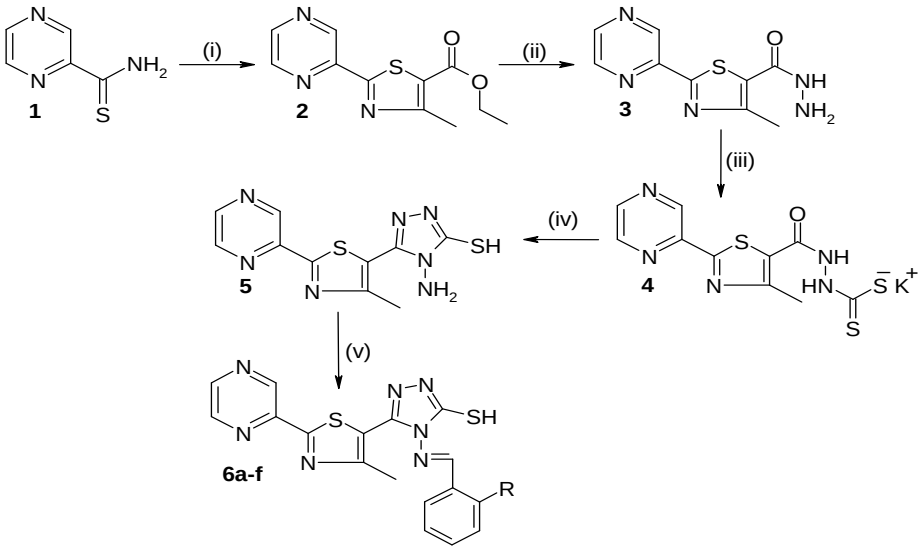
The title compounds, 4-(benzylidene-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4] triazole-3-thiol and its derivatives **(6a-f)** have been synthesized by a five-step and convenient synthetic route (Scheme 1) with pyrazine-2-carbothioic amide **(1)** as starting material. Thus, the raw compound through cyclization was reacted with ethyl-2-choloroacetoacetate in ethanol at reflux temperature for 4 h on water bath with uniform stirring to offer the initial intermediate, 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid ethyl ester **(2)** in good yield. The evidence for the formation of compound **2** can be obtained by ^{1}H-NMR, IR, mass spectroscopic methods and elemental analysis.

The IR spectrum showed a band at 1738 cm^{-1} due to the C=O bond and CH_3 group band at 2966 cm^{-1} associated with CH_3 group. These two groups were absent in its precursor. There is no signal of NH_2 group in its ^{1}H NMR spectrum which is present in the raw material. In addition to this, three additional signals have been observed at corresponding t δ-chemical shifts of CH_3 and CH_2 groups. The base peak of compound **2** is situated at m/z 249 confirmed the chemical structure.

After that, 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid hydrazide **(3)** was gained from the reaction of compound **2** with hydrazine hydrate in ethanol at reflux for 3 h on water bath with constant stirring. The structure of this compound was established by elemental analysis and on the basis of their mass spectra, infrared spectra, nuclear magnetic resonance spectra. In the IR spectrum, a broad and sharp signals were observed in the range of 3342-3165 cm^{-1} for N-H and NH_2 groups and the characteristic amide C=O group absorption band appeared at 1685 cm^{-1}. New signals in H NMR spectrum derived from the NH_2 group located at δ 7.71 ppm (D_2O exchange) and NH_2 group was identified at δ 4.47 ppm. In addition, relatively stable molecular ion peaks in the corresponding mass spectrum is detected at m/z 235.

Further, compound **3** on reaction with carbon disulfide and potassium hydroxide in absolute ethanol at room temperature for 4 h with steady stirring afforded the third intermediate, potassium *N*-(4-methyl-2-pyrazine-2-yl-thiazole-5-carbonyl)-hydrazine carbodithioate **(4)** in quantitative yield. The chemical structure of compound **4** was confirmed on the basis of IR, NMR, and mass spectroscopic methods and elemental analysis. IR spectrum revealed the absence of the NH_2 group at 3165 cm^{-1} which is present in its parent IR spectrum. Different from compound **3**, the ^{1}H-NMR spectrum of compound **4** exhibited no signal of NH_2 group. The mass spectrum showed M^+ peak at m/z 349 in agreement with their molecular formula.

The final intermediate, 3-methyl-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-[1,2,4]-triazol-4-ylamine **(5)** has been achieved from the solution of compound **4** and hydrazine hydrate in ethanol at reflux temperature with stable stirring on water bath for 3 h. From the spectral and elemental analysis data, the synthesized compound was in conformity with structure envisaged. A broad band at 3228 cm^{-1} was obtained due to the NH_2 stretching vibration and the S-H group was observed as a sharp band at 2572 cm^{-1}. In the ^{1}H NMR spectrum, two additional signals were found as singlet at δ 10.89 ppm and δ 4.56 ppm proved the presence of S-H and NH_2 groups. In the MS spectrum, the molecular ion peak at m/z 291 indicated the formation of intermediate **5**.



(i) Ethyl-2-choloroacetoacetate, ethanol, reflux, 4 h, (ii) Hydrazine hydrate, ethanol, reflux, 3 h, (iii) CS_2, KOH, ethanol, 4 h, (iv) Hydrazine hydrate, ethanol, reflux, 3 h, (v) Aromatic aldehyde, H_2SO_4, ethanol, reflux, 5-6 h.

Finally, the title compounds, 4-(benzylidene-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol and its derivatives **(6a-f)** have been prepared in moderate to good yields through condensation between intermediate **5** and aromatic aldehyde and catalytic amount of concentrated sulphuric acid in ethanol on reflux with constant stirring on water bath for 5-6 h. The spectral data of all the newly synthesized compounds were in accordance with the proposed structures. All protons were seen in the ^{1}H-NMR spectrum with the expected chemical shifts and integral values. One additional signal belonging to the CH group was noticed at δ 5.26 ppm in case of compound **6a**. The mass spectrum contains the peak corresponding at m/z 379 to its molecular weight.

Table 1: Physical data of synthesized compounds.

Entry	R	M. P (^{0}C)	Yield (%)	M. Formula	Mass
2	–	125-127	75	$C_{11}H_{11}N_3O_2S$	249
3	–	133-135	79	$C_9H_9N_5OS$	235
4	–	108-110	81	$C_{10}H_8KN_5OS_3$	349
5	–	144-146	77	$C_{10}H_9N_7S_2$	291
6a	H	129-131	73	$C_{17}H_{13}N_7S_2$	379
6b	CH_3	103-105	77	$C_{18}H_{15}N_7S_2$	393
6c	Cl	130-132	79	$C_{17}H_{12}ClN_7S_2$	414
6d	Br	102-104	76	$C_{17}H_{12}BrN_7S_2$	457
6e	OH	140-142	80	$C_{17}H_{13}N_7OS_2$	395
6f	NO_2	139-141	78	$C_{17}H_{12}N_8O_2S_2$	424

3. Antifungal Activity

The newly prepared compounds, 4-(benzylidene-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol and its derivatives **(6a-f)** have been used to screen for their antifungal activity against four representative fungal organisms such as *C. albicans*, *A. niger*, *T. rubrum* and *E. vitae* in dimethyl sulfoxide (DMSO) by broth dilution method[13] to measure the minimum inhibitory concentration (MIC, μg/mL) compared with the standard drug Amphotericin B and the screening results reported in the Figure 1.

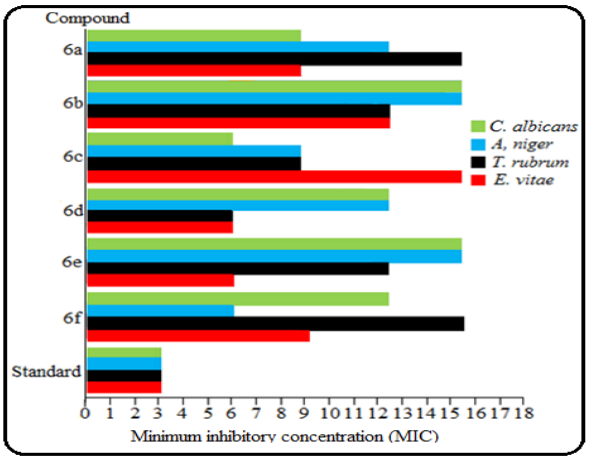


Figure 1: Antifungal activity of compounds 6a-f.

As per the activity results, all the tested compounds have been performed moderate to good activity with a degree of difference. Compound **6c** against *C. albicans*, **6d** towards both *T. rubrum* and *E. vitae*, **6e** in the direction of *E. vitae*, product **6f** opposed to *A. niger* exhibited highest activity at minimum inhibitory concentration 6.24 μg/mL in contrast to the reference drug. Compound **6a** against *T. rubrum*, **6b** towards both *C. albicans* and *A. niger*, **6c** in the direction of *E. vitae*, product **6e** opposed to *C. albicans* and *A. niger* and finally compound **6f** averse to *T. rubrum* performed least activity at minimum inhibitory concentration 15.6 μg/mL as compared to the standard drug. It is clear that remaining activity study indicates moderate activity between the MIC 6.24 μg/mL and 15.6 μg/mL. None of the title compound displayed equal or more antifungal activity as compared to the reference drug Amphotericin B.

4. Experimental

4. 1. Preparation of 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid ethyl ester (2): A mixture of pyrazine-2-carbothioic amide **(1)** (0.01 mol), ethyl 2-chloroacetoacetate (0.01 mol) in ethanol (15 ml) was refluxed on water bath with uniform stirring for 4 h. After completion of the reaction (monitored by the TLC), the resulted solution was cooled, poured in ice-cold water and thus obtained precipitate was filtered, washed with distilled water and recrystallized from absolute ethanol to offer 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid ethyl ester **(2)** in pure form. IR (KBr): 3020 (C-H, pyrazine ring), 2966 (C-H, CH_3), 1738 (C=O), 1572 (C=C, pyrazine ring), 1440 (C=N), 1131 (C-O) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.49 (d, 1H, J = 7.5 Hz, pyrazine ring), 7.36 (d, 1H, J = 7.5 Hz, pyrazine ring), 7.31 (s, 1H, pyrazine ring), 3.39 (q, 2H, J = 5.4 Hz, CH_2), 3.21 (s, 3H, CH_3), 2.48 (t, 3H, J = 5.4 Hz, CH_3); Elemental analysis: Calculated: C-53.00, H-4.45, N-16.86, O-12.84, S-12.86. Found: C-52.58, H-4.44, N-16.79, O-12.80, S-12.83.

4.2. Preparation of 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid hydrazide (3): To a solution of 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid ethyl ester **(2)** (0.01 mol) and ethanol (10 ml) was added hydrazine hydrate (0.03 mol). The resulted mixture was

stirred constantly on water bath for 3 h. After realization of the reaction (scanned by the TLC), the solution was poured in ice-cold water and the precipitate was filtered, dried and recrystallized from ethyl acetate to get pure 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid hydrazide **(3)**. IR (KBr): 3342 (N-H), 3165 (N-H, NH_2), 3032 (C-H, pyrazine ring), 2958 (C-H, CH_3), 1658 (C=O), 1582 (C=C, pyrazine ring), 1446 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.71 (s, 1H, NH), 7.52 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.39 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.36 (s, 1H, pyrazine ring), 4.47 (s, 2H, NH_2), 3.25 (s, 3H, CH_3); Elemental analysis: Calculated: C-45.95, H-3.86, N-29.77, O-6.80, S-13.63. Found: C-45.36, H-3.85, N-29.62, O-6.79, S-13.59.

4.3. Preparation of potassium *N*-(4-methyl-2-pyrazine-2-yl-thiazole-5-carbonyl)-hydrazine carbodithioate (4).

The solution of potassium hydroxide (0.02 mol) and CS_2 (0.02 mol) in ethanol (10 ml) was drop wise added to mixture of 4-methyl-2-pyrazin-2-yl-thiazole-5-carboxylic acid hydrazide **(3)** (0.01 mol) in ethanol (10 ml). Thus, obtained solution was stirred well at room temperature for 4 h. After fulfilment of the reaction (inspected by the TLC), the generated solid was filtered, washed with ethanol, dried and recrystallized from ethyl acetate to obtain potassium *N*-(4-methyl-2-pyrazine-2-yl-thiazole-5-carbonyl)-hydrazine carbodithioate **(4)** in pure form. IR (KBr): 3328 (N-H), 3171 (N-H), 3052 (C-H, pyrazine ring), 2948 (C-H, CH_3), 1652 (C=O), 1575 (C=C, pyrazine ring), 1552 (C=S), 1438 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.75 (s, 1H, NH), 7.55 (d, 1H, J = 7.4 Hz, pyrazine ring), 7.42 (d, 1H, J = 7.4 Hz, pyrazine ring), 7.34 (s, 1H, pyrazine ring), 4.52 (s, 2H, NH), 3.35 (s, 3H, CH_3); Elemental analysis: Calculated: C-34.37, H-2.31, K-11.19, N-20.04, O-4.58, S-27.52. Found: C-34.12, H-2.30, K-11.11, N-19.89, O-4.57, S-27.32.

4.4 Preparation of 3-methyl-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-[1,2,4]-triazol-4-yl-amine (5): A solution of potassium *N*-(4-methyl-2-pyrazine-2-yl-thiazole-5-carbonyl)-hydrazine carbodithioate **(4)** (0.01 mol) and hydrazine hydrate (0.03 mol) in ethanol (15 ml) was maintained at reflux temperature with steady stirring on water bath for 3 h. After execution of the reaction (scrutinized by the TLC), cooled the mixture, acidified with dilute HCl and generated solid was filtered and washed with ethanol and recrystallized from ethanol to achieve 3-methyl-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-[1,2,4]-triazol-4-yl-amine **(5)** in pure form. IR (KBr): 3228 (N-H, NH_2), 3065 (C-H, pyrazine ring), 2962 (C-H, CH_3), 2572 (S-H), 1568 (C=C, pyrazine ring), 1442 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.89 (s, 1H, SH), 7.59 (d, 1H, J = 7.6 Hz, pyrazine ring), 7.48 (d, 1H, J = 7.6 Hz, pyrazine ring), 7.39 (s, 1H, pyrazine ring), 4.56 (s, 2H, NH_2), 3.40 (s, 3H, CH_3); Elemental analysis: Calculated: C-41.22, H-3.11, N-33.65, S-22.01. Found: C-40.96, H-3.10, N-32.87, S-21.89.

4.5 Preparation of benzylidene-[3-methyl-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-[1,2,4]-triazol-4-yl]-amine (6a): A suspension of benzaldehyde (0.01 mol), ethanol (15 ml) and catalytic amount of concentrated H_2SO_4 (3 drops) has been added to the mixture of 3-methyl-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-[1,2,4]-triazol-4-yl-amine **(5)** (0.01 mol) and ethanol (10 ml). Then the solution was stirred at reflux temperature for 5 h with static stirring. The obtained solid was cooled, filtered, washed with ethanol, dried and recrystallized from ethyl acetate. The remaining compounds **(6b-f)** of this series were prepared by following the similar procedure. IR (KBr): 3055 (C-H, Ar), 2958 (C-H, CH_3), 2568 (S-H), 1570 (C=C, Ar), 1452 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.78 (s, 1H, SH), 7.55 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.51-7.36 (m, 5H, Ar-H), 7.43 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.36 (s, 1H, pyrazine ring), 5.26 (s, 1H, CH), 3.35 (s, 3H, CH_3); Elemental analysis: Calculated: C-53.81, H-3.45, N-25.84, S-16.90. Found: C-52.98, H-3.44, N-25.65, S-16.78.

4-[2-Methyl-benzylidene)-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol (6b): IR (KBr): 3062 (C-H, Ar), 2955 (C-H, CH_3), 2570 (S-H), 1578 (C=C, Ar), 1460 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.82 (s, 1H, SH), 7.62 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.57-7.31 (m, 5H, Ar-H), 7.46 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.39 (s, 1H, pyrazine ring), 5.32 (s, 1H, CH), 3.39 (s, 3H, CH_3), 3.21 (s, 3H, CH_3); Elemental analysis: Calculated: C-54.94, H-3.84, N-24.92, S-16.30. Found: C-54.71, H-3.83, N-24.26, S-16.18.

4-[2-Chloro-benzylidene)-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol (6c): IR (KBr): 3050 (C-H, Ar), 2942 (C-H, CH_3), 2556 (S-H), 1580 (C=C, Ar), 1455 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.78 (s, 1H, SH), 7.58 (d, 1H, J = 7.1 Hz, pyrazine ring), 7.55-7.37 (m, 5H, Ar-H), 7.42 (d, 1H, J = 7.1 Hz, pyrazine ring), 7.35 (s, 1H, pyrazine ring), 5.28 (s, 1H, CH), 3.44 (s, 3H, CH_3); Elemental analysis: Calculated: C-49.33, H-2.92, Cl-8.57, N-23.69, S-15.49. Found: C-48.95, H-2.91, Cl-8.56, N-23.46, S-15.29.

4-[2-Bromo-benzylidene)-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol (6d): IR (KBr): 3042 (C-H, Ar), 2952 (C-H, CH_3), 2560 (S-H), 1575 (C=C, Ar), 1463 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.68 (s, 1H, SH), 7.60 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.60-7.35 (m, 5H, Ar-H), 7.46 (d, 1H, J = 7.3 Hz, pyrazine ring), 7.38 (s, 1H, pyrazine ring), 5.32 (s, 1H, CH), 3.48 (s, 3H, CH_3); Elemental analysis: Calculated: C-44.55, H-2.64, Br-17.43, N-21.39, S-13.99. Found: C-43.85, H-2.63, Br-17.31, N-21.18, S-13.75.

4-[2-Hydroxy-benzylidene)-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol (6e): IR (KBr): 3056 (C-H, Ar), 2966 (C-H, CH_3), 2564 (S-H), 1582 (C=C, Ar), 1468 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.37 (s, 1H, OH), 10.76 (s, 1H, SH), 7.55 (d, 1H, J = 7.5 Hz, pyrazine ring), 7.64-7.40 (m, 5H, Ar-H), 7.42 (d, 1H, J = 7.5 Hz, pyrazine ring), 7.35 (s, 1H, pyrazine ring), 5.42 (s, 1H, CH), 3.50 (s, 3H, CH_3); Elemental analysis: Calculated: C-51.63, H-3.31, N-24.79, O-4.05, S-16.22. Found: C-50.86, H-3.30, N-24.59, O-4.04, S-16.06.

4-[2-Nitro-benzylidene)-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4]triazole-3-thiol (6f): IR (KBr): 3072 (C-H, Ar), 2970 (C-H, CH_3), 2559 (S-H), 1592 (C=C, Ar), 1474 (C=N) cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 10.65 (s, 1H, SH), 7.59 (d, 1H, J = 7.5 Hz, pyrazine ring), 7.60-7.42 (m, 5H, Ar-H), 7.39 (d, 1H, J = 7.5 Hz, pyrazine ring), 7.33 (s, 1H, pyrazine ring), 5.46 (s, 1H, CH), 3.54 (s, 3H, CH_3); Elemental analysis: Calculated: C-48.10, H-2.85, N-26.40, O-7.54, S-15.11. Found: C-47.68, H-2.84, N-26.22, O-7.53, S-15.02.

5. SUMMARY

The series of new derivatives of 4-(benzylidene-amino)-5-(4-methyl-2-pyrazin-2-yl-thiazol-5-yl)-4*H*-[1,2,4] triazole-3-thiol and its derivatives **(6a-f)** was prepared. Considering the structure of the obtained compounds it can be expected that these compounds can reveal antifungal activity.

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