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Synthesis, Characterization and Biological Screening of Some Novel Pyrazine Associated 1,2,4-Triazoles

PRASADA REDDY MEKA[1], PULLA RAO BOLLAM[2], RAVI CHANDRA BABU[1,*] and SIVA RAMI REDDY[3]

[1]Department of Chemistry, Institute of Science, GITAM University, Visakhapatnam-530 045, India
[2]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur-522 510, India
[3]Maithili Life Sciences Private Limited, Plot No. 02, Gajulamandyam, APIIC, Tirupati- 517 520, India

*Corresponding author: E-mail: prasadreddy.m83@gmail.com

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A new and efficient method has been developed for the synthesis of some novel pyrazine associated 1,2,4-triazoles *e.g.*, 2-methyl-5-(4-alkyl/aryl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (**5a-c**). This novel method involves 5-methyl-2-pyrazinecarboxylic acid (**1**) as raw material and 5-methyl-2-pyrazinecarboxylic acid hydrazide (**2**), 5-methyl-2-pyrazinecarboxylic acid hydrazide *N*-alkyl/aryl carbothioamide (**3a-c**) and 4-alkyl/aryl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (**4a-c**) as intermediates in good to excellent yields. The chemical structures of all these compounds are identified by IR, ^{1}H NMR, mass spectroscopy and elemental analysis. The antimicrobial activity of the target compounds has been also evaluated.

Keywords: Pyrazine, 1,2,4-Triazoles, Biological screening.

INTRODUCTION

1,2,4-Triazoles and their derivatives are important group of heterocyclic compounds. The biological activity of 1,2,4-triazoles have been demonstrated by various studies [1,2]. The 1,2,4-triazole nucleus posses a wide range of pharmacological activities such as analgesic [3], antibacterial [4], antifungal [5], anti-inflammatory [6] and antioxidant [7] properties. Therefore, there is a continuing need for new antimicrobial agents with more selectivity and lower side effect.

EXPERIMENTAL

All reagents and solvents were used as purchased 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 Perkin Elmer 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.

5-Methyl-2-pyrazinecarboxylic acid hydrazide (2): A mixture of 5-methyl-2-pyrazinecarboxylic acid (**1**) (0.01 mol) and hydrazine hydrate (0.025 mol) in ethanol (20 mL) was refluxed for 9 h on consistent stirring. After completion of the reaction (monitored by the TLC), the reaction mixture is cooled to room temperature, poured in ice-cold water (20 mL). Thus the solid separated was filtered off and the crude product was recrystallized from ethyl acetate to give pure 5-methyl-2-pyrazinecarboxylic acid hydrazide (**2**).

5-Methyl-2-pyrazinecarboxylic acid hydrazide *N*-alkyl/aryl carbothioamide (3a-c): A mixture of 5-methyl-2-pyrazinecarboxylic acid hydrazide (**2**) (0.01 mol) and alkyl or phenyl isocyanate in ethanol was refluxed on uniform stirring for 4 h. After realization of the reaction (observed by the TLC), the reaction mixture is cooled to ambient temperature, poured in ice-cold water (20 mL) and the resulted solid was filtered and the crude product was recrystallized from ethanol to offer 5-methyl-2-pyrazinecarboxylic acid hydrazide *N*-alkyl/aryl carbothioamide (**3a-c**) in pure form.

4-Alkyl/aryl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (4a-c): A mixture of 5-methyl-2-pyrazinecarboxylic acid hydrazide *N*-alkyl/aryl carbothioamides (**3a-c**) (0.01 mol) and 10 % sodium hydroxide (15 mL) in ethanol was heated at reflux temperature on constant stirring for 3 h. After completion of the reaction (scanned by the TLC), the reaction mixture is cooled to ambient temperature and decanted in crushed ice (25 g). The resulted solution was neutralized with conc. HCl and formed solid was isolated by filtration, washed with ice-cold water, dried and recrystallized

from ethanol to get 4-alkyl/aryl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (**4a-c**) in pure form.

2-Methyl-5-(4-alkyl/aryl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (5a-c): A solution of iodomethane (0.01 mol) and methanol (5 mL) is gradually added to a mixture of 4-alkyl/phenyl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thiones (**4a-c**) (0.01 mol) and sodium hydroxide (10 %, 10 mL) in methanol (10 mL) on steady stirring. The overall mixture refluxed for 3 h. After completion of the reaction (examined by the TLC), the reaction mixture is cooled and neutralized with conc. HCl and extracted with chloroform and resulted solid was dried $CaCl_2$ to obtain pure 2-methyl-5-(4-alkyl/aryl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (**5a-c**).

Spectral data

5-Methyl-2-pyrazinecarboxylic acid hydrazide (2): Colour: White; Yield: 73 %; m.p.: 156-158 °C; IR (KBr, ν_{max}, cm^{-1}): 3255 (N-H), 3020 (C-H, Ar), 2966 (C-H, CH_3), 1670 (C=O), 1572 (C=C, Ar), 1445 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.74 (s, 1H, NH), 7.77 (s, 1H, Ar-H), 7.36 (s, 1H, Ar-H), 4.45 (s, 2H, NH_2), 2.50 (s, 3H, CH_3); MS: *m/z* 152 (M^+); Elemental analysis: Calcd. for $C_6H_8N_4O$: C 47.36, H 5.30, N 36.82. Found: C 47.02, H 5.24, N 36.08.

5-Methyl-2-pyrazinecarboxylic acid hydrazide *N*-methyl carbothioamide (3a): Colour: Yellow; Yield: 75 %; m.p.: 141-143 °C; IR (KBr, ν_{max}, cm^{-1}): 3270 (N-H), 3030 (C-H, Ar), 2966 (C-H, CH_3), 1684 (C=O), 1577 (C=C, Ar), 1432 (C=N), 1210 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.74 (s, 1H, CONH), 7.72 (s, 1H, Ar-H), 7.43 (s, 1H, CSNH), 7.36 (s, 1H, Ar-H), 4.85 (s, 1H, NH-CH_3), 3.28 (s, 3H, N-CH_3), 2.62 (s, 3H, CH_3); MS: *m/z* 225 (M^+); Elemental analysis: Calcd. for $C_8H_{11}N_5OS$: C 42.65, H 4.92, N 31.09, S 14.23. Found: C 42.14, H 4.88, N 30.87, S 14.12.

5-Methyl-2-pyrazinecarboxylic acid hydrazide *N*-ethyl carbothioamide (3b): Colour: Pale yellow; Yield: 70 %; m.p.: 138-140 °C; IR (KBr, ν_{max}, cm^{-1}): 3278 (N-H), 3044 (C-H, Ar), 2984 (C-H, CH_3), 1670 (C=O), 1574 (C=C, Ar), 1428 (C=N), 1220 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.74 (s, 1H, CONH), 7.68 (s, 1H, Ar-H), 7.52 (s, 1H, CSNH), 7.29 (s, 1H, Ar-H), 4.79 (s, 1H, NH-CH_3), 3.30 (q, 2H, J = 5.4 Hz, CH_2), 3.22 (t, 3H, J = 5.4 Hz, CH_3), 2.58 (s, 3H, CH_3); MS: *m/z* 239 (M^+); Elemental analysis: Calcd. for $C_9H_{13}N_5OS$: C 45.17, H 5.48, N 29.27, S 13.40. Found: C 44.87, H 5.43, N 29.11, S 13.28.

5-Methyl-2-pyrazinecarboxylic acid hydrazide *N*-phenyl carbothioamide (3c): Colour: Yellow; Yield: 71 %; m.p.: 130.132 °C; IR (KBr, ν_{max}, cm^{-1}): 3284 (N-H), 3040 (C-H, Ar), 2977 (C-H, CH_3), 1658 (C=O), 1560 (C=C, Ar), 1444 (C=N), 1224 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.89 (s, 1H, CONH), 7.58-7.21 (m, 5H, Ar-H), 7.52 (s, 1H, Ar-H), 7.49 (s, 1H, Ar-H), 7.34 (s, 1H, CSNH), 4.88 (s, 1H, NH-CH_3), 2.50 (s, 3H, CH_3); MS: *m/z* 287 (M^+); Elemental analysis: Calcd. for $C_{13}H_{13}N_5OS$: C 54.34, H 4.56, N 24.37, S 11.16. Found: C 53.98, H 4.52, N 24.12, S 11.04.

4-Methyl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (4a): Colour: White; Yield: 74 %; m.p.: 162-164 °C; IR (KBr, ν_{max}, cm^{-1}): 3274 (N-H), 3034 (C-H, Ar), 2968 (C-H, CH_3), 1555 (C=C, Ar), 1440 (C=N), 1230 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.79 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.46 (s, 1H, NH), 3.22 (s, 3H, CH_3), 2.57 (s, 3H, CH_3); MS: *m/z* 207 (M^+); Elemental analysis: Calcd. for $C_8H_9N_5S$: C 46.36, H 4.38, N 33.79, S 15.47. Found: C 46.08, H 4.36, N 33.12, S 15.25.

4-Ethyl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (4b): Colour: Pale yellow; Yield: 77 %; m.p.: 155-157 °C; IR (KBr, ν_{max}, cm^{-1}): 3262 (N-H), 3028 (C-H, Ar), 2975 (C-H, CH_3), 1548 (C=C, Ar), 1434 (C=N), 1227 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.72 (s, 1H, Ar-H), 7.36 (s, 1H, Ar-H), 7.49 (s, 1H, NH), 3.36 (q, 2H, J = 5.2 Hz, CH_2), 3.21 (s, 3H, CH_3), 2.48 (t, 3H, J = 5.2 Hz, CH_3); MS: *m/z* 221 (M^+); Elemental analysis: Calcd. for $C_9H_{11}N_5S$: C 48.85, H 5.01, N 31.65, S 14.49. Found: C 47.98, H 4.99, N 31.12, S 14.35.

4-Phenyl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (4c): Colour: Yellow; Yield: 76 %; m.p.: 123-125 °C; IR (KBr, ν_{max}, cm^{-1}): 3255 (N-H), 3018 (C-H, Ar), 2981 (C-H, CH_3), 1559 (C=C, Ar), 1448 (C=N), 1225 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.55-7.25 (m, 5H, Ar-H), 7.52 (s, 1H, Ar-H), 7.41 (s, 1H, NH), 7.45 (s, 1H, Ar-H), 2.58 (s, 3H, CH_3); MS: *m/z* 269 (M^+); Elemental analysis: Calcd. for $C_{13}H_{11}N_5S$: C 57.97, H 4.12, N 26.00, S 11.91. Found: C 57.36, H 4.08, N 25.87, S 11.58.

2-Methyl-5-(4-methyl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (5a): Colour: White; Yield: 72 %; m.p.: 170-172°C; IR (KBr, ν_{max}, cm^{-1}): 3262 (N-H), 3032 (C-H, Ar), 2958 (C-H, CH_3), 1562 (C=C, Ar), 1428 (C=N), 615 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.58 (s, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 3.23 (s, 3H, N-CH_3), 3.15 (s, 3H, S-CH_3), 2.62 (s, 3H, CH_3); MS: *m/z* 221 (M^+); Elemental analysis: Calcd. for $C_9H_{11}N_5S$: C 48.85, H 5.01, N 31.65, S 14.49. Found: C 48.41, H 4.99, N 31.36, S 14.40.

2-Methyl-5-(4-ethyl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (5b): Colour: Pale yellow; Yield: 74 %; m.p.: 153-155 °C; IR (KBr, ν_{max}, cm^{-1}): 3020 (C-H, Ar), 2966 (C-H, CH_3), 1572 (C=C, Ar),1448 (C=N), 621 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.65 (s, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 3.28 (q, 2H, J = 5.6 Hz, CH_2), 3.18 (s, 3H, S-CH_3), 2.52 (t, 3H, J = 5.6 Hz, CH_3), 2.39 (s, 3H, CH_3); MS: *m/z* 235 (M^+); Elemental analysis: Calcd. for $C_{10}H_{13}N_5S$: C 51.04, H 5.57, N 29.67, S 13.67. Found: C 50.68, H 5.49, N 29.38, S 13.42.

2-Methyl-5-(4-phenyl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (5c): Colour: Yellow; Yield: 70 %; m.p.: 149-151 °C; IR (KBr, ν_{max}, cm^{-1}): 3035 (C-H, Ar), 2959 (C-H, CH_3), 1584 (C=C, Ar),1429 (C=N), 628 (C-S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.85-7.42 (m, 5H, Ar-H), 7.69 (s, 1H, Ar-H), 7.45 (s, 1H, Ar-H), 3.42 (s, 3H, S-CH_3), 2.85 (s, 3H, CH_3); MS: *m/z* 283 (M^+); Elemental analysis: Calcd. for $C_{14}H_{13}N_5S$: C 59.34, H 4.62, N 24.72, S 11.32. Found: C 58.99, H 4.55, N 24.58, S 11.28.

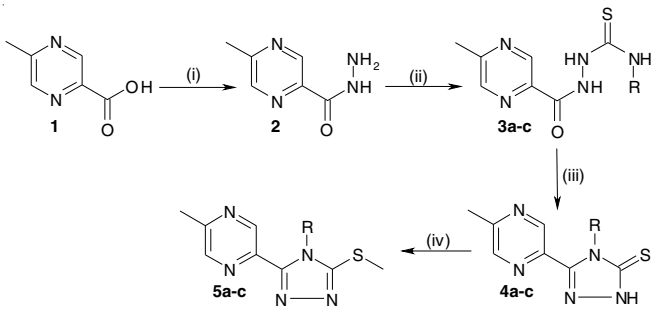
RESULTS AND DISCUSSION

The synthetic path of the target compounds, 2-methyl-5-(4-alkyl/aryl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (**5a-c**) is involved 5-methyl-2-pyrazinecarboxylic acid (**1**), 5-methyl-2-pyrazinecarboxylic acid hydrazide (**2**), 5-methyl-2-pyrazinecarboxylic acid hydrazide *N*-alkyl/aryl

TABLE-1
ANTIMICROBIAL ACTIVITY OF COMPOUNDS **4a-c** AND **5a-c** (ZONES OF INHIBITION IN mm)

Compound	Antibacterial activity				Antifungal activity
	S. aureus	*S. pyogenes*	*P. aureginosa*	*E. coli*	*C. albicans*
4a	13.0	11.4	10.5	15.6	10.4
4b	16.6	13.8	12.9	14.8	16.9
4c	13.8	13.6	11.2	15.1	13.2
5a	17.3	12.6	12.8	13.6	12.5
5b	16.4	12.8	13.1	15.2	17.4
5c	11.6	11.9	12.6	12.3	13.4
Ampicilline	18.0	14.0	14.0	16.0	–
Fuoconazole	–	–	–	–	18.0

carbothioamide (**3a-c**) and 4-alkyl/aryl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (**4a-c**) as intermediates (**Scheme-I**). Thus the initial intermediate, 5-methyl-2-pyrazinecarboxylic acid hydrazide (**2**) has been prepared from 5-methyl-2-pyrazinecarboxylic acid (**1**) on reaction with hydrazine hydrate in refluxing ethanol on constant stirring for 9 h. Then compound **2** was turned into the next intermediate, 5-methyl-2-pyrazinecarboxylic acid hydrazide *N*-alkyl/aryl carbothioamide (**3a-c**) on reaction with isocyante in refluxing ethanol under uniform stirring for 4 h. Further the final intermediate, 4-alkyl/aryl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (**4a-c**) was obtained in better yield when the cyclization reaction performed between compound **3** and sodium hydroxide solution on steady stirring for 3 h. Finally the target compounds, 2-methyl-5-(4-alkyl/aryl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (**5a-c**) have been achieved in good to excellent yields from the reaction occurred between compound **4** with methyl iodide and sodium hydroxide solution on steady stirring for 3 h. The chemical structures of all the intermediates and products are readily identified from their IR, ^{1}H NMR, mass spectroscopy and elemental analysis. Eventually, the target compounds were evaluated for their antimicrobial activity.



Scheme-I: (i) $NH_2NH_2.H_2O$, EtOH, reflux, 9 h; (ii) RNCS, EtOH, reflux, 4 h; (iii) NaOH, reflux, 3 h; (iv) CH_3I, NaOH, reflux, 3h. **3-5 R** a) = CH_3, b) = CH_2CH_3 c) = C_6H_5

Antimicrobial activity: The antimicrobial activity of the newly prepared 4-alkyl/aryl-5-(5-methyl-pyrazin-2-yl)-2,4-dihydro-[1,2,4]-triazole-3-thione (**4a-c**) and 2-methyl-5-(4-alkyl/aryl-5-methylsulfanyl-4*H*-[1,2,4]-triazol-3-yl)-pyrazine (**5a-c**) has been carried out with cup plate method [8] using nutrient agar medium against four bacterial strains such as *Stapylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aureginosa* and towards one fungal organism like *Candida albicans*. Ampicilline and fluoconazole were used as reference drugs for antibacterial and antifungal study respectively. DMSO was used as sample solution and the size of sample of all compounds was fixed at 0.1 mL and the concentration is restricted at 100 µg/mL. The test compound solution (0.1 mL) was added in the cups and the petri dishes were subsequently incubated at 37 °C for 48 h. Zone of inhibition produced by each compound was measured in mm and the results are listed in Table-1. According to the results, compounds **4b, 5a** and **5b** are highly active against all types of tested bacteria. Compounds like **4a** and **5b** are also highly active against *C. albicans*. Compounds **4a** and **4c** are highly active against *S. pyogenes* and *E. coli* while compound **5c** is highly active against *S. pyogenes* and *P. aeruginosa*. The rest of products were found to be moderately active against the tested organisms. It is interesting to note that, none of the compound is inactive towards any microorganism and this outstanding property may be obtained to the target compound by incorporating pyrazine rings into 1,2,4-triazole moiety.

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