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Pyrazine Associated Novel 1,2,4-Triazolo Thiadazines: Synthesis, Characterization and as Antibacterial Agents

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5-(5-Methyl-pyrazin-2-yl)-[1,3,4]-oxadiazole-2-thiol **(4)** has been synthesized form the starting material 5-methyl-2-pyrazinecarboxylic acid **(1)** on esterification with ethanol and condensation with hydrazine hydrate followed by cyclization with carbon disulfide. Compound **4** further converted into the next intermediate, 4-amino-5-(5-methyl-pyrazin-2-yl)-4*H*-[1,2,4]-triazole-2-thiol **(5)** when reacts with hydrazine hydrate. Finally, the target compounds, 3-(5-methyl-pyrazin-2-yl)-6-aryl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine **(6a-g)** have been synthesized successfully from the condensation reaction performed using compound **5** and a variety of phenacyl bromides. The chemical structures of all the newly synthesized intermediates and products were confirmed by IR, ^{1}H NMR, mass spectral studies and elemental analysis. Additionally all the target compounds have been screened for antibacterial activity.

Keywords: 1,2,4-Triazolo thiadiazines, Pyrazine, Antibacterial activity.

INTRODUCTION

The development of drug resistance towards the clinically used antibacterial agents has increased the demand for the design and synthesis of new chemical scaffolds possessing antimicrobial activity. 1,2,4-Triazole derivatives have consistently attracted scientific and practical interest because of their widely varying chemical properties, synthetic versatility and pharmacological activities, such as antibacterial [1], antifungal [2], antitubercular [3], analgesic [4], anti-inflammatory [5], anticancer [6], anticonvulsant [7], antiviral, [8], insecticidal [9] and antidepressant [10] properties.

EXPERIMENTAL

All the reagents and solvents were used as purchased from Sigma Aldrich without further purification. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60-120 mesh. IR spectra were obtained on a PerkinElmer BX series FT-IR 5000 spectrometer using KBr pellet. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer. The chemical shifts were reported as ppm down field using TMS as an internal standard. Mass spectra were recorded on a VG-Micromass 7070H spectrometer operating at 70 eV.

Preparation of 5-methyl-2-pyrazine carboxylic acid ethyl ester (2): To the solution of 5-methyl-2-pyrazine carboxylic acid **(1)** (0.01 mol) in absolute ethyl alcohol (10 mL), conc. H_2SO_4 (2 mL) was added. The mixture was refluxed for 4 h with constant stirring. After completion of the reaction (monitored by the TLC), the mixture was poured into ice-cold water. Crude product was collected by filtration, washed with 10 % $NaHCO_3$ solution, dried and recrystallized from ethyl alcohol to get pure 5-methyl-2-pyrazine carboxylic acid ethyl ester **(2)**.

Preparation of 5-methyl-2-pyrazine carboxylic acid hydrazide (3): A mixture of 5-methyl-2-pyrazinecarboxylic acid ethyl ester **(2)** (0.01 mol) and hydrazine hydrate (0.02 mol) in ethyl alcohol (20 mL) was refluxed for 3 h with consistent stirring. After realization of the reaction (examined by the TLC), the reaction mixture is cooled to room temperature and filtered. The crude product was recrystallized from ethanol to offer pure 5-methyl-2-pyrazine carboxylic acid hydrazide **(3)**.

Preparation of 5-(5-methyl-pyrazin-2-yl)-[1,3,4]-oxadiazole-2-thiol (4): A mixture of 5-methyl-2-pyrazine carboxylic acid hydrazide **(3)** (0.01 mol), potassium hydroxide (0.02 mol) and carbon disulfide (0.01 mol) in ethanol (25 mL) was heated under reflux with steady stirring for 9 h. After completion of the reaction (scanned by the TLC), the solvent was distilled *in vacuo*, the residual mass was poured over crushed ice and neutralized the alkaline solution with 10 % hydrochloric acid. The precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol to achieve pure 5-(5-methyl-pyrazin-2-yl)-[1,3,4]-oxadiazole-2-thiol **(4)**.

Preparation of 4-amino-5-(5-methyl-pyrazin-2-yl)-4*H*-[1,2,4]-triazole-2-thiol (5): To a warm solution of 5-(5-methyl-pyrazin-2-yl)-[1,3,4]-oxadiazole-2-thiol **(4)** (0.01 mol) in ethanol (15 mL), 80 % hydrazine hydrate (0.02 mol) was added drop wise and the reaction mixture was heated under reflux for 5 h with constant stirring. After fulfillment of the reaction (check by the TLC), the solvent was distilled off *in vacuo,* cooled and the crystals separated were filtered, washed with cold ethanol and recrystallized from chloroform to give pure 4-amino-5-(5-methyl-pyrazin-2-yl)-4*H*-[1,2,4]-triazole-2-thiol **(5)**.

Preparation of 3-(5-methyl-pyrazin-2-yl)-6-aryl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6a-g): A mixture of 4-amino-5-(5-methyl-pyrazin-2-yl)-4*H*-[1,2,4]-triazole-2-thiol **(5)** (0.01 mol) and corresponding phenacyl bromide (0.01 mol) in absolute ethanol (15 mL) was refluxed for 6-7 h with constant stirring. After completion of the reaction (checked by the TLC), the reaction mixture was concentrated and cooled to room temperature and the remaining solvent was removed under reduced pressure, then diethyl ether (10 mL) was added and the reaction mixture was left at 0 °C for overnight. The precipitated solid was filtered off; the crude product thus obtained was purified by column chromatography on silica gel with hexane-ethyl acetate as eluent to afford pure 3-(5-methyl-pyrazin-2-yl)-6-aryl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine **(6a-g)**.

Physical and spectral data

5-Methyl-2-pyrazine carboxylic acid ethyl ester (2): White solid; Yield 74 %; m.p.: 132-134 °C; IR (KBr, ν_{max}, cm^{-1}): 3024 (C-H, pyrazine), 2945 (C-H, CH_3), 1695 (C=O), 1640 (C=N) 1560 (C=C, pyrazine), 1145 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29 (t, 3H, *J* = 5.4 Hz, CH_3), 2.42 (s, 3H, CH_3), 4.12 (q, 2H, *J* = 5.4 Hz, CH_2), 7.12 (s, 1H, pyrazine-H), 7.45 (s, 1H, pyrazine-H); MS *m/z* 166 (M^+); Elemental analysis: Calculated for $C_8H_{10}N_2O_2$: C-57.82, H-6.07, N-16.86. Found: C-57.25, H-6.00, N-16.74.

5-Methyl-2-pyrazinecarboxylic acid hydrazide (3): Yellow solid; Yield 72 %; m.p.: 142-144 °C; IR (KBr, ν_{max}, cm^{-1}): 3240 (N-H), 3035 (C-H, pyrazine), 2954 (C-H, CH_3), 1640 (C=O), 1630 (C=N), 1568 (C=C, pyrazine); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.59 (s, 3H, CH_3), 5.28 (s, 2H, NH_2), 7.21 (s, 1H, pyrazine-H), 7.45 (s, 1H, pyrazine-H), 7.58 (s, 1H, NH); MS *m/z* 152 (M^+); Elemental analysis: Calculated for $C_6H_8N_4O$: C-47.36, H-5.30, N-36.82. Found: C-47.02, H-5.27, N-36.65.

5-(5-Methyl-pyrazin-2-yl)-[1,3,4]-oxadiazole-2-thiol (4): Pale yellow solid; Yield 76 %; m.p.: 145-147 °C; IR (KBr, ν_{max}, cm^{-1}): 3041 (C-H, pyrazine), 2936 (C-H, CH_3), 2836 (S-H), 1642 (C=N), 1610 (C=C, pyrazine), 1128 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3), 7.32 (s, 1H, pyrazine-H), 7.52 (s, 1H, pyrazine-H), 10.85 (s, 1H, S-H); MS *m/z* 194 (M^+); Elemental analysis: Calculated for $C_7H_6N_4OS$: C-43.29, H-3.11, N-28.85, S-16.51. Found: C-43.06, H-3.07, N-28.68, S-16.46.

4-Amino-5-(5-methyl-pyrazin-2-yl)-4*H*-[1,2,4]-triazole-2-thiol (5): Orange solid; Yield 71 %; m.p.: 155-157 °C; IR (KBr, ν_{max}, cm^{-1}): 3365 (N-H, NH_2), 3038 (C-H, pyrazine), 2928 (C-H, CH_3), 2848 (S-H), 1635 (C=N), 1575 (C=C, pyrazine); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 2H, NH_2), 2.66 (s, 3H, CH_3), 7.33 (s, 1H, pyrazine-H), 7.59 (s, 1H, pyrazine-H), 10.21 (s, 1H, S-H); MS *m/z* 208 (M^+); Elemental analysis: Calculated for $C_7H_8N_6S$: C-40.37, H-3.87, N-40.36, S-15.40. Found: C-40.14, H-3.82, N-40.28, S-15.35.

3-(5-Methyl-pyrazin-2-yl)-6-phenyl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6a): Yellow solid; Yield 77 %; m.p.: 120-122 °C; IR (KBr, ν_{max}, cm^{-1}): 3036 (C-H, Ar), 2974 (C-H, CH_3), 1634 (C=N), 1623 (C=C, Ar), 1285 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.42 (s, 3H, CH_3), 3.84 (s, 2H, CH_2-S), 7.19 (s, 1H, pyrazine-H),), 7.35-7.54 (m, 5H, Ar-H), 7.52 (s, 1H, pyrazine-H); MS *m/z* 308 (M^+); Elemental analysis: Calculated for $C_{15}H_{12}N_6S$: C-58.43, H-3.92, N-27.25, S-10.40. Found: C-58.12, H-3.90, N-27.18, S-10.34.

3-(5-Methyl-pyrazin-2-yl)-6-*p*-tolyl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6b): Orange solid; Yield 71 %; m.p.: 139-141 °C; IR (KBr, ν_{max}, cm^{-1}): 3038 (C-H, Ar), 2968 (C-H, CH_3), 1642 (C=N), 1624 (C=C), 1280 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3), 2.44 (s, 3H, CH_3), 3.95 (s, 2H, CH_2-S), 7.28 (s, 1H, pyrazine-H), 7.49 (s, 1H, pyrazine-H), 7.51 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.65 (d, 2H, *J* = 7.0 Hz, Ar-H); MS *m/z* 322 (M^+); Elemental analysis: Calculated for $C_{16}H_{14}N_6S$: C-59.61, H-4.38, N-26.07, S-9.95. Found: C-59.18, H-4.35, N-25.99, S-9.94.

6-(2-Methoxy-phenyl)-3-(5-methyl-pyrazin-2-yl)-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6c): Yellow solid; Yield 70 %; m.p.: 158-160 °C; IR (KBr, ν_{max}, cm^{-1}): 3051 (C-H, Ar), 2964 (C-H, CH_3), 1648 (C=N), 1623 (C=C, Ar),1270 (C-S), 1148 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.71 (s, 3H, CH_3), 3.54 (s, 3H, O-CH_3), 3.87 (s, 2H, S-CH_2), 7.32 (s, 1H, pyrazine-H), 7.34-7.59 (m, 4H, Ar-H), 7.52 (s, 1H, pyrazine-H); MS *m/z* 338 (M^+); Elemental analysis: Calculated for $C_{16}H_{14}N_6OS$: C-56.79, H-4.17, N-24.84, S-9.48. Found: C-56.48, H-4.15, N-24.48, S-9.40.

6-(4-Methoxy-phenyl)-3-(5-methyl-pyrazin-2-yl)-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6d): Orange solid; Yield 73 %; m.p.: 114-116 °C; IR (KBr, ν_{max}, cm^{-1}): 3048 (C-H, Ar), 2968 (C-H, CH_3), 1660 (C=N), 1625 (C=C, Ar), 1275 (C-S), 1158 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.61 (s, 3H, CH_3), 3.51 (s, 2H, S-CH_2), 3.74 (s, 3H, O-CH_3), 7.25 (s, 1H, pyrazine-H), 7.39 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.49 (s, 1H, pyrazine-H), 7.68 (d, 2H, *J* = 7.8 Hz, Ar-H); MS *m/z* 338 (M^+); Elemental analysis: Calculated for $C_{16}H_{14}N_6OS$: C-56.79, H-4.17, N-24.84, S-9.48. Found: C-56.48, H-4.15, N-24.48, S-9.40.

6-(4-Chloro-phenyl)-3-(5-methyl-pyrazin-2-yl)-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6e): Brown solid; Yield 70 %; m.p.: 130-132 °C; IR (KBr, ν_{max}, cm^{-1}): 3048 (C-H, Ar), 2974 (C-H, CH_3), 1634 (C=N), 1610 (C=C, Ar), 1275 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.68 (s, 3H, CH_3), 3.81 (s, 2H, S-CH_2), 7.32 (s, 1H, pyrazine-H), 7.42 (d, 2H, *J* = 7.4 Hz, Ar-H), 7.51 (s, 1H, pyrazine-H), 7.74 (d, 2H, *J* = 7.4 Hz, Ar-H); MS *m/z* 342 (M^+); Elemental analysis: Calculated for $C_{15}H_{11}N_6SCl$: C-52.55, H-3.23, Cl-10.34, N-24.52, S-9.35. Found: C-52.12, H-3.21, Cl-10.28, N-24.47, S-9.32.

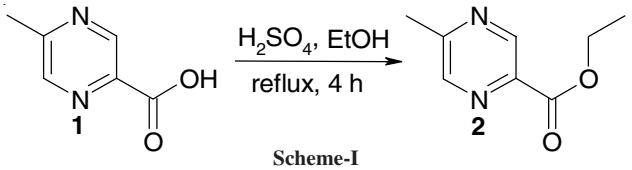
6-(4-Bromo-phenyl)-3-(5-methyl-pyrazin-2-yl)-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6f): Pale yellow

solid; Yield 74 %; m.p.: 150-152 °C; IR (KBr, ν_{max}, cm^{-1}): 3032 (C-H, Ar), 2971 (C-H, CH_3), 1631 (C=N), 1578 (C=C, Ar), 1265 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (s, 3H, CH_3), 3.92 (s, 2H, CH_2-S), 7.19 (s, 1H, pyrazine-H), 7.39 (s, 1H, pyrazine-H), 7.42 (d, 2H, *J* = 7.4 Hz, Ar-H), 7.59 (d, 2H, *J* = 7.4 Hz, Ar-H); MS *m/z* 387 (M^+); Elemental analysis: Calculated for $C_{15}H_{11}N_6SBr$: C-46.52, H-2.86, Br-20.63, N-21.70, S-8.28. Found: C-46.02, H-2.83, Br-20.54, N-21.51, S-8.24.

6-(4-Nitro-phenyl)-3-(5-methyl-pyrazin-2-yl)-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine (6g): Brown solid; Yield 73 %; m.p.: 118-120 °C; IR (KBr, ν_{max}, cm^{-1}): 3028 (C-H, Ar), 2965 (C-H, CH_3), 1630 (C=N), 1584 (C=C, Ar), 1248 (C-S); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.65 (s, 3H, CH_3), 3.74 (s, 2H, CH_2-S), 7.32 (s, 1H, pyrazine-H), 7.39 (d, 2H, *J* = 7.0 Hz, Ar-H), 7.54 (s, 1H, pyrazine-H), 7.56 (d, 2H, *J* = 7.0 Hz, Ar-H); MS *m/z* 353 (M^+); Elemental analysis: Calculated for $C_{15}H_{11}N_7O_2S$: C-50.99, H-3.14, N-27.75, S-9.07. Found: C-50.02, H-3.12, N-27.58, S-9.01.

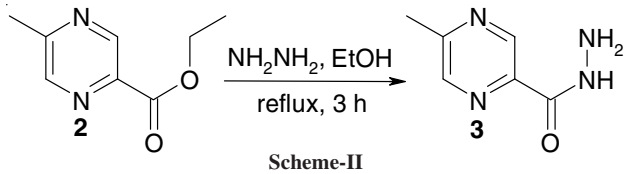
RESULTS AND DISCUSSION

The biological and medicinal characteristics of triazole derivatives stimulated us to synthesize some new biologically active heterocycles, we integrated thiadiazine and triazole moieties into pyrazine molecule, since these systems possess well documented antimicrobial activity. Accordingly, in this paper, we reported the synthesis of 3-(5-methyl-pyrazin-2-yl)-6-aryl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine **(6a-g)**. The syntheses of the target compounds **6a-g** commenced from commercially available 5-methyl-2-pyrazine carboxylic acid **(1)**. The initial intermediate, 5-methyl-2-pyrazine carboxylic acid ethyl ester **(2)**, has been prepared in excellent yield by boiling of a mixture of compound **1** and absolute ethyl alcohol in the presence of catalytic amount of conc. H_2SO_4 for 4 h with constant stirring (**Scheme-I**).



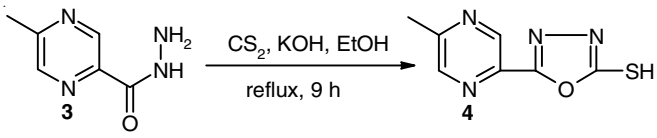
Scheme-I

Then the intermediate **2** is turned into next intermediate, 5-methyl-2-pyrazine carboxylic acid hydrazide **(3)** when reacts with hydrazine hydrate in ethyl alcohol at reflux for 3 h with uniform stirring (**Scheme-II**).



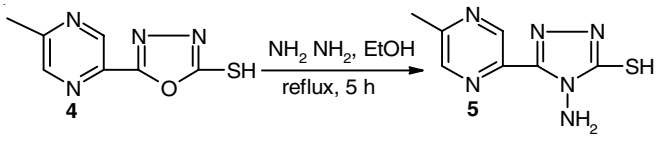
Scheme-II

Further intermediate, 5-(5-methyl-pyrazin-2-yl)-[1,3,4]-oxadiazole-2-thiol **(4)** has been achieved in good yield from the reaction occurred between compound **3** and carbon disulfide in presence of potassium hydroxide in ethanol at reflux for 9 h on steady stirring followed by acidification (**Scheme-III**).



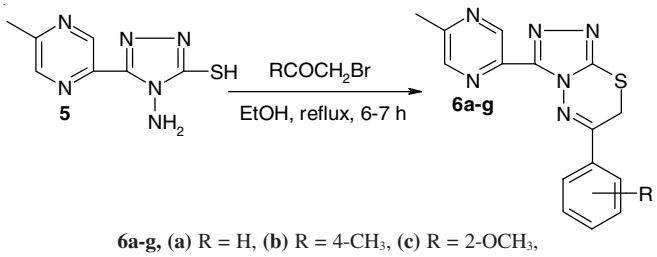
Scheme-III

Then this intermediate **4** is converted into final intermediate, 4-amino-5-(5-methyl-pyrazin-2-yl)-4*H*-[1,2,4]-triazole-2-thiol **(5)** in excellent yield when reacts with hydrazine hydrate in ethanol at reflux for 5 h with consistent stirring (**Scheme-IV**).



Scheme-IV

Finally, the target compounds, 3-(5-methyl-pyrazin-2-yl)-6-aryl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine **(6a-g)** have been synthesized in good to excellent yields successfully from the condensation reaction performed between compound **5** and a variety of phenacylbromides in ethanol under reflux for 6-7 h on uniform stirring (**Scheme-V**). The chemical structures of all the newly synthesized intermediates and products were confirmed by IR, ^{1}H NMR, mass spectral studies and elemental analysis. Antibacterial activity of the final compounds has been evaluated and all the compounds have shown significant inhibition of bacterial growth.



6a-g, (a) R = H, **(b)** R = 4-CH_3, **(c)** R = 2-OCH_3, **(d)** R = 4-OCH_3, **(e)** R = 4-Cl, **(f)** R = 4-Br, **(g)** R = 4-NO_2

Scheme-V

Antibacterial activity: The newly synthesized compounds, 3-(5-methyl-pyrazin-2-yl)-6-aryl-7*H*-[1,2,4]-triazole-[3,4-*b*][1,3,4]-thiadazine **(6a-g)** were used to evaluate for their *in vitro* antibacterial activity against four representative bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli* with broth dilution method [11] using roxythromycin as standard drug. The assay was performed in duplicates and the mean diameters of the zone of inhibition in mm were recorded. The results of the *in vitro* antibacterial activity of the target compounds are reported in Table-1. Compound **6g** with *para* nitro derivative was performed highest activity against *B. subtilis* with zone of inhibition 20 mm. The same **6g** was exhibited prominent antibacterial activity towards *S. aureus* with zone of inhibition 21 mm. Compound **6f** with *para* bromo group showed eminent activity against *S. typhimurium* with zone of inhibition 23 mm. Same compound **6f** executed significant activity towards *E. coli* with

TABLE-1
in vitro ANTIBACTERIAL ACTIVITY OF NEW COMPOUNDS **6a-g** (ZONE OF INHIBITION IN mm)

Entry	*B. subtilis*	*S. aureus*	*S. typhimurium*	*E. coli*
6a	06	07	09	10
6b	12	13	14	09
6c	18	16	15	17
6d	16	14	13	15
6e	17	19	20	20
6f	11	21	23	21
6g	20	21	18	17
Roxythromycin	26	25	28	27

21 mm. The remaining compounds of this series disclosed moderate to good antibacterial activity. The outstanding properties of this new class of antibacterial substances deserve further investigation in order to clarify the mode of action at molecular level, responsible for the activity observed. More extensive study is also warranted to determine additional physico-chemical and biological parameters to have a deeper insight into structure-activity relationship (SAR) and to optimize the effectiveness of this series of molecules.

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