SYNTHESIS AND IN VITRO ANTIMICROBIAL ACTIVITY OF SOME NEW 1-THIAZOLYL-2-PYRAZOLINE DERIVATIVES

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ABSTRACT:

In this present study, some new 1-thiazolyl-2-pyrazoline derivatives were prepared by the base catalyzed condensation of 4-(2'-hydroxy-5'-chlorophenyl)-2-hydrazino-thiazole and pyrazole containing chalcones in polyethylene glycol (PEG-400) as a green reaction medium. All the synthesized compounds were tested for their antimicrobial activities. Most of the compounds showed very good antibacterial and antifungal activity.

Keywords: 1-thizolyl-2-pyrazoline; Polyethylene glycol-400; Antimicrobial activity

INTRODUCTION

Resistance to antibacterial agents is a significant problem since last three decades.[1,2] This emerging resistance has resulted in the development of a wide variety of antibiotics. The pharmaceutical industry rapidly took advantage of the wealth of novel targets available as a result of genomic revolution. Despite the expectation that these new targets would decrease the hurdle in identifying novel classes of antimicrobial agents, discovery of compounds that act via novel mechanisms remains a significant challenge. The major obstacle appears to be the identification of novel drug able chemical matter.[3] In addition, primary and opportunistic fungal infections continue to increase rapidly because of the increased number of immunocompromised patients (AIDS, cancer and transplants). Several reviews have appeared illustrating the problems encountered by today's infectious disease clinicians.[4-6]

To overcome this rapid development of drug resistance, new agents should preferably consist of chemical characteristics that clearly differ from those of existing agents. In the process of drug designing an essential component of the search for new leads is the synthesis of novel molecules, which are biologically active by the virtue of the presence of critical structural features. Electron-rich nitrogen heterocycles play an important role in diverse biological activities. Introducing a pyrazolidinone ring[7,8] in place of the β-lactam ring (in penicillins and cephalosporins[9] results in enhanced activity). A second nitrogen in the five-membered ring also influences the antibacterial or pharmacokinetic properties.[10,11] Pyrazoline derivatives have also been reported in the literature to exhibit various pharmacological activities such as antiinflammatory,[12] antihypertensive,[13] and antimicrobia.[14] On the other hand, sulfur and/or nitrogen heterocycles having pharmaceutical activities are widely occur in nature in the form of alkaloids, vitamins, pigments and as a constituents of plant and animal cells. Penicillins containing a thiazole ring system (thiazolidine)[15] are also important naturally occurring products. Thiazoles and their derivatives are found to possess various biological activities such as antituberculosis,[16] anti-HIV,[17] and antimicrobial.[18]

In view of above mentioned data of pyrazolines and thiazoles, we planned to synthesize a system like thiazolyl pyrazolines that contains two liable components of pyrazolines and thiazoles by applying the principles of green chemistry.[19]

Chemistry

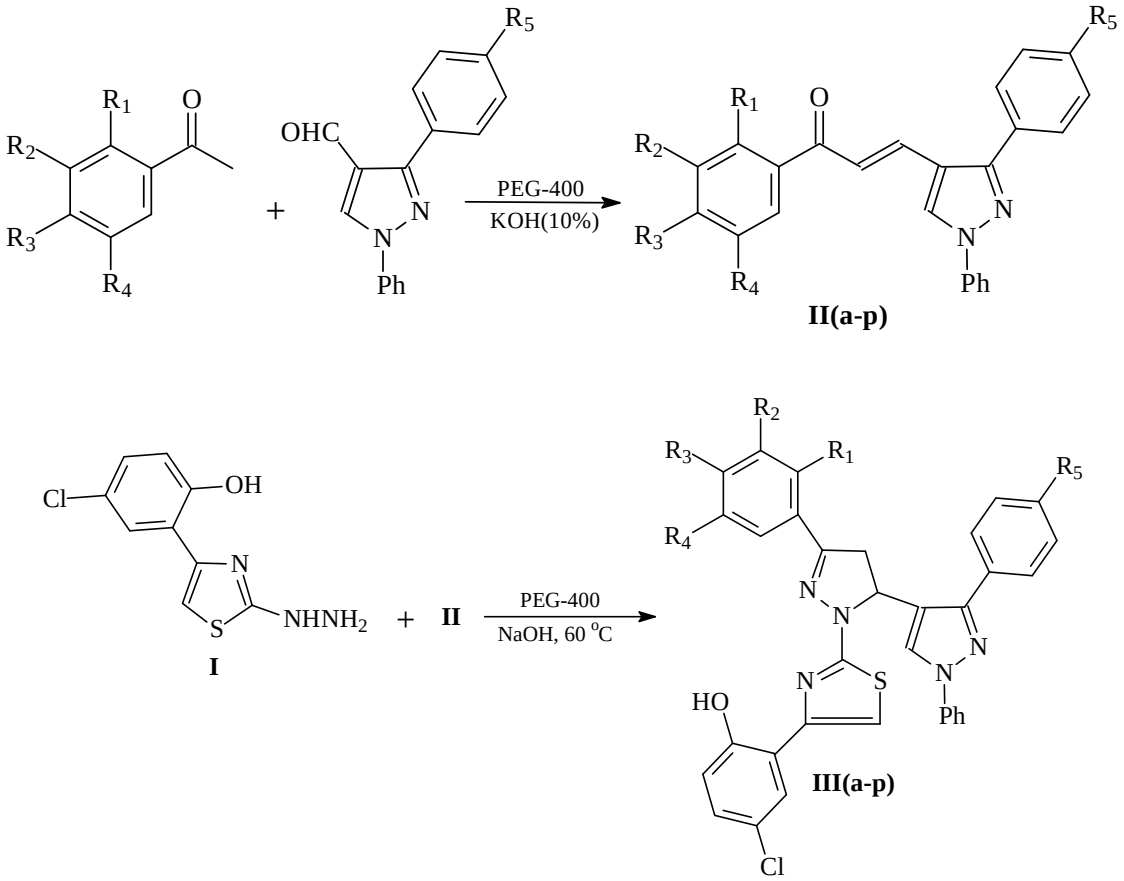
The synthetic route of compounds is presented in Scheme-1. Initially, the starting 4-(2'-hydroxy-5'-chlorophenyl)-2-hydrazino-thiazole (I) was prepared from the reaction of 2'-hydroxy-5'-chloro-α-haloketone and thiosemicarbazide in PEG-400 (10 mL) at 40 °C by reported method,[20] while novel chalcones were synthesized by the reported method.[21] Finally, the 1-thiazolyl-2-pyrazoline derivatives (IIIa-p) were prepared by condensation of 4-(2'-hydroxy-5'-chlorophenyl)-2-hydrazino-thiazole and chalcones (II) using solid NaOH in polyethylene glycol (PEG-400) as reaction solvent under mild reaction condition as shown in Scheme-1.

Structures of the all newly synthesized products were confirmed by the spectral and elemental analysis. The IR spectra of the products showed a characteristic band between 1590-1610 cm^{-1} referring to C=N double band between the N-2 and C-3 atoms of the pyrazoline ring. In the ^1H NMR spectra, HA, HB, HX protons of the pyrazoline ring wee seen as a doublet of doublets at about δ3.34-3.62, 3.92-4.21 and 5.23-5.71 ppm, respectively. The phenolic proton appeared as a singlet near δ11.0-13.0 ppm due to the hydrogen bonding, while other aromatic and aliphatic protons were observed at excepted regions. The mass spectra (EIMS) of compounds were also in agreement with their corresponding molecular formula.

Antimicrobial activity

The antimicrobial activities of the synthesized 1-thiazolyl-2-pyrazoline derivatives (**IIIa-p**) were determined by agar diffusion method.[22] The compounds were evaluated for antimicrobial activity against bacteria *viz. Escherichia coli* (MTCC 2939), *Salmonella typhi* (MTCC 98), *Proteus*

vulgaris (MTCC 1771), *Pseudomonas auriginosa* (MTCC 1688), *Staphylococus aureus* (MTCC 96), *Bacillus megaterium* (MTCC 1684), *Bacillus subtilis* (MTCC 441) and *Serratia marcescens* (MTCC 97) and antifungal activity against various fungi *viz. Aspergillus niger* (MTCC 281), *Trichoderma viridae* (MTCC 167), *Penicillium chrysogenum* (MTCC 160), *Microsporum cannis* (MTCC 2820), *Candida albicans* (MTCC 183), *Fusarium moniliformc* (MTCC 156). The antibiotic Tetracycline and Nystatin are used as reference antibacterial and antifungal substances, respectively under similar conditions for comparison. Dimethyl sulphoxide (1%, DMSO) was used a control. The minimum inhibitory concentration (MIC) value was determined at a concentration of 25 μg/mL, using dimethyl sulfoxide (DMSO) as solvent against bacteria as well as fungal strains.



Scheme-1: Synthesis of chalcones (II) and 1-thiazolyl-2-pyrazolines (III)

The culture strains of bacteria were maintained on nutrient agar slant at 37±0.5°C for 24 h. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 10^5 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of target compound dilution ranging from 25 to 1000 μg/mL separately for each bacterial strain. All the plates were incubated at 37±0.5°C for 24 h.

For antifungal activity, all the culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27±0.2°C for 24-48 hrs, till sporulation. Spore of strains were transferred in to 5 mL of sterile distilled water containing 1% Tween-80 (to suspend the spore properly). The spores were counted by haemocytometer (10^6 CFU/mL). Sterile PDA plate was prepared containing 2% agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27±0.2 °C for 12 hrs. After incubation well prepared using sterile cork borer and each agar well was filled with 0.1 mL compound solution of concentrations 25 to 1000 μg/mL separately to get minimum inhibitory concentration value of 1-thiazolyl-2-pyrazoline derivatives. The plates were kept in refrigerator for 20 minutes for diffusion and then incubated at 27±0.2 °C for 24-28 hrs. The results of antifungal studies are given in Table-3.

The results of antibacterial studies are given in Table-2. Compound **IIIa** showed maximum activity against *Escherichia Coli*, *Staphylocous aureus* and *Bacillus megaterium*, where as the substitution of hydroxyl group in position 2 of compound (**IIIg**) increases its activity against *Proteus vulgaris*. Moreover, compound **IIIh** also showed significant antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, *Psendomonas auriginosa*, *Bacillus megaterium*. Compound **IIIo** was active maximally against *Escherichia coli*, *Salmonella typhi* and *Proteus vulgaris*. Compound **IIIp** showed maximum activity against *Proteus vulgaris*,

Escherichia coli, *Pseudomonas auriginosa*, *Bacillus subtilis*, and *Serratia marcescens*.

Table-1: Synthesis of some new 1-thiazolyl-2-pyrazolines using PEG- 400

Product	R_1	R_2	R_3	R_4	R_5	Time[a]	Yield(%)[b]	M.P.(°C)
IIIa	OH	H	H	Cl	OH	36	89	158
IIIb	OH	Br	H	Cl	OH	25	90	165
IIIc	OH	I	H	Cl	OH	35	88	142
IIId	OH	H	Cl	H	OH	30	90	175
IIIe	OH	H	CH_3	Cl	OH	30	88	128
IIIf	OH	I	CH_3	Cl	OH	35	88	151
IIIg	OH	H	OH	H	OH	35	88	144
IIIh	OH	Cl	OH	Cl	OH	25	89	168
IIIi	OH	H	H	Cl	Cl	30	86	135
IIIj	OH	Br	H	Cl	Cl	35	88	168
IIIk	OH	I	H	Cl	Cl	35	88	182
IIIl	OH	H	Cl	H	Cl	40	89	140
IIIm	OH	H	CH_3	Cl	Cl	35	88	126
IIIn	OH	I	CH_3	Cl	Cl	35	88	172
IIIo	OH	H	OH	H	Cl	30	86	152
IIIp	OH	Cl	OH	Cl	Cl	35	90	180

[a] Time in minutes, [b] Pure isolated yields of products.

Table-2: Antibacterial activities of synthesized compounds (**IIIa-p**)

Compound	Bacteria (zone of inhibition in mm)							
	Ec	St	Pr	Pa	Sa	Bm	Bs	Sm
IIIa	20	10	—	12	22	18	—	12
IIIb	12	—	15	10	—	15	10	16
IIIc	18	12	—	12	18	20	10	10
IIId	—	—	—	—	10	—	—	—
IIIe	—	—	10	10	12	18	—	—
IIIf	20	—	12	15	18	—	10	10
IIIg	18	16	22	18	16	14	12	18
IIIh	20	20	18	20	10	18	16	16
IIIk	08	—	16	15	18	—	13	—
IIIn	10	10	12	—	—	—	10	—
IIIo	18	18	20	16	14	16	12	12
IIIp	20	12	22	18	20	20	18	18
Control	—	—	—	—	—	—	—	—
Tetracycline	—	32	25	33	34	27	29	20

Ec = *Escherichia coli* St = *Salmonella typhi* Pr = *Proteus vulgaris*
Pa = *Pseudomonas aeruginosa* Sa = *Staphylococus aureus*
Bm = *Bacillus subtillis* Sm = *Serratia marcescens*
— = Not detected

Table-3: Antifungal activities of synthesized compounds (**IIIa-p**)

Compound	Fungi germination (zone of inhibition in mm)					
	An	Tv	Pc	Mc	Ca	Fm
IIIa	16	20	16	15	18	12
IIIb	10	12	10	08	16	—
IIIc	12	14	—	12	12	—
IIIe	—	12	15	10	16	14
IIIf	10	—	—	10	12	12
IIIg	20	22	16	18	18	16
IIIh	18	20	15	16	20	18
IIIk	10	08	12	10	12	10
IIIn	15	12	16	12	—	—
IIIo	20	18	22	16	18	16
IIIp	22	24	20	18	18	16
Control	—	—	—	—	—	—
Nystatin	14	18	17	10	17	10

An = *Aspergillus niger* Tv = *Trichoderma viridae*
Pc = *Penicillium chrysogenium* Mc = *Microsporum cannis*
Ca = *Candida albicans* Fm = *Fusarium moniliforme*
— = Not detected

The results of in vitro antifungal activities are summarized Table 3. Compounds IIIa, IIIg, IIIh, IIIn, IIIo, and IIIp exhibited equal or stronger antifungal activities against all tested fungi viz. *Aspergillus niger*, *Trichoderma viridae*, *Penicillium chrysogenum*, *Microsporum cannis*, *Candida albicans*, *Fusarium moniliformc* than that of standard drug nystatin. The antifungal activities of IIIb, IIIc, IIIf, and IIIk were lower than that of Nystatin. Considering the results obtained from antifungal and antibacterial tests together, it is noteworthy to mention that tested compounds are more active towards fungi than bacteria.

In conclusion, we have prepared some new 1-thiazolyl-2-pyrazoline derivatives under environmentally benign conditions and their in vitro antimicrobial activities were evaluated. Compounds IIIa, IIIg, IIIh, IIIn, IIIo, and IIIp were identified as promising leads for antifungal activities.

Experimental

Melting points were determined by in an open capillary method and are uncorrected. The chemicals and solvents used for laboratory grade and were purified. IR spectra were recorded (in KBr pallets) on Shimadzu spectrophotometer. ^{1}H NMR spectra were recorded (in DMSO-d_6) on Avance-300 MHz spectrometer using TMS as an internal standard. The mass were recorded on EI-Shimadzu-GC-MS spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer.

General procedure for the synthesis of chalcones (IIa-p):[20]

A mixture of substituted acetophenone (1 mmol), 1-phenyl-3-(4-sustituted phenyl) pyrazol-4-carboxaldehyde (1 mmol), KOH (2 mmol) and PEG-400 (10 mL) was stirred at 40°C for 1 hr. After completion of reaction

(TLC), the reaction mixture was cooled and poured into ice cold water (100 mL). The obtained solid product was filtered and washed with 2 x 5 mL water and recrystallized by aqueous acetic acid to give corresponding product.

(IIa): MP: 204 ^{o}C; IR (KBr): 3284, 3070, 1639, 1591 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.01 - 8.02 (m, 13H, Ar-H + CH=CH), 9.11 (s, 1H, 5H of pyrazole), 10.25 (s, 1H, OH), 12.21 (s, 1H, OH); EIMS: m/z = 416 (M^+).

Typical procedure for the synthesis of 1- thiazolyl-2-pyrazoline (IIIa-p):

A mixture of chalcone **(II)** (1 mmol), 4-(2'-hydroxy-5'-chlorophenyl)-2-hydrazino-thiazole **(I)** (1 mmol), NaOH (1.5 mmol) and PEG-400 (10 mL) was stirred at room temperature for 5 minutes and then temperature raised to 60 ^{o}C for the appropriate time (Table-1). After completion of reaction (monitored by TLC), the reaction mixture was cooled and poured into ice-cold water (100 mL). The obtained solid product was filtered and washed with 2 x 5 mL water and recrystallized by aqueous acetic acid to give pure product. The PEG-400 was recovered from water by direct distillation and reused for second run by charging the same substrates.

IIIa: IR (KBr): 3285, 3156, 1602 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.40 (m, 1H, H_A), 4.22 (dd, 1H, H_B), 5.60 (t, 1H, H_X), 6.80-8.00 (m, 16H, Ar-H), 8.61 (s, 1H, 5H of pyrazole), 10.28 (s, 1H, Ar-OH), 10.91 (s, 1H, Ar-OH); EMIS (m/z): 639 (M^+), 641 (M+2), 643 (M+4); Anal Calcd for $C_{33}H_{23}O_3N_5Cl_2S$: C, 61.88; H, 3.62; N, 10.93; Found: C, 61.96; H, 3.68; N, 10.98.

IIIb: IR (KBr): 3320, 3168, 1605 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.45 (m, 1H, H_A), 4.31 (dd, 1H, H_B), 5.48 (t, 1H, H_X), 6.95-8.12 (m, 15H, Ar-H), 8.52 (s, 1H, 5H of pyrazole), 10.34 (s, 1H, Ar-OH), 11.26 (s, 1H, Ar-OH); Anal Calcd for $C_{33}H_{22}O_3N_5Cl_2SBr$: C, 55.09; H, 3.08; N, 9.73; Found: C, 55.16; H, 3.14; N, 9.61.

IIIi: IR (KBr): 3196, 1599 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.38 (m, 1H, H_A), 4.18 (dd, 1H, H_B), 5.54 (t, 1H, H_X), 7.08-8.15 (m, 16H, Ar-H), 8.58 (s, 1H, 5H of pyrazole), 10.98 (s, 1H, Ar-OH); Anal Calcd for $C_{33}H_{22}O_2N_5Cl_3S$: C, 60.15; H, 3.36; N, 10.63; Found: C, 60.22; H, 3.31; N, 10.72.

IIIm: IR (KBr): 3225, 1605 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.32 (m, 1H, H_A), 4.25 (dd, 1H, H_B), 5.46 (t, 1H, H_X), 6.92-8.05 (m, 15H, Ar-H), 8.46 (s, 1H, 5H of pyrazole), 11.21 (s, 1H, Ar-OH); Anal Calcd for $C_{34}H_{24}O_2N_5Cl_3S$: C, 60.68; H, 3.59; N, 10.41; Found: C, 60.81; H, 3.65; N, 10.52.

IIIn: IR (KBr): 3268, 1602 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.42 (m, 1H, H_A), 4.32 (dd, 1H, H_B), 5.38 (t, 1H, H_X), 7.12-8.16 (m, 14H, Ar-H), 8.52 (s, 1H, 5H of pyrazole), 11.82 (s, 1H, Ar-OH); Anal Calcd for $C_{34}H_{23}O_2N_5Cl_3S$: C, 51.92; H, 2.91; N, 15.85; Found: C, 51.98; H, 2.96; N, 15.72.

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