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Note

Synthesis and antimicrobial activity of some new pyrazolo[3,4-*d*]pyrimidines and thiazolo[4,5-*d*]pyrimidines

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The desired fused ring system 3-isopropyl-4-aryl-1,4,5,7-tetrahydropyrazolo[3,4-*d*] pyrimidin-6-ones **4a-d** have been synthesized by the reaction of 5-isopropyl-2,4-dihydro-3-pyrazolone **1**, urea and different aromatic aldehydes, while 7-aryl-6,7-dihydro-3*H*,4*H*-thiazolo[4,5-*d*]pyrimidine-2,5-diones **7a-d** have been synthesized by using 2,4-thiazolidine **5** instead of 5-isopropyl-2,4-dihydro-3-pyrazolone **1**. The structures of the compounds have been characterized by elemental analysis, IR, ^{1}H NMR, and mass spectroscopy. The antibacterial activity of the newly synthesized compounds have been tested against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 1539; antifungal activity against *Candida albicans* ATCC 10231 have been tested using the disk diffusion method. Compounds **4b, 4c, 4d, 7c** and **7d** are found to be active against *S. aureus* ATCC 6538 (MIC: 185, 78, 156, 72 and 102 µg/mL respectively) and compounds **4d** and **7d** against *C. albicans* ATCC 10231 (MIC: 312.5 µg/mL). The minimum inhibitory concentrations of these compounds have been determined using the micro dilution method.

Keywords: Pyrazolo[3,4-*d*]pyrimidines, thiazolo[4,5-*d*]pyrimidines, antimicrobial activity

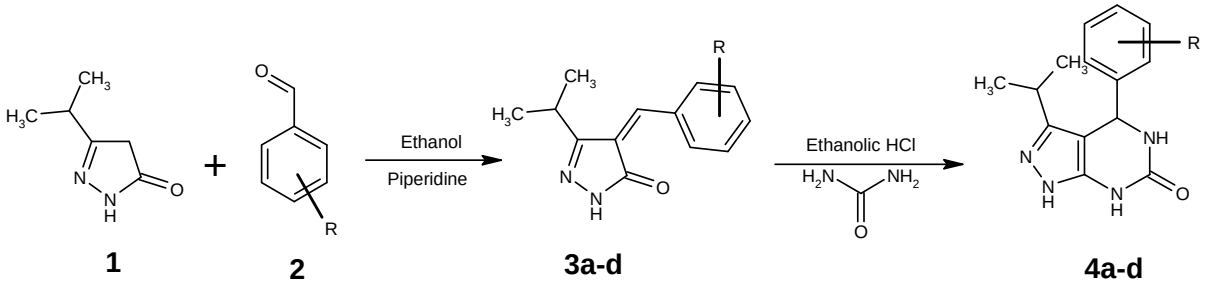
Pyrimidine and their derivatives are well known for their potential biological activity such as fungicide[1], algaecide[2] and antibiotic[3]. Similarly thiazoles have shown a wide range of applications[4] in drug development[5] against inflammation[6] bacterial[7] and HIV infection[8] and pyrazolones are used as starting materials for the synthesis of biologically active compounds[9], as well as for the construction of condensed heterocyclic systems[10,11]. This inspired the synthesis of 3-isopropyl-4-aryl-1,4,5,7-tetrahydropyrazolo[3,4-*d*]pyrimidin -6-ones **4a-d** and 7-aryl-6,7-dihydro-3*H*,4*H*-thiazolo[4,5-*d*]pyrimidine-2,5-diones **7a-d**. In the literature it was shown that new derivatives are formed[12,13], when pyrazolone or rhodanine reacts with different aromatic aldehydes and urea at reflux temperature. In this work, in line with literature findings, 5-isopropyl-2,4-dihydro-3-pyrazolone[14,15] or 2,4-thiazolidine[16], when reacted with different aromatic aldehydes **2** and urea, afforded the desired products. The antibacterial activity of the new compounds were investigated against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 1539; antifungal activity was tested against *Candida albicans* ATCC 10231.

Results and Discussion

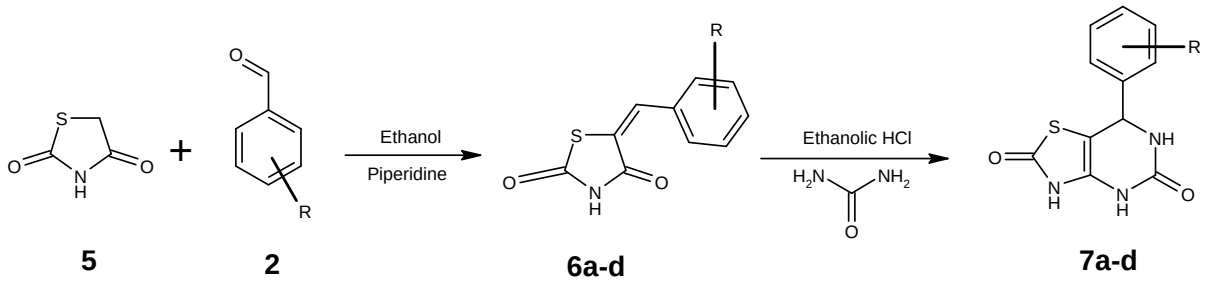
The synthesis of 3-isopropyl-4-aryl-1,4,5,7-tetrahydropyrazolo[3,4-*d*]pyrimidin -6-ones **4a-d** and 7-aryl-6,7-dihydro-3*H*,4*H*-thiazolo[4,5-*d*]pyrimidine-2,5-diones **7a-d** was carried out in two steps, first by the condensation of 5-isopropyl-2,4-dihydro-3-pyrazolone **1** or 2,4-thiazolidine **5** with different aromatic aldehydes by Knoevenagel condensation in the presence of piperidine at reflux temperature to give 4-benzylidene-5-isopropyl-2, 4-dihydro-3-pyrazolone **3a-d** or benzylidenethiazolidine-2,4-dione **6a-d**, which on reflux with urea in ethanolic HCl yielded 3-isopropyl-4-aryl-1,4,5,7-tetrahydropyrazolo[3, 4-*d*]-pyrimidin-6-ones **4a-d** and 7-aryl-6,7-dihydro-3*H*,4*H*-thiazolo[4,5-*d*]pyrimidine-2,5-diones **7a-d** respectively (**Schemes I** and **II**).

The molecular formulae of the compounds were confirmed by the elemental analysis and their structures were determined from IR, ^{1}H NMR, and mass spectral data. The IR spectra of the compounds displayed the characteristic N-H stretching vibration at 3374-3448 cm^{-1}. The ^{1}H NMR spectra of a few selected compounds gave characteristic peaks in the expected regions. MS of the compounds **4a**, **4b, 4c, 7a, 7b** and **7d** showed the molecular ion peak (M^+) with low intensity, while the MS of compounds **4d** and **7c** did not show any molecular ion peak but showed the peaks due to fragments that supported the expected structures.

Experiments were performed to evaluate the antibacterial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC



Scheme I — Synthesis of substituted pyrazolo[3,4-*d*]pyrimidine



where, R = 4-OCH_3, 2-OCH_3, 3,4-$(OCH_3)_2$

Scheme II — Synthesis of substituted thiazolo[4,5-*d*]pyrimidine

12228, *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 1539; antifungal activity against *Candida albicans* ATCC 10231 were tested using the disk diffusion method. Compounds **4b, 4c, 4d, 7c** and **7d** were found to be active against *S. aureus* ATCC 6538, and compounds **4d** and **7d** against *C. Albicans* ATCC 10231. The minimum inhibitory concentrations of these compounds were determined using the micro dilution method. As a result, four compounds were found to be active. The most active compound was compound **4b**, which had a methoxy group at the 4th position of the phenyl ring, while the least active one was **7c**. The compounds **4a, 7a,** and **7b** did not show any activity. The MIC values of compounds **4b, 4c, 4d, 7c** and **7d** against *S. aureus* ATCC 6538 were 185, 78, 156, 78 and 102 µg/mL respectively and of compounds **4d** and **7d** against *C. albicans* ATCC 10231 were both 312.5 µg/mL **Table I**.

Table I — MIC values (µg/mL) of compounds **4b, 4c, 4d, 7c** and **7d**

Compd	*S. aureus* ATTC 6535	*C. albicans* ATCC 10231
4b	185	-
4c	78	-
4d	156	312.5
7c	72	-
7d	102	312.5
Cefuroxim Na	1.2	2.4

Microbiology

Derivatives **4a-d** and **7a-d** were tested *in vitro* for antimicrobial activity against *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 1539, and antifungal activity against *C. albicans* ATCC 10231 using the disk diffusion method where each disc contained 200 µg of the test compound. For this method, Mueller-Hinto agar was melted at 100°C and after cooing to 56°C, was poured into Petri plates of 9 cm diameter in portions of 20 mL volume, and left on a flat surface to solidify and the surface of the medium was dried at 37°C. Then, the cultures of each bacteria and yeast strain, after being incubated in Mueller-Hinton broth at 37°C for 18-24 hr and diluted with Mueller-Hinton broth to 10^5 cfu/mL, were pipetted into the Mueller-Hinton agar plate prepared as described above. The surface of the medium was allowed to dry. The 10,000 µg/mL (in DMSO) compound impregnated discs were applied to the surface of inoculated plates. The Petri plates were placed in an incubator at 37°C. After 10-24 hr of incubation, the Petri plates were examined and it was found that compounds **4b, 4c, 4d, 7c** and **7d** were active against *S. aureus* ATCC 6539 and compounds **4d** and **7d** against *C. albicans* ATCC 10231.

The minimum inhibitory concentrations (MIC) of these compounds were determined by the microbroth dilution technique using Mueller-Hinton broth. Serial two-fold dilution ranged from 2500 to 2.4 mg/L for all the compounds.

The inoculum was prepared in broth, which had been diluted with Mueller-Hinton broth to give a final concentration of 10^5 cfu/mL in the test tray. The trays were covered and placed in plastic bags to prevent drying. After incubation at 37°C for 18-24 hr, the MIC was defined as the lowest concentration of compound giving complete inhibition of visible growth. MIC values of the compounds are given in **Table I**.

Experimental Section

Melting points were estimated in open capillaries and are uncorrected. Elemental analyses were performed on a Carlo Erba EA 1108 elemental analyzer. IR spectra were recorded on KBr discs, using FTIR-8400 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AVANCE II 400 spectrometer (^{1}H NMR 400 MHz, in DMSO-d_6). Mass spectra were determined using direct inlet probe on a GCMS-QP2010 mass spectrometer. The homogeneity of the compounds was checked by TLC using silica gel "G" as absorbent and visualization was effected by UV light and iodine vapours.

General procedure for the preparation of 3-isopropyl-4-aryl-1, 4, 5, 7-tetrahydro-pyrazolo[3,4-*d*]pyrimidin-6-ones, 4a-d: A mixture of 5-isopropyl-2,4-dihydro-3-pyrazolone (0.01 mol) **1** and aromatic aldehyde **2** (0.01 mol) was taken in a round bottom flask (100 mL) containing 10 mL of ethanol, heated under reflux for 30 min and pyridine (1 mL) was add to the reaction mixture. After 5 hr, when a solid products separated, the reaction mixture was cooled, the solid product was filtered and washed with a cold mixture of ethanol:water (1:1) to give the product. The crude product was purified by recrystallization from 95% ethanol to afford the pure product.

Each product **3a-d** (0.01 mol), urea (0.011 mol) and ethanolic HCl (10 mL, 0.01N) was heated at reflux for 5-6 hr, the separated solid was isolated from the RBF, washed with ethanol (5mL) and purified by recrystallization from ethanol to get **4a-d**.

3-isopropyl-4-phenyl-1,4,5,7-tetrahydro-pyrazolo[3,4-*d*]pyrimidin-6-one, 4a: Yellow crystalline solid; yield 38%; m.p. 245-48°C; IR (KBr): 3447 (-NH), 3232 (ArH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.12 (d, 3H, *J*=6.9 Hz, CH_3), 1.19 (d, 3H, *J*=6.8 Hz, CH_3), 2.95 (m, 1H, *J*=6.8 Hz, CH), 6.50-7.42 (m, 6H, ArH), 7.84 (s, 1H, NH-pyrazole), 8.58 (s, 1H, NH pyrimidine), 8.84 (s, 1H, NH pyrimidine); MS: *m/z* $[M+H]^+$ 257. Anal. Calcd. for $C_{14}H_{16}N_4O$: C, 65.61; H, 6.29; N, 21.8. Found: C, 65.22; H, 6.56; N, 21.74%.

3-isopropyl-4-(4-methoxyphenyl)-1, 4, 5, 7-tetrahydro-pyrazolo[3,4-*d*]pyrimidin-6-one, 4b: Yellow powder; yield 42%; m.p. 221-22°C; IR (KBr): 3447 (-NH), 3243 (ArH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.14 (d, 3H, *J*=7.0 Hz, CH_3), 1.23 (d, 3H, *J*=6.9 Hz, CH_3), 2.99 (m, 1H, *J*=6.9 Hz, CH), 3.83 (s, 3H, OCH_3), 6.57-7.35 (m, 5H, ArH), 7.03 (s, 1H, NH-pyrazole), 8.55 (s, 1H, NH pyrimidine), 8.82 (s, 1H, NH pyrimidine); MS: *m/z* $[M+H]^+$ 287. Anal. Calcd. for $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.57. Found: C, 62.89; H, 6.63; N, 19.77%.

3-isopropyl-4-(2-methoxyphenyl)-1, 4, 5, 7-tetrahydro-pyrazolo[3,4-*d*]pyrimidin-6-one, 4c: Light yellow powder; yield 32%; m.p. 233-35°C; IR (KBr): 3420 (-NH), 3309 (ArH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.12 (d, 3H, *J*=6.9 Hz, CH_3), 1.21 (d, 3H, *J*=6.9 Hz, CH_3), 2.97 (m, 1H, *J*=6.9 Hz, CH), 3.82 (s, 3H, OCH_3), 6.52-7.30 (m, 5H, ArH), 7.44 (s, 1H, NH-pyrazole), 8.79 (s, 1H, NH pyrimidine), 9.01 (s, 1H, NH pyrimidine); MS: *m/z* $[M+H]^+$ 287. Anal. Calcd. for $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.57. Found: C, 62.71; H, 6.12; N, 19.32%.

3-isopropyl-4-(3, 4-dimethoxyphenyl)-1,4,5,7-tetrahydro-pyrazolo[3, 4-*d*]pyrimidin-6-one, 4d: Orange crystalline solid; yield 47%; m.p. 241-42°C; IR (KBr): 3421 (-NH), 3276 (ArH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.15 (d, 3H, *J*=6.9 Hz, CH_3), 1.23 (d, 3H, *J*=6.9 Hz, CH_3), 3.00 (m, 1H, *J*=6.9 Hz, CH), 3.83 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.53-6.92 (m, 4H, ArH), 7.47 (s, 1H, NH-pyrazole), 7.82 (s, 1H, NH pyrimidine), 8.01 (s, 1H, NH pyrimidine). Anal. Calcd. for $C_{16}H_{20}N_4O_3$: C, 60.75; H, 6.37; N, 17.71. Found: C, 60.54; H, 6.45; N, 17.45%.

General procedure for the preparation of 7-aryl-6,7-dihydro-3*H*,4*H*-thiazolo[4,5-*d*]pyrimidine-2,5-diones, 7a-d: A mixture of 2,4-thiazolidinone **5** (0.01 mol) and aromatic aldehyde **2** (0.01 mol) was taken in a round bottom flask (100 mL) containing 10 mL of ethanol, heated under reflux for 30 min and pyridine (1 mL) was added to the reaction mixture. After 5 hr, when a solid products separated, the reaction mixture was cooled, the solid product was filtered and washed with cold mixture of ethanol:water (1:1) to give the product. The crude product was purified by recrystallization from 95% ethanol to afford the pure product.

Each product **6a-d** (0.01 mol), urea (0.011 mol) and ethanolic HCl (10 mL, 0.01N) was heated at reflux for 5-6 hr, the separated solid was isolated from the RBF, washed with ethanol (5mL) and purified by recrystallization from ethanol to get **7a-d**.

7-phenyl-6, 7-dihydro-3*H*,4*H*-thiazolo[4, 5-*d*]pyrimidine-2,5-dione, 7a: Light yellow crystalline solid; yield 41%; m.p. 219-20°C; IR (KBr): 3474 (-NH), 3232 (ArH), 1733 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 6.50-7.90 (m, 6H, ArH), 10.03 (s, 1H, NH-thiazole), 10.84 (s, 1H, NH pyrimidine), 11.15 (s, 1H, NH pyrimidine); MS: *m/z* $[M+H]^+$ 248. Anal. Calcd. for $C_{11}H_9N_3O_2S$: C, 53.43; H, 3.67; N, 16.99; Found: C, 53.32; H, 3.45; N, 17.15%.

7-(4-methoxyphenyl)-6, 7-dihydro-3*H*, 4*H*-thiazolo[4,5-*d*]pyrimidine-2,5-dione, 7b: Orange crystalline solid; yield 50%; m.p. 215-16°C; IR (KBr): 3448 (-NH), 3232 (ArH), 1742 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.86 (s, 3H, CH_3), 6.98-7.72 (m, 5H, ArH), 10.03 (s, 1H, NH-thiazole), 11.04 (s, 1H, NH pyrimidine), 11.15 (s, 1H, NH pyrimidine); MS: *m/z* $[M+H]^+$ 278. Anal. Calcd. for $C_{12}H_{11}N_3O_3S$: C, 51.98; H, 4.00; N, 15.15. Found: C, 51.82; H, 3.88; N, 15.21%.

7-(2-methoxyphenyl)-6,7-dihydro-3*H*,4*H*-thiazolo[4,5-*d*]pyrimidine-2,5-dione, 7c: Orange crystalline solid; yield 28%; m.p. 196-97°C; IR (KBr): 3416 (-NH), 3232 (ArH), 1734 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.82 (s, 3H, CH_3), 6.52-7.30 (m, 5H, ArH), 10.03 (s, 1H, NH-thiazole), 11.22, (s, 1H, NH pyrimidine), 11.32 (s, 1H, NH pyrimidine). Anal. Calcd. for $C_{12}H_{11}N_3O_3S$: C, 51.98; H, 4.00; N, 15.15. Found: C, 51.86; H, 4.12; N, 15.29%.

7-(3, 4-dimethoxyphenyl)-6, 7-dihydro-3*H*, 4*H*-thiazolo[4, 5-*d*]pyrimidine-2,5-dione, 7d: Yellow crystalline solid; yield 40%; m.p. 214-15°C; IR (KBr): 3448 (-NH), 3232 (ArH), 1727 (C=O) cm^{-1}; MS: *m/z* $[M+H]^+$ 308. Anal. Calcd. for $C_{13}H_{13}N_3O_4S$: C, 50.81; H, 4.26; N, 13.67. Found: C, 50.87; H, 4.22; N, 13.76%.

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