



Synthesis and Antimicrobial Activity Analysis of Substituted Thiadiazepines

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ABSTRACT

Several 7-methyl-3-substitutedphenyl-9- (pyridin-2-yl)-7, 8, 9, 9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4) triazolo (3,4-b) (1,3,4)-thiadiazepines 4 (a-f) have been synthesised by using conventional methodology. The synthesized derivatives were characterized by IR, ¹H-NMR, Mass and elemental analysis (C, H, N). Furthermore the synthesized substituted thiadiazepines 4(a-f) were biologically evaluated for antimicrobial activities

Keywords: Antibacterial, antifungal, thiazolidin-4-one, acute toxicity.

INTRODUCTION

Sulphur linkage bearing compounds viz. thiols (RSH) and thioethers (R-S-R') possessed better biological approach [1]. Literature study explored diverse biological activities of 1,2,4-triazoles ring possessing systems viz. antiseptic, analgesic, anti-convulsant [2-13], antibiotic [2], antiallergic [2], anti-inflammatory [2-11,14], diuretic [2,6,9], fungicidal [4,5,11-14], insecticidal [4,11,14], herbicidal [4,11,14], antibacterial [4-7,12,13], antiviral [3-6,8,9,11], antidepressant [3,6,10], antimicrobial [3-6,8,11-13], antitumor [4,7,10,11], antihypertensive [6,9,10], and antimigraine compounds [8]. In addition several well known substituted triazole group bearing drugs as are under therapeutic uses for various disease anastrozole, rizatriptan, nefazodone, vorozole, ribavirin, fluconazole, letrozole and uniconazole. On the other hand, substituted pyrazoles have been reported to possess anti-inflammatory [15], antibacterial [16-17], anti-convulsant [18], anticancer [19-20], anti-depressant [21], anti-hyperglycemic [22], antiviral [23], antipyretic [24], antioxidant [25], antitubercular [26], fungicides [27] activities. Herein we presented an synthetic approach to design several 7-methyl-3-substitutedphenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo(4,3-f)(1,2,4)triazolo(3,4-b)(1,3,4) thiadiazepines 4(a-f) and their antimicrobial testing.

RESULTS AND DISCUSSION

The synthetic work is outlined in scheme-1. 3-Aryl-4-amino-5-mercapto triazoles 3(a-f) were prepared according to the reported method [23-26]. Ethyl acetoacetate and 2-hydrazinylpyridine undergo cyclo-condensation reaction to furnish 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1) which on chloroformylated yielded 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (2). Condensation reaction of 3-phenyl-4-amino-5-mercapto triazoles 3(a-f) with

5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde in catalytic presence of p-toluene sulphonic acid afforded the target compounds

7-methyl-3-substitutedphenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4)triazolo (3,4-b) (1,3,4)-thiadiazepines 4(a-f) (Scheme-1).

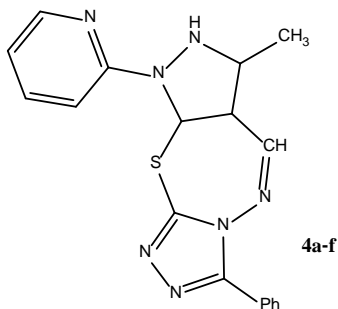
ANTIMICROBIAL TEST

All the newly synthesized compounds were screened for their antibacterial and antifungal activity. All the bacterial and fungal strains were clinical isolates, identified with conventional morphological and biochemical methods. Microorganisms employed antibacterial studies were *Staphylococcus aureus*, *Escherichia coli*, *Klasiella pneumoniae* and *Proteus vulgaris*. Disk diffusion method [30-31] was used for determination of the preliminary antibacterial activity. Disks measuring 6.25 mm in diameter were punched from Whatman no. 1 filter paper. Batches of 100 disks were dispensed to each screw-capped bottle and sterilized by dry heat at 140 °C for an h. The test compounds were prepared with different concentrations using DMF. One milliliter containing 100 times the amount of chemical in each disk was added to each bottle, which contained 100 disks. Disks of each concentration were for placed in triplicate in nutrient agar medium seeded with fresh bacteria separately. The incubation was carried out at 37 °C for 24 h. Ampicillin trihydrate was used as a standard drug. Solvent and growth controls were kept and zones of inhibition were noted. The inhibition zone values of the tested compounds against the tested bacteria strains summarized in Table 1. On the other hand, the newly prepared compounds were screened for their in vitro antifungal activity against *Aspergillus fumigates* (plant isolate), *Candida glabrata*, *Candida albicans* and *Candida krusei* in DMSO by the serial plate dilution method [32-33]. Fluconazole (antifungal) was used as reference drug. Sabouraud's agar media were prepared by dissolving peptone (1

g), D-glucose (4 g), and agar (2 g) in distilled water (100 ml) and adjusting the pH to 5.7. Normal saline was used to make a suspension of the spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 ml saline to get a suspension of the corresponding species. Agar media (20 ml) was poured into each petri dish. Excess suspension was decanted and the plates were dried by placing in an incubator at 37 °C for 1 h.

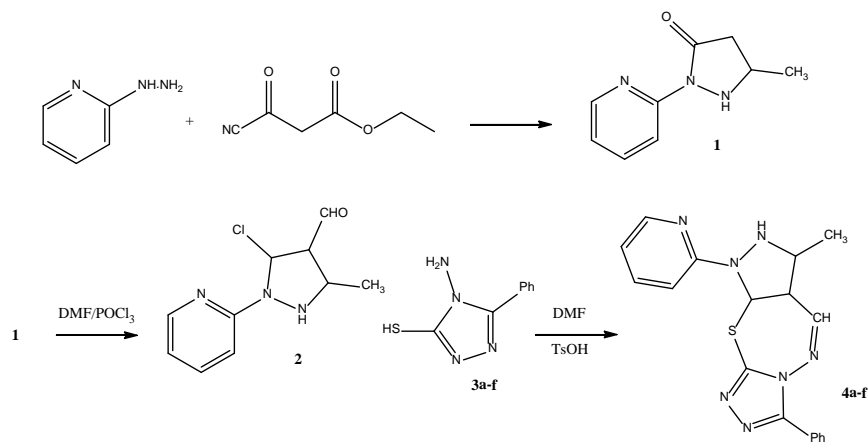
Using an agar punch wells were made into each well labelled. A control was also prepared in triplicate and maintained at 37 °C for 3-4 days. Antifungal activity was determined by measuring the diameter of the inhibition zone. The inhibitory data of the tested compounds against the tested fungal strains were recorded in Table 1.

Table 1: Antimicrobial evaluation of 7-methyl-3-substitutedphenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4)triazolo(3,4-b) (1,3,4)-thiadiazepines (4a-f).



Compounds	Antibacterial inhibition (mm)				Antifungal inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>A. fumigatus</i>	<i>A. niger</i>	<i>C. albicans</i>	<i>C. krusei</i>
@ Control	-	-	-	-	-	-	-	-
Ampicillin trihydrate	16	16	18	20	-	-	-	-
Fluconazole	-	-	-	-	29	25	15	-
4a.	-	-	-	-	05	05	-	-
4b.	10	14	06	08	-	-	-	05
4c.	15	-	10		10	10	06	08
4d.	10	10	-	08	10	12	08	-
4e.	12	-	-	-	-	10	-	08
4f.	15	12	20	22	14	20	8	10

- indicates no activity



Ph = C₆H₅, 2-OH.C₆H₄, 4-OH.C₆H₄, 4-C₂H₅O.C₆H₄, 4-Cl.C₆H₄

Scheme-1

EXPERIMENTAL

All the chemicals used for the preparation of desired derivatives, were obtained from Sisco Research Laboratories (SRL), Mumbai, India; Qualigen Fine Chemicals, Mumbai, India; E. Merck Ltd., New Delhi, India. The reference drugs Ampicillin trihydrate and fluconazole were procured from Ind-Swift, Pharmaceutical, Punjab, India and Dr. Reddy Lab., Hyderabad, India. The melting points of the compounds were determined in open glass capillaries with the help of thermionic melting points apparatus (Campbell Electronics, Mumbai, India) and are uncorrected. The homogeneity of all the newly synthesized compounds were routinely checked by TLC on silica gel G plates and spots were located by using iodine chamber. Elemental analysis was performed in Heraeus CHN rapid analyzer. The results were found within the $\pm 0.4\%$ of theoretical values. Infrared spectra were recorded on KBr pellets on a Perkin Elmer system 2000 FTIR spectrometer and $^1\text{H-NMR}$ spectra on Bruker DPX 200 using TMS as internal standard.

PROCEDURE

General method of preparation of 3-Methyl-1-phenyl-1H-pyrazol-5(4H)-one 1.

An ethanolic solution of the ethyl acetoacetate (0.001 mol) and 2-hydrazinylpyridine (0.001 mol) was stirred at room temperature for 1 hr. The resulting reaction mixture was refluxed for 6 h. On completion of reaction, the reaction mixture was allowed to cool in ice bath to precipitate the solid. The solid filtered, dried and recrystallized from ethanol to give compound 1. Yield: 77%, R_f: 0.68. m.p. 127–129 °C. Anal. Calcd. For C₆H₁₁N₃O: C, 61.00; H, 6.26; N, 23.71%. Found: C, 60.91; H, 6.25; N, 23.76%. IR (KBr, cm⁻¹): 3170 (NH), 3140 (C...H aromatic), 2960 (C-H aliphatic), 1680 (C=O), 1611 (C...C of aromatic ring), 1572 (C=N), 1280 (N-N), 1231 (C-N). $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 6.86-7.20 (m, 4H, Ar-H), 6.14 (brs, 1H, NH exchangeable with D₂O), 5.20 (s, 1H, CH of pyrazole ring), 4.45 (d, 2H, CH₂ of pyrazole ring), 1.12 (s, 3H, -CH₃). MS (m/z): 177.09 (M)⁺.

Preparation of 5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde 2.

Dimethylformamide solution of compound 1 (0.001 mol) cooled and followed by the dropwise addition of phosphorus oxychloride (0.002 mol). After completion of the addition the resulting mixture was refluxed for 2 h. On completion of the reaction, the reaction mixture was cooled, poured into crushed ice-water, filtered, dried and recrystallised from ethanol to furnish yellowish brown solid compound 2. Yield: 75%, m.p. 142–144 °C. Anal. Calcd. For C₁₀H₁₂N₃OCl: C, 53.22; H, 5.36; N, 18.62%. Found: C, 53.29; H, 5.37; N, 18.60%. IR (KBr, cm⁻¹): 3162, 3146, 2964, 1688, 1620, 1565, 1277, 1237. $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 9.70 (s, 1H), 6.80-7.15 (m, 4H), 6.10 (brs, 1H), 4.15-5.13 (m, 3H), 1.03 (s, 3H). MS (m/z): 225.07 (M)⁺.

General method of synthesis of 7-methyl-3-substitutedphenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4)triazolo(3,4-b) (1,3,4)-thiadiazepines 4(a-f).

The solution of compound 3(a-f) (0.001 mol) in n-butanol was refluxed with 5-chloro-3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (0.001

mol) for 2-3 h. Excess of solvent was distilled off and the reaction mixture thus obtained was cooled, poured into ice cold water, filtered, dried, triturated with petroleum ether (40-60 °C) and recrystallised with appropriate solvents to furnish the products 4(a-f).

7-Methyl-3-phenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4) triazolo(3,4-b) (1,3,4)-thiadiazepines 4a. Yield: 62%, m.p. 119–121 °C. Anal. Calcd. For C₁₈H₁₇N₇S: C, 59.49; H, 4.71; N, 26.98%. Found: C, 59.42; H, 4.82; N, 27.00%. IR (KBr, cm⁻¹): 3156, 3130, 2961, 1627, 1558, 1270, 1223, 670. $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 6.80-7.40 (m, 9H, Ar-H), 6.22 (s, 1H), 5.65 (brs, 1H), 4.10-4.75 (m, 3H), 1.03 (s, 3H). MS (m/z): 363.44 (M)⁺.

7-Methyl-3-(2-hydroxy)phenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4)triazolo (3,4-b) (1,3,4)-thiadiazepines 4b. Yield: 56%, m.p. 143–145 °C. Anal. Calcd. For C₁₈H₁₇N₇SO: C, 56.98; H, 4.52; N, 25.84%. Found: C, 57.12; H, 4.50; N, 26.07%. IR (KBr, cm⁻¹): 3161, 3133, 2965, 1622, 1562, 1273, 1222, 675. $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 12.45 (s, 1H), 6.68-7.36 (m, 8H), 6.26 (s, 1H), 5.60 (brs, 1H), 4.15-4.70 (m, 3H), 1.10 (s, 3H). MS (m/z): 379.44 (M)⁺.

7-Methyl-3-(3-hydroxy)phenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4)triazolo (3,4-b) (1,3,4)-thiadiazepines 4c. Yield: 52%, m.p. 155–157 °C. Anal. Calcd. For C₁₈H₁₇N₇SO: C, 56.98; H, 4.52; N, 25.84%. Found: C, 57.07; H, 4.58; N, 25.92%. IR (KBr, cm⁻¹): 3158, 3130, 2962, 1625, 1566, 1276, 1224, 671. $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 12.52 (s, 1H), 6.72-7.41 (m, 8H), 6.20 (s, 1H), 5.73 (brs, 1H), 4.11-4.67 (m, 3H), 1.06 (s, 3H). MS (m/z): 379.44 (M)⁺.

7-Methyl-3-(4-hydroxy)phenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4)triazolo (3,4-b) (1,3,4)-thiadiazepines 4d. Yield: 60%, m.p. 126–128 °C. Anal. Calcd. For C₁₈H₁₇N₇SO: C, 56.98; H, 4.52; N, 25.84%. Found: C, 56.86; H, 4.49; N, 26.00. IR (KBr, cm⁻¹): 3163, 3136, 2965, 1634, 1570, 1270, 1229, 678. $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 12.40 (s, 1H), 6.70-7.48 (m, 8H), 6.15 (s, 1H), 5.60 (brs, 1H), 4.15-4.69 (m, 3H), 1.02 (s, 3H). MS (m/z): 379.44 (M)⁺.

7-Methyl-3-phenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4) triazolo(3,4-b) (1,3,4)-thiadiazepines 4e. Yield: 63%, m.p. 169–171 °C. Anal. Calcd. For C₂₀H₂₁N₇SO: C, 58.95; H, 5.19; N, 24.06%. Found: C, 59.10; H, 5.12; N, 24.17%. IR (KBr, cm⁻¹): 3165, 3130, 2959, 1620, 1569, 1271, 1220, 669. $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 6.65-7.32 (m, 8H), 6.32 (s, 1H), 5.65 (brs, 1H), 4.20-4.73 (m, 3H), 3.60 (q, 2H), 1.65 (d, 3H), 1.00 (s, 3H). MS (m/z): 407.49 (M)⁺.

7-Methyl-3-phenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4) triazolo(3,4-b) (1,3,4)-thiadiazepines 4f. Yield: 49%, m.p. 131–133 °C. Anal. Calcd. For C₁₈H₁₆N₇SCl: C, 54.34; H, 4.05; N, 24.64%. Found: C, 54.40; H, 4.02; N, 24.70%. IR (KBr, cm⁻¹): 3159, 3134, 2962, 1629, 1566, 1267, 1228, 674. $^1\text{H-NMR}$ (DMSO-*d*₆ /ppm): 6.80-7.37 (m, 8H, Ar-H), 6.25 (s, 1H), 5.76 (brs, 1H), 4.14-4.69 (m, 3H), 1.08 (s, 3H). MS (m/z): 397.88 (M)⁺.

CONCLUSION

In the present study, our attention was focused on the synthesis and antimicrobial evaluation of a series of 7-methyl-3-substitutedphenyl-9-(pyridin-2-yl)-7,8,9,9a-tetrahydro-6aH-pyrazolo (4,3-f) (1,2,4)triazolo(3,4-b)

(1,3,4)-thiadiazepines **4(a-f)**. Based on the resulting biological evaluation data, all the compound showed mild to moderate antimicrobial potential. Among all the synthesized thiadiazepines, compound **4f** possessing 3-chlorophenyl substitution showed broad antimicrobial activity against the all tested microbes.

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