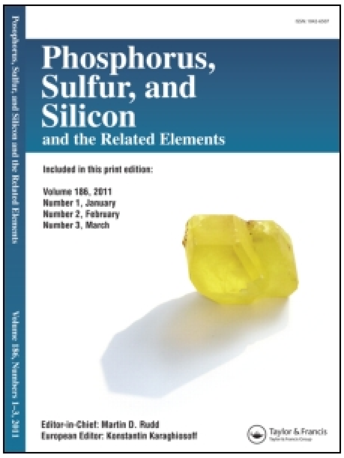

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Synthesis of Some New Pyrazolo[3,4-d]pyrimidines and Thiazolo [4,5-d]pyrimidines and Evaluation of Their Antimicrobial Activities

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Synthesis of Some New Pyrazolo[3,4-d]pyrimidines and Thiazolo [4,5-d]pyrimidines and Evaluation of Their Antimicrobial Activities

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The desired fused ring system 3-isopropyl-4-aryl-1,4,5,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-6-thiones ***4a–d*** *were synthesized by the reaction of 5-isopropyl-2,4-dihydro-3-pyrazolone* ***1****, thiourea and different aromatic aldehydes, while 7-aryl-5-thioxo-4,5,6,7-tetrahydro-3H-thiazolo[4,5-d]pyrimidin-2-ones* ***7a–d*** *were synthesized by using 2,4-thiazolidine* ***5*** *instead of 5-isopropyl-2,4-dihydro-3-pyrazolone* ***1****. The structure of the compounds was assigned on the basis of elemental analysis, IR, ^{1}H-NMR, and mass spectroscopy. The antibacterial activity of the newly synthesized compounds were tested against* Staphylococcus aureus *ATCC 6538,* Staphylococcus epidermidis *ATCC 12228,* Escherichia coli *ATCC 8739,* Pseudomonas aeruginosa *ATCC 1539, and their 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 (MIC: 185; 78; 156; 78; and 102 µg/Ml, respectively) and compounds* ***4d*** *and* ***7d*** *against* C. albicans *ATCC 10231 (MIC: 312.5 µg/mL) . The minimum inhibitory concentration of these compounds was determined using the micro dilution method.*

Keywords Antimicrobial activities; pyrazolo[3,4-d]pyrimidines; thiazolo[4,5-d]pyrimidines

INTRODUCTION

Pyrimidine and their derivatives are well known for their potential biological activity such as fungiside[1], algaecide,[2] and as antibiotic.[3] Similarly thiazoles have shown wide range of applications,[4] in drug

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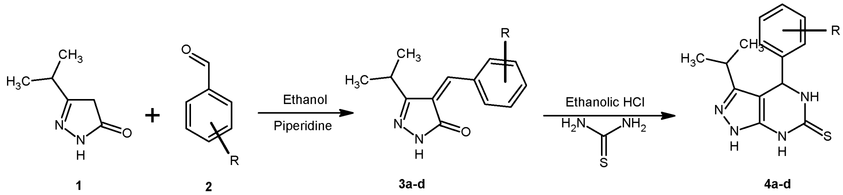
development,[5] against inflammation,[6] bacterial,[7] and HIV infection,[8] and pyrazolones are also used as starting materials for the synthesis of biologically active compounds,[9] as well as for the construction of condensed heterocyclic systems.[10,11] This inspired us to synthesize 3-isopropyl-4-aryl-1,4,5,7-tetrahydro-pyrazolo[3,4-*d*]pyrimidine-6-thiones **4a–d** and 7-aryl-5-thioxo-4,5,6,7-tetrahydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ones **7a–d**. In the literature it was shown that when pyrazolone or rhodanine react with different aromatic aldehydes and thiourea at reflux temperature, new derivatives are formed.[12,13] In this work, in line with literature findings, 5-isopropyl-2,4-dihydro-3-pyrazolone[14,15] or 2,4-thiazolidine[16] when react with different aromatic aldehydes **2** and thiourea afforded desired product. The antibacterial activity of the new compounds against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 1539, and antifungal activity against *Candida albicans* ATCC 10231 were investigated.

RESULTS AND DISCUSSION

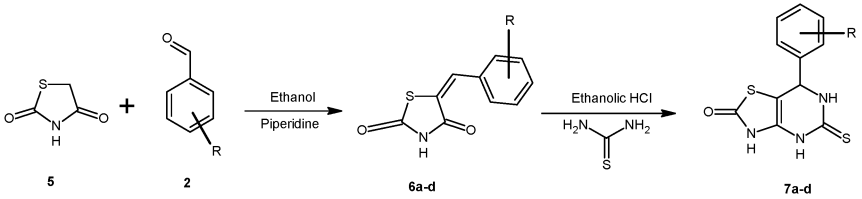
Condensation of 5-isopropyl-2,4-dihydro-3-pyrazolone **1** or 2,4-thiazolidine **5** with different aromatic aldehydes by Knoevenagel condensation in the presence of piperidine at reflux temperature to give 4-benzylidene-5-isopropyl-2,4-dihydro-3-pyrazolone **3a–d** or benzylidene-thiazolidine-2,4-dione **6a–d** respectively. Which were on reflux with thiourea in ethanolic HCl to yield 3-isopropyl-4-aryl-1,4,5,7-tetrahydro-pyrazolo[3,4-*d*]pyrimidine-6-thiones **4a–d** and 7-aryl-5-thioxo-4,5,6,7-tetrahydro-3*H*-thiazolo[4,5-*d*]pyrimidin-2-ones **7a–d** respectively (Schemes 1 and 2).



Where R = 4-OCH$_3$, 2-OCH$_3$, and 3,4-(OCH$_3$)$_2$

SCHEME 1 Synthesis of substituted pyrazolo[3,4-d]pyrimidine.

The formulas of the compounds were confirmed by the elemental analysis and their structures were determined by IR, ^{1}H-NMR, and mass spectral data. The IR spectra of the compounds displayed the characteristic N-H stretching vibration at 3474–3448 cm^{-1}. The ^{1}H-NMR spectrums of selected compounds give its characterized peak in



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

SCHEME 2 Synthesis of substituted thiazolo[4,5-d]pyrimidine.

particular region. Mass of all compounds was taken, and it was shown molecular ion peak (M^+) with low intensity.

Experiment were performed to evaluate the antibacterial activity against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *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 concentration of these compounds was determined using the micro dilution method. As a result, four compounds were found to be active. The most active compound was compound **4c**, which had a methoxy group on 2nd position of the phenyl ring, while the least active one, **4b**. The compounds **4a, 7a,** and **7b** did not show any activity. The MIC values of the compounds **4b**, **4c**, **4d**, **7c**, and **7d** against *S. aureus* ATCC 6538 were 185; 78; 156; 78; and 102 μg/mL, respectively, and the compounds **4d** and **7p** 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

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

EXPERIMENTAL SECTION

General Procedures

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 spectrophotometer. ^{1}H-NMR spectra were taken on a Bruker AVANCE II 400 (^{1}H: 400 Mz, [d$_6$] DMSO) spectrometer. Mass spectra were determined on a GCMS-QP2010 mass spectrometer.

General Procedure for the Preparation of 3-Isopropyl-4-aryl-1,4,5,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-6-thiones (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 round bottom flask (100 mL) containing 10 mL of ethanol, heated under reflux for 30 min and add pyridine (1 mL) in reaction mixture. Further refluxed it for 5 h; thus, the solid product was separated, filtered off and washed with cold mixture of ethanol:water (1:1) to give product **3a**–**d**. The crude products were purified by crystallization from 95% ethanol to afford pure product.

Product **3a**–**d** (0.01 mol), thiourea (0.011 mol) and ethanolic HCl 10 mL (0.01N) was reflux for 5–6 h and separated solid was isolate from RBF, washed with ethanol (5mL) and crystallized from ethanol to get **4a**–**d.**

3-Isopropyl-4-phenyl-1,4,5,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-6-thione (4a)

Yellow crystals (1.03 g, 38%); m.p. 245=248°C. IR (KBr): v 3447 (—NH), 3232 (ArH), 2925 (C—H str.), 1439 (C—H i.p.def.) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.12 (3H, d, $J = 6.92$ Hz, CH$_3$), 1.19 (3H, d, $J = 6.8$ Hz, CH$_3$), 2.95 (1H, m, —CH), 6.50–7.42 (6H, m, ArH), 7.84 (1H, s, NH-pyrazole), 8.58 (1H, s, NH pyrimidine), 8.84 (1H, s, NH pyrimidine). Mass M^{+1}273. Anal. calcd. for $C_{14}H_{16}N_4S$: C, 61.74; H, 5.92, N, 20.57. Found: C, 61.22; H, 5.56, N, 20.74.

3-Isopropyl-4-(4-methoxyphenyl)-1,4,5,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-6-thione (4b)

Yellow powder (1.26 g, 42%); m.p. 221=222°C. IR (KBr): v 3447 (-NH), 3243 (ArH), 2934 (C—H str.), 1436 (C—H i.p.def.) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.14 (3H, d, $J = 7$ Hz, CH$_3$), 1.23 (3H, d, $J = 6.88$ Hz, CH$_3$), 2.99 (1H, m, —CH), 3.83 (3H, s, OCH$_3$), 6.57=7.35 (5H, m, ArH), 7.03 (1H, s, NH-pyrazole), 8.55 (1H, s, NH pyrimidine), 8.82

(1H, s, NH pyrimidine). Mass M^+303. Anal. calcd. for $C_{15}H_{18}N_4OS$: C, 59.58; H, 6.00, N, 18.25. Found: C, 60.89; H, 5.83, N, 18.77.

3-Isopropyl-4-(2-methoxyphenyl)-1,4,5,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-6-thione (4c)

Light yellow powder (0.96 g, 32%); m.p. 233=235°C. IR (KBr): υ 3420 (-NH), 3309 (ArH), 2925 (C—H str.), 1461 (C—H i.p.def.) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.12 (3H, d, $J = 6.96$ Hz, CH_3), 1.21 (3H, d, $J = 6.88$ Hz, CH_3), 2.97 (1H, m, —CH), 3.82 (3H, s, OCH_3), 6.52-7.30 (5H, m, ArH), 7.44 (1H, s, NH-pyrazole), 8.79 (1H, s, NH pyrimidine), 9.01 (1H, s, NH pyrimidine). Mass M^+303. Anal. calcd. for $C_{15}H_{18}N_4OS$: C, 59.58; H, 6.00, N, 18.25. Found: C, 59.31; H, 5.82, N, 18.32.

3-Isopropyl-4-(3,4-dimethoxyphenyl)-1,4,5,7-tetrahydro-pyrazolo[3,4-d]pyrimidine-6-thione (4d)

Orange crystals (1.56 g, 47%); m.p. 241=242°C. IR (KBr): υ 3421 (-NH), 3276 (ArH), 2932 (C—H str.), 1433 (C—H i.p.def.) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 1.15 (3H, d, $J = 6.96$ Hz, CH_3), 1.23 (3H, d, $J = 6.88$ Hz, CH_3), 3.00 (1H, m, -CH), 3.83 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 6.53-6.92 (4H, m, ArH), 7.47 (1H, s, NH-pyrazole), 7.82 (1H, s, NH pyrimidine), 8.01 (1H, s, NH pyrimidine). Mass M^+316. Anal. calcd. for $C_{16}H_{20}N_4O_2S$: C, 57.18; H, 6.06, N, 16.85. Found: C, 57.34; H, 5.97, N, 16.77.

General Procedure for the Preparation of 7-Aryl-5-thioxo-4,5,6,7-tetrahydro-*3H*-thiazolo[4,5-*d*]pyrimidin-2-ones (7a–d)

A mixture of 2,4-thiazolidinone (0.01 mol) **5** and aromatic aldehyde **2** (0.01 mol) was taken in round bottom flask (100 mL) containing 10 mL of ethanol, heated under reflux for 30 min and add pyridine (1 mL) in reaction mixture. Further refluxed it for 5 h, thus the solid product was separated, filtered off and washed with cold mixture of ethanol:water (1:1) to give product **6a–d**. The crude products were purified by crystallization from 95% ethanol to afford pure product.

Product **6a–d** (0.01 mol), thiourea (0.011 mol) and ethanolic HCl 10 mL (0.01N) was reflux for 5–6 h and separated solid isolate from RBF, washed with ethanol (5mL) and crystallized from ethanol to get **7a–d.**

7-Phenyl-5-thioxo-4,5,6,7-tetrahydro-3H-thiazolo[4,5-d]pyrimidin-2-one (7a)

Light yellow crystals (1.08 g, 41%); m.p. 219–220°C. IR (KBr): υ 3474 (-NH), 3232 (ArH), 1733 (C=O) cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): 6.50–7.90 (6H, m, ArH), 10.03 (1H, s, NH-thiazole), 10.84 (1H, s, NH pyrimidine), 11.15 (1H, s, NH pyrimidine). Mass M$^+$264. Anal. calcd. for $C_{11}H_9N_4OS_2$: C, 50.17; H, 3.44, N, 15.96. Found: C, 49.88; H, 3.32, N, 15.85.

7-(4-Methoxyphenyl)-5-thioxo-4,5,6,7-tetrahydro-3H-thiazolo [4,5-d]pyrimidin-2-one (7b)

Orange crystals (1.46 g, 50%); m.p. 215–216°C. IR (KBr): v 3448 (-NH), 3232 (ArH), 1742 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 3.86 (3H, s, CH_3), 698–7.72 (5H, m, ArH), 10.03 (1H, s, NH-thiazole), 11.04 (1H, s, NH pyrimidine), 11.15 (1H, s, NH pyrimidine). Mass M$^+$294. Anal. calcd. for $C_{12}H_{11}N_3O_2S_2$: C, 49.13; H, 3.78, N, 14.32. Found: 49.32; H, 3.88, N, 14.21.

7-(2-Methoxyphenyl)-5-thioxo-4,5,6,7-tetrahydro-3H-thiazolo [4,5-d]pyrimidin-2-one (7c)

Orange crystals (0.82 g, 28%); m.p. 196–197°C. IR (KBr): v 3416 (-NH), 3232 (ArH), 1734 (C=O) cm^{-1}. ^{1}H NMR (400 MHz, DMSO-d_6): 3.82 (3H, s, CH_3), 6.52–7.30 (5H, m, ArH), 10.03 (1H, s, NH-thiazole), 11.22, s, NH pyrimidine), 11.32 (1H, s, NH pyrimidine). Mass M$^+$294. Anal. calcd. for $C_{12}H_{11}N_3O_2S_2$: C, 49.13; H, 3.78, N, 14.32. Found: C, 49.21; H, 3.75, N, 14.45.

7-(3,4-Dimethoxyphenyl)-5-thioxo-4,5,6,7-tetrahydro-3H-thiazolo[4,5-d]pyrimidin-2-one (7d)

Yellow crystals (1.29 g, 40%); m.p. 214–215°C. IR (KBr): v 3448 (-NH), 3232 (ArH), 1727 (C=O) cm^{-1}. Mass M$^+$324. Anal. calcd. for $C_{13}H_{13}N_3O_3S_2$: C, 48.28; H, 4.05, N, 12.99. Found: C, 48.41; H, 4.22, N, 12.76.

Microbiology

Derivatives **4a–d** and **7a–d** were tested in vitro for antimicrobial activity *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 tested compound. For this method, Mueller-Hinto agar (Difco) was melted at 100°C and after cooing to 56°C, was poured into Petri plates of 9 cm diameter in quantities of 20 mL, 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 kept in Mueller-Hinton broth (Difco) at 37°C for 18–24 h and diluted with Mueller-Hinton broth to 10^5cfu/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 h 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 compounds.

The inoculum was prepared in broth, which had been diluted with Mueller-Hinton broth to give a final concentration of 10^5cfu/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 h, the MIC was defi3ed as the lowest concentration of compound giving complete inhibition of visible growth. MIC values of the compounds are given in Table I.

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