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Synthesis of a series of pyrazoline derivatives carrying tosyl substitution and evaluation of their antimicrobial properties

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ABSTRACT

A series of pyrazoline derivatives with tosyloxy substitution were synthesized and characterized. They were evaluated for antimicrobial potential against *Mycobacterium smegmatis*, gram positive *Staphylococcus aureus*, gram negative *Escherichia coli*, and a fungi *Candida albicans*. The initial screening using zones of inhibition at 50µL concentrations revealed that they are moderately active against the tested strains, and therefore further studies have not been carried out.

INTRODUCTION

The antimicrobial agents with novel structural characteristics are increasingly in demand due to antimicrobial resistanceto the prescribed drugs (Bogatcheva *et al.*, 2006).Various strategies are adopted to find newer drugs among them are synthesis of small chemical entities, complex molecular structures, and metal complexes of organic ligands or isolation of structural lead from natural plant products or from marine resources (Muller, 2003).

Other strategies are to synthesize novel molecules based on hybrid pharmacophores, where two or more therapeutically useful structural units are combined with appropriate chemical linkages (Kalaria *et al.*, 2014). Pyrazoline compounds are having significant biological activities as they are structurally related to the pyrazolone family of drugs. Among isomeric pyrazoline derivatives, 1,2-pyrazolines are the most thermodynamically stable and therefore are explored widely for a range of biological applications, including antitubercular, antioxidant, anticancer and as antidepressant agents. Due to their biological significance, a variety of structural modifications have been achieved around pyrazoline ring (Ghorab *et al.*, 2010; Rostom *et al.*, 2003; Yeu *et al.*, 2001; Amnerkar *et al.*, 2010). We have recently explored the synthesis and antimicrobial activity of a series of compounds having aryloxy substituents attached to pyrazoline ring providing moderate antimicrobial action.

Introduction of tosyl group at an aromatic ring gives new molecules that can be utilized to probe their biological potential as well as further derivatization of such molecules are possible as tosyl group is easily replaceable with many nucleophiles. In one example, we have described the synthesis and antimicrobial properties of newer pyrimidinethione derivatives possessing tosyl substituents (Shubhalaxmi *et al.*, 2016).In this paper, we report the synthesis, characterization and antimicrobial screening of a series of pyrazoline derivatives.

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MATERIALS AND METHODS

All reagents were used as procured from commercial suppliers and used without further purification. The melting points were determined in open capillaries, using Thomas Hoover melting point apparatus and expressed in °C. The reactions were monitored using TLC for completion and compounds were checked for purity by TLC on silica gel-G (Merck grade). Infrared spectra (IR) were recorded on Schimadzu 8400S Infrared Spectrophotometer using potassium bromide (KBr) pellets and the values are expressed in cm^{-1}. The ^1H NMR spectra of the compounds were recorded on Bruker Ascend 400 MHz NMR spectrophotometer using TMS as an internal standard and the values are expressed in δ ppm.

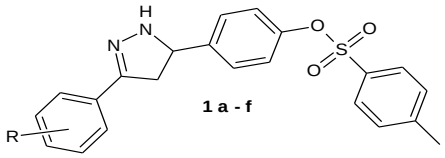
EXPERIMENTAL

Procedure for the synthesis of chalcones

The required chalcones were synthesized by aldol condensation of the corresponding aldehydes and ketones. The detailed synthetic procedure and their characterization data were given in our earlier publications (Shubhalaxmi *et al*., 2016; Shubhalaxmi, Manjunatha *et al*., 2016)

Procedure for the synthesis of pyrazoline derivatives (1a-f, 2 a-f)

The various pyrazoline derivatives are synthesized by reacting chalcone derivatives with excess of hydrazine hydrate in presence of catalytic amount glacial acetic acid for 3-6 hours at 70 -80^{0}C.The formation of the products were identified by thin layer chromatography and completion of the reaction is monitored. The products were recovered by pouring the reaction mixture into ice cold water or in few cases crystals were developed after keeping the mother liquor overnight. The products were isolated by filtration, dried and recrystallized from suitable solvent. The R_f values were reported using mobile phase of hexane: ethylacetate (2:0.9).



R = 1a: 4-H; 1b: 4-Br; 1c: 2 Br; 1d: 4-OCH_3; 1e: 4-Br-2-CH_3; 1f: 4-Br-2-OCH_3.

R = 2a: 4-H; 2b: 4-Cl; 2c: 4-NO_2; 2d: 4-OCH_3; 2e: 3,4-$(OCH_3)_2$; 2f: 2,5-$(OCH_3)_2$.

1a:4-(3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl) pheny l-4-methylbenzene-1-sulfonate: yield: 70 %, m.p.:98-100 ºC, R_f= 0.63; IR (cm^{-1}): 3340 (N-H str), 3070 (C-H str), 3039 and 2823 (asym, sym C-H str of CH_3), 1504 (C=N str), 1157 and 1200 (asym, sym SO_2str);^{1}H NMR (DMSO, δ ppm): 2.472 (s, 3H, CH_3), 2.76-2.83 (q, 1H, H of pyrazoline ring), 4.81-4.86 (t,2H, H of pyrazoline ring), 7.00 – 7.76 (m, 14H, aromatic H).

1b : 4-[3-(4-bromophenyl) -4,5-dihydro-1*H*-pyrazol-5-yl]phenyl-4-methylbenzene-1-sulfonate: yield - 63 %, m. p.: 82-84 ºC, R_f = 0.6;IR (cm^{-1}): 3355 (N-H str), 3062 (arC-H str), 2885 (C-H str) 1504 (C=N str), 1195 and 1157 (asym, sym SO_2str); ^{1}H-NMR (DMSO, δ ppm): 2.425 (s, 3H, CH_3), 2.51-3.359 (q, 1H, H of pyrazoline ring), 4.89 – 4.93 (t, 2H, H of pyrazoline ring), 7.00 – 7.76 (m, 1H, 13H, aromatic H).

1c: 4-[3-(2-bromophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]phenyl-4-methylbenzene-1-sulfonate: yield - 63 %, m. p. : 76-78 ºC, R_f = 0.6; IR(cm^{-1}): 3363 (N-H str), 3224 (C-H str), 2893 and 3062 (asym,symC-H str) 1504(C=N str), 1172 and 1149 (asym,sym SO_2str); ^{1}H- NMR (DMSO, δ ppm): 2.425 (s, 3H, CH_3), 2.51-3.359 (q, 1H, H of pyrazoline ring), 4.89 – 4.93 (t, 2H, H of pyrazoline ring), 7.00 – 7.76 (m,13H,aromatic H).

1d: 4-[3-(4-methoxyphenyl) -4,5-dihydro-1*H*-pyrazol-5-yl]phenyl-4-methylbenzene-1-sulfonate: yield - 59 %, m. p.: 68-70 ºC, R_f = 0.59;IR(cm^{-1}): 3340 (N-H str), 3047 (arC-H str), 2916 and 2839 (asym, sym C-H str) 1504 (C=N str), 1172 and 1149 (asym,sym SO_2str); ^{1}H- NMR (DMSO, δ ppm): 2.425 (s, 3H, CH_3), 2.51-3.359 (q,1H, H of pyrazoline ring), 4.28 (s, 3H, OCH_3), 4.89 – 4.93 (t, 2H, H of pyrazoline ring), 7.00 – 7.76 (m,13H,aromatic H).

1e: 4-[3-(2-bromo-4-methylphenyl) -4,5-dihydro-1*H*-pyrazol-5-yl]phenyl-4-methylbenzene-1-sulfonate:yield - 67 %, m. p. – 112 ºC, R_f = 0.6;IR (cm^{-1}): 3356 (N-H str), 3210 (C-H str), 2889 and 3060 (asym, sym C-H str) 1504(C=N str), 1172 and 1149 (asym, sym SO_2str); ^{1}H- NMR (DMSO, δ ppm):1.21 (s, 3H, CH_3), 2.425 (s, 3H, CH_3), 2.51-3.359 (q, 1H, H of pyrazoline ring), 4.89 – 4.93 (t, 2H, H of pyrazoline ring), 7.00 – 7.76 (m,12H, aromatic H).

1f: 4-[3-(2-bromo-4-methoxyphenyl) -4,5-dihydro-1*H*-pyrazol-5-yl]phenyl-4-methylbenzene-1-sulfonate:yield - 78 %, m. p. – 120 ºC, R_f =0.62;IR (cm^{-1}): 3340 (N-H str), 3047 (arC-H str), 2916 and 2839 (asym, sym C-H str) 1504 (C=N str), 1172 and 1149 (asym,sym SO_2str); ^{1}H- NMR (DMSO, δ ppm): 2.425 (s, 3H, CH_3), 2.51-3.359 (q,1H, H of pyrazoline ring), 4.312 (s, 3H, OCH_3), 4.89 – 4.93 (t, 2H, H of pyrazoline ring), 7.00 – 7.76 (m,12H,aromatic H).

2a:4-(5-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl) phenyl-4-methylbenzene-1-sulfonate: yield - 78 %, m. p. – 91-93 ºC, R_f = 0.45; IR(cm^{-1}): 3348 (N-H str), 3193 (ar C-H str), 2923 and 2854 (asym, sym C-H str) 1504 (C=N str), 1365 and 1203 (asym,sym SO_2str); ^{1}H- NMR (DMSO,δ ppm): 2.425 (s, 3H, CH_3), 2.78-2.84(q, 1H, H of pyrazoline ring), 4.82 – 4.87 (t,2H, H of pyrazoline ring), 7.01 – 7.76 (m, 14H,Ar-H).

2b: 4-[5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenyl-4-methylbenzene-1-sulfonate: yield - 59 %, m. p. – 96-98 ºC, R_f = 0.5; IR(cm^{-1}): 3362 (N-H str), 3055 (ar C-H str), 2939 and 2792 (asym,sym C-H str), 1504 (C=N str), 1342 and 1195 (asym, sym SO_2str); ^{1}H- NMR (DMSO, δ ppm): 2.402 (s, 3H, CH_3), 2.51-2.79 (q, 1H, H of pyrazoline ring), 3.739 (s, 3H, OCH_3), 4.81-4.83 (t, 2H, H of pyrazoline ring), 7.01- 7.75 (m, 13H, Ar-H).

2c. 4-[5-(4-nitrophenyl) -4,5-dihydro-1*H*-pyrazol-3-yl]phenyl-4-methylbenzene-1-sulfonate: M. W. – 437, yield - 61 %, m. p. – 120-122 ºC, R_f = 0.42; IR(cm^{-1}): 3355 (N-H str), 3078 (ar C-H str), 2846 and 2800 (asym, sym C-H str), 1519 (C=N stretching), 1365 and 1342 (asym, symSO2 str); ^{1}H- NMR (DMSO, 400 MHz, δ ppm): 2.421 (s, 3H, CH_3), 2.50-2.87 (q, 1H, H of pyrazoline ring), 5.00-5.03 (t, 2H, H of pyrazoline ring), 7.01-8.23 (m, 13H, Ar-H).

2d: 4-[5-(4-methoxyphenyl) -4,5-dihydro-1*H*-pyrazol-3-yl]phenyl-4-methylbenzene-1-sulfonate:yield - 42 %, m. p. – 153 ºC, R_f = 0.3;IR(KBr, cm^{-1}): 3362 (N-H str), 3055 (ar C-H str), 2939 and 2792 (asym,sym C-H str), 1504 (C=N str), 1342 and 1195 (asym, sym SO_2str); ^{1}H- NMR (DMSO, δ ppm): 2.402 (s, 3H, CH_3), 2.51-2.79 (q, 1H, H of pyrazoline ring), 3.739 (s, 3H, OCH_3), 4.81-4.83 (t, 2H, H of pyrazoline ring), 7.01- 7.75 (m, 13H, Ar-H).

2e: 4-[5-(3,4-dimethoxyphenyl) -4,5-dihydro-1*H*-pyrazol-3-yl]phenyl-4-methylbenzene-1-sulfonate:yield - 64 %, m. p. – 94-96 ºC, R_f = 0.3; IR(KBr, cm^{-1}): 3317 (N-H str), 2970 (arC-H str), 2846 and 2800 (asym, sym C-H str) 1592 (C=N str), 1255 and 1226 (asym,sym SO_2str); ^{1}H- NMR (DMSO, δ ppm): 2.503 (s, 3H, CH_3), 2.50-2.82 (q, 1H, H of pyrazoline ring), 3.351 (s,3H, OCH_3), 3.739 (s, 3H, OCH_3), 4.71-4.76 (t, 2H, H of pyrazoline ring), 6.82-8.25 (m, 12H, Ar-H).

2f: 4-[5-(2,5-dimethoxyphenyl) -4,5-dihydro-1*H*-pyrazol-3-yl]phenyl-4-methylbenzene-1-sulfonate:yield - 74 %, m. p. – 112-114 ºC, R_f = 0.3;IR(KBr,cm^{-1}): 3325 (N-H str), 2962 (arC-H str), 2862 and 2810 (asym, sym C-H str) 1596 (C=N str), 1256 and 1222 (asym,sym SO_2str); ^{1}H- NMR (DMSO, δ ppm): 2.508 (s, 3H, CH_3), 2.46-2.878 (q, 1H, H of pyrazoline ring), 3.351 (s,3H, OCH_3), 3.739 (s, 3H, OCH_3), 4.72-4.768 (t, 2H, H of pyrazoline ring), 6.82- 8.25 (m, 12H, Ar-H).

Antimicrobial Activity

The antimicrobial property of the synthesized compounds **1 a-f** and **2a-f** was evaluated by well diffusion method in nutrient agar media. Inhibition zones of the test compounds against microorganisms considered qualitatively suggest the antimicrobial potential (Sathish *et al.*, 2012). 12 h old bacterial culture of a tuberculosis variant bacteria *Mycobacterium smegmatis* (MTCC 944), gram positive bacteria *Staphylococcus aureus* (MTCC 3160), and gram negative bacteria *Escherichia coli* (MTCC 1687) were taken for the i*n vitro* antibacterial evaluation of compounds (Palomino *et al.*, 2002). Antifungal activity of the compounds were tested against pathogenic fungi *Candida albicans* (MTCC 7253). The bacterial and fungal cultures were obtained from the microbial type culture collection, IMTECH, Chandigarh, India and maintained the cultures as per the standard protocol. About 15-20 mL of nutrient agar media was poured into each petri plate and allowed to solidify for 15 minutes inside laminar air flow chamber. About 100 µL of 0.5 McFarland standard of bacterial/fungal suspension was inoculated on the agar media and spread on the whole surface by swabbing with sterile cotton buds. Than 5 mm wells were dig on the seeded agar plates with a sterile cork borer. Working solutions of the test compounds is prepared in DMSO at 10 mg/mL as stock and were poured at different concentrations (25 and 50 µg mL^{-1}) in to the wells in triplicates. The test plates were incubated at 37 °C for 12 h before observing for the zone of inhibition, which is measured in millimeter. DMSO was used as a negative control. Ciprofloxacin was used as antibacterial standard and fluconozole as antifungal standard (10 mcg discs).

RESULTS AND DISCUSSION

Chemistry

The title compounds were synthesized starting from hydroxy acetophenones and hydroxy aldehydes by introducing tosyl substitution, as per the procedure described in detail in our previous publication (Shubhalaxmi *et al*., 2016). The structures of all the compounds were confirmed using FT-IR and ^{1}H NMR. The absence of the carbonyl peak of chalcones around 1650 $cm^{-1}$and the appearance of additional peaks due to NH stretch at around 3200 cm^{-1} along with C=N stretch at around 1550 cm^{-1} confirms the formation of pyrazoline ring. The presence of S=O asymmetric and symmetric stretching bands in FTIR spectra of the compounds also prove that tosyl substitution is unaffected under the experimental conditions. The PMR spectrum of the compound **1a** show peaks at 2.4 for CH_3 group of tosyl moiety, 2.76-2.83 due to H of pyrazoline ring, 4.81-4.86 for 2H of pyrazoline ring, and the remaining aromatic protons appeared as multiplets in the range 7.00-7.76.In many cases, the NH protons were merged in the aromatic range and therefore not assigned separately.

Biological Evaluation

The zones of inhibition revealed that the compounds screened are moderately active towards all the tested gram positive bacteria. There were no inhibition zones exhibited against gram negative bacteria, *E.coli*, implying that these compounds are inactive against similar bacteria. Few of the compounds showed moderate zones of inhibition against the fungi considered for the study, *C.albicans*. It may be noted that the compounds without substitution at 4 of the aromatic ring, **2a,** showed highest inhibition zone against *M. smegmatis*. The compound **2b,** with4-chloro substitution in aromatic ring showed remarkably higher inhibition zone against *S. aureus*. The compounds **1a**, **2a** and **2e** without substitution at aromatic rings and 3,4-dimethoxy substitution gave highest inhibition zones against *C. albicans* at 50 µL concentrations.

Table 1: Antimicrobial activity of synthesized compounds by well diffusion assay.

Synthesized compounds	Inhibition Zone in mm; Mean±SD					
	M.smegmatis		*S.aureus*		*C.albicans*	
	50µL	25µL	50µL	25µL	50µL	25µL
1a	-	-	14.66±1.52	7.66±1.52	17.00±1.00	13.33±2.08
1b	-	-	10.66±0.57	-	12.0±00	11.0±0
1d	-	-	-	-	12.33±0.57	12.0±0
1e	12.33±0.57	10.33±0.57	12.66±0.57	12.33±0.57	16.66±1.15	13.66±0.57
2a	16.66±0.57	14.33±0.57	13.66±0.57	11.66±0.57	22.33±0.57	18.66±0.57
2c	15.66±0.57	13.33±0.57	17.33±0.57	-	16.00±0	11.33±0.57
2d	-	-	10.33±0.57	-	9.33±0.57	-
2e	-	-	16.66±0.57	11.66±0.57	17.66±0.57	13.00±0
2f	-	-	-	-	16.66±1.15	9.66±0.57
ABS/AFS	46.67±0.58		33.33±0.58		30.27±1.55	

ABS; antibacterial standard Ciprofloxacin; AFS; anti-fungal standard Fluconazole; both standards used are 10 µg discs; --: not detected inhibition; control; dimethylsulfoxide. Note: Any of the tested compounds showed no significant inhibition zones against gram negative *E.coli*. Compound **1c**, **1f**, **2b** showed no inhibition against any of the tested organisms.

CONCLUSION

A series of 2-pyrazoline derivatives of different tosyloxy substituted chalcones were synthesized. Some of these compounds are positional isomers of one another. The synthesized compounds were characterized by spectral techniques and structures were assigned. The compounds were subjected to qualitative screening for their antimicrobial potential using well diffusion assay with drug Ciprofloxacin, and Fluconazole for antibacterial and antifungal action as standards respectively. Some of the compounds showed moderate zones of inhibition at 50 µL concentration. We intend to extend the scope of the present work by replacing tosyl group with better biologically active substituents by nucleophilic substitution.

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Conflict of Interests: There are no conflicts of interest.

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