Hindawi Publishing Corporation
Journal of Chemistry
Volume 2015, Article ID 982404, 6 pages
http://dx.doi.org/10.1155/2015/982404

Research Article

Synthesis and Antibacterial Activity of New Spiro[thiadiazoline-(pyrazolo[3,4-d]pyrimidine)] Derivatives

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Received 24 February 2015; Revised 16 April 2015; Accepted 20 April 2015

Academic Editor: Marco Radi

New heterocyclic compounds spiroderivatives of allopurinol of biological interest were prepared from allopurinol via thionation and 1,3-dipolar cycloaddition and were produced in high to excellent yields. These compounds were characterized on the basis of spectral and spectroscopic data (^{1}H NMR, ^{13}C, IR, and MS). The antibacterial activity of the synthesized products was studied using bacterial strains: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Compounds having an ethyl group showed the best activity with MIC value of 31.25 μg/mL against *Staphylococcus aureus* and *Streptococcus fasciens*.

1. Introduction

Heterocycles are widely distributed in nature and play a key role in the metabolism of all living cells. Among the many heterocyclic compounds containing nitrogen, the pyrazolopyrimidine ring is very interesting and versatile scaffold for the synthesis of potential drugs or molecular tools (Figure 1). Among their many applications, pyrazolo[3,4-d]pyrimidines (Figure 2) were used as inhibitor kinases [1, 2] antiviral agents [3, 4], adenosine antagonists [5–7], glutamate modulators [8], antituberculosis agents [3], and as antibiotics inhibit bacterial growth [9, 10].

In light of the great importance of spirocyclic systems containing one carbon atom common to two rings [11–13], in recent years efforts have been made in developing methodologies for the synthesis of these compounds especially by cycloaddition reactions [14–22].

1,3-Dipolar cycloaddition is a subject of intense research during the last decade due to its great synthetic value. The cycloaddition is a method of synthesis of five membered heterocycles, which are difficult to prepare by other means.

Generally, reactions of 1,3-dipoles with true heterocyclic thiones having the thione form **A** proceed via 1,3-dipolar cycloaddition to the C=S double bond to form the spirocycloadducts, namely, spirothiadiazoles. The reaction of heterocyclic thiones **A** with nitrilimines, generated *in situ* by base-catalyzed dehydrohalogenation of hydrazonoyl halides, has been described for synthesis of various derivatives of spiro[heterocycle-n,2′-3H-1,3,4-thiadiazole] **B** (Figure 3).

In continuation of our work on the synthesis of the excess of allopurinol [23–28], compounds **2** and **2a-b** have been synthesized by using reported methods [29–31]. Herein we report simple efficient synthesis of spiro thiadiazoline-(pyrazolo[3,4-d]pyrimidine) derivatives **4a-b** by 1,3-dipolar cycloaddition of diphenyl hydrazonoyl chloride **3** with an equimolecular amount of pyrazolo[3,4-d]pyrimidin-4(5H)-thione derivatives **2a-b**. All the synthesized compounds were evaluated for their antibacterial activity.

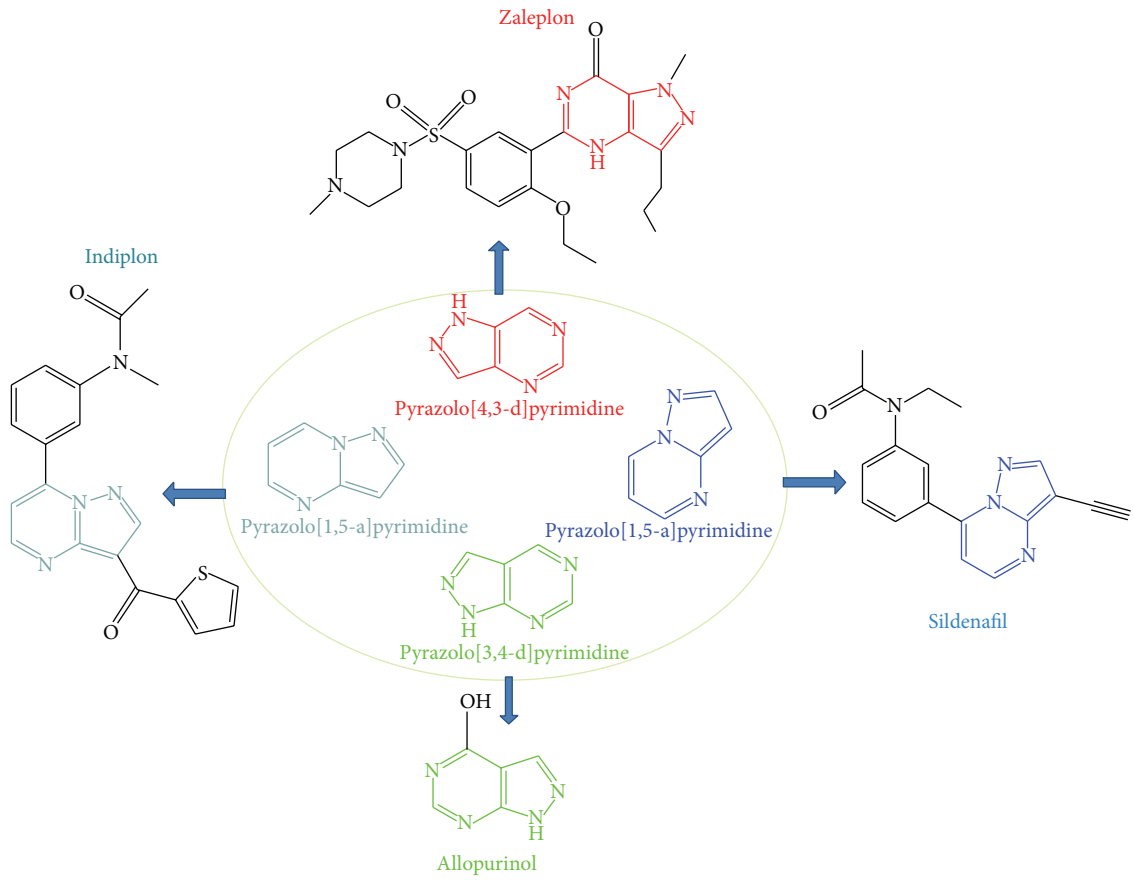


Figure 1: Pyrazolopyrimidine containing drugs.

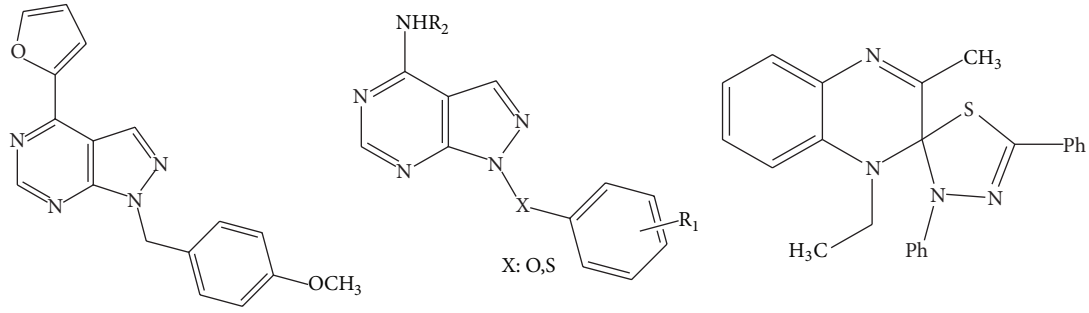


Figure 2: Example of bioactive molecules derived from pyrazolo[3,4-d]pyrimidine and thiadiazole.

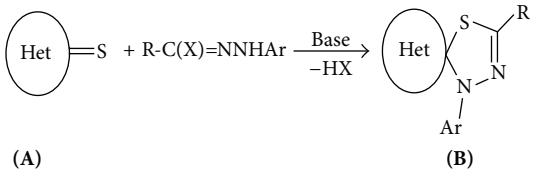


Figure 3: Synthesis of spiroheterocycles.

2. Materials and Methods

Generally the melting points were taken on an electrothermal capillary melting point apparatus. Infrared spectra (ν-cm^{-1}) were recorded on a Perkin Elmer 577, using KBr disks. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 300 NMR Spectrometer in DMSO-d_6. Spectra were internally referenced to TMS. Peaks are reported in ppm downfield of TMS. Mass spectra are recorded in a SYNAPT G2 HDMS (Waters) Spectrometer in electrospray ionization (ESI).

2.1. Thionation. 3.67 mmol of pyrazolo[3,4-d]pyridine is refluxed in pyridine with 3.67 mmol of phosphorus pentasulfide for 4 hours. Then the solvent is evaporated under reduced pressure, and the precipitate formed is washed with hot water to remove residual dimerized P_2S_5 until there is colorless filtrate.

*1H-Pyrazolo[3,4-d]pyrimidine-4(5H)-thione (**2**).* Yield = 90%; mp: 151°C. IR: ϑ = 1585 cm^{-1} ^{1}H NMR (DMSO-d_6): δppm: 8.11 (1H, s, CH); 8.22 (1H, s, CH); 13.47 (1H, s, NH); 13.92 (1H, s, NH). ^{13}C NMR (DMSO-d_6) δppm: 105.05; 134.05 (Cq); 150.77 (CH); 151.20 (CH); 156.79 (Cq C=S). HRMS (ESI) [M + H]: m/z = 153.17.

*1,5-Diethyl-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-thione (**2a**).* Yield = 75%; mp: 150°C. ^{1}H NMR (DMSO-d_6) δ ppm: 1.3 (3H, t, J = 7.2 Hz. CH_3); 1.36 (3H, t, J = 7.2, CH_3); 4.30 (2H, q, J = 7.2 Hz, CH_2); 4.51 (2H, q, J = 7.2 Hz, CH_2); 8.15 (1H, s, CH); 8.72 (1H, s, CH). ^{13}C NMR (DMSO-d_6) δppm: 14.56 (CH_3); 15.15 (CH_3); 42.38 (CH_2); 46.34 (CH_2); 118.17; 137.09 (Cq); 145.09 (CH); 149.99 (CH); 179.11 (Cq, C=S). HRMS (ESI) [M + H]: m/z = 209.07.

*1,5-Dibenzyl-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-thione (**2b**).* Yield = 70%; mp: 160°C. ^{1}H NMR (DMSO-d_6) δ ppm: 3.25 (2H, s, CH_2); 3.78 (2H, s, CH_2); 6.85–7.12 (q, $10H_{Ar}$); 8.21 (1H, s, CH), 8.89 (1H, s, CH). ^{13}C NMR (DMSO-d_6) δppm: 51.83 (CH_2); 54.31 (CH_2); 118.24; 127.94 (Cq); 128.11–137.89 (CH_{Ar}); 145.54 (CH); 150.95 (CH); 179.82 (Cq, C=S). HRMS (ESI) [M + H]: m/z = 33.10.

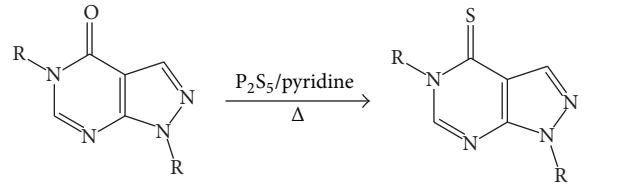
2.2. 1,3-Dipolar Cycloaddition. To a solution of 1,5-diethyl-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-thione (10 mmol) and diphenylnitrilimine (1.3 × 10 mmol) in THF (30 mL) triethylamine (2 mL) was added. The mixture was refluxed for 24 hours. The precipitate was collected by filtration and was separated by silica gel chromatography (hexane/ethyl acetate: 8/2).

*2,5-Diethyl-3′,5′-diphenyl-2,5-dihydro-3′H-spiro[pyrazolo[3,4-d]pyrimidine-1,2′-[1,3,4]thiadiazole] (**4a**).* Yield = 60%; mp: 165°C. ^{1}H NMR (DMSO-d_6) δ ppm 1.12 (3H, t, J = 7.2 Hz, CH_3); 1.28 (3H, t, J = 7.2 Hz, CH_3); 3.37 (2H, q, J = 7.2 Hz. CH_2); 4.11 (2H, q, J = 7.2 Hz, CH_2); 6.83–7.67 (q, J = 7.7 Hz, $10H_{Ar}$); 7.69 (1H, s, CH); 7.69 (1H, s, CH). ^{13}C NMR (DMSO-d_6) δppm: 15.61 (CH_3); 16.03 (CH_3); 41.88 (CH_2); 42.28 (CH_2); 99.70; 100.79; 117.03; 126.38 (Cq); 129.26–142.20 (CH_{Ar}); 143.87 (CH); 148.01 (CH). HRMS (ESI) [M + H]: m/z = 403.16.

*2,5-Dibenzyl-3′,5′-diphenyl-2,5-dihydro-3′H-spiro[pyrazolo[3,4-d]pyrimidine-1,2′-[1,3,4]thiadiazole] (**4b**).* Yield = 60%; mp: 185°C. ^{1}H NMR (DMSO-d_6) δ ppm: 3.65 (2H, s, CH_2); 4.28 (2H, s, CH_2); 7.25–7.32 (q, J = 7.2 Hz, $20H_{Ar}$); 8.81 (1H, s, CH); 8.99 (1H, s, CH). ^{13}C NMR (DMSO-d_6) δppm: 50.83 (CH_2); 53.21 (CH_2); 77.2; 118.24; 121.02; 125.13; 127.94; 128.04; 128.15; 128.46; 128.79 (Cq); 129.12–137.9 (CH_{Ar}); 145.54 (CH); 150.95 (CH). HRM (ESI) [M + H]: m/z = 527.39.

3. Results and Discussion

We first prepared 1,5-diethyl-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-thiones **2a-b** from **1a-b** by refluxing phosphorus pentasulfide in pyridine. The identification of the product was determined by ^{1}H NMR, ^{13}C NMR, IR, and mass spectra.



1: R = H
1a: R = ethyl
1b: R = benzyl

2: R = H (90%)
2a: R = ethyl (65%)
2b: R = benzyl (67%)

Scheme 1: Thionation of 1,5-dialkyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one **1a-b**.

IR-spectra of compounds **2a-d** did not contain C=O-group signals, the signal of C=S group (1500cm^{-1}) was present. The spectra of ^{13}C NMR corroborated the results of IR. In addition, its mass spectrum shows a molecular ion peak at m/z 152 (M+) corresponding to the molecular formula $C_5H_4N_4S$ compound **2**, molecular ion at m/z 208.07 (M+) corresponding to the molecular formula $C_9H_{12}N_4S$ compound **2a** and molecular ion at m /z 332.10 (M+) corresponding to the molecular formula $C_{19}H_{16}N_4S$ compound **2b** (Scheme 1, Figure 4) [23].

We investigated the reaction of 1,3-dipolar cycloaddition of diphenyl hydrazonoyl chloride **3** with equimolecular amount of 1,5-diethyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-thione **2a** in dry tetrahydrofuran in presence of triethylamine (Scheme 2); one cycloadduct was obtained as a result of 1,3-dipolar cycloaddition of diphenyl nitrile imine ylide generated *in situ* from diphenyl hydrazonoyl chloride and triethylamine, on the dipolarophilic group C=S.

The structure of these compounds was confirmed on the basis of their spectroscopic characteristics. The ^{1}H NMR spectra of **4a** (in DMSO-d_6) showed an aromatic multiplet in the region of 6.83 to 7.67 ppm corresponding to the aromatic protons. Two downfield singlets were observed in the region of 7691–7968 ppm representing the protons for CH in pyrimidine ring and CH in pyrazole ring because of the high incidence of the aromatic ring system deshielding protons CH pyrimidine CH pyrazole. 1H NMR spectra of **4a** also showed the CH_2 and CH_3 signals as triplets and multiplets and between 1.12 and 1.27 ppm and between 3.37 and 4.11 ppm, respectively. ^{13}C NMR spectra of **4a** exhibit in signal spirocarbon to 99.70 ppm, aromatic carbons 100.79 to 129.38 ppm, and the imine carbon to 142.2 and 139.93 ppm (HC=N). The mass spectrum shows a peak at m/z 403.16 corresponding to [M + H].

In order to examine the N-substitution effect of alkyl group on the 1,3-dipolar cycloaddition, 1,5-dibenzyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-thione **2a** was chosen to be employed to react with DPNI. So in this case it was found that C=S group underwent the 1,3-dipolar cycloaddition reaction and formed of novel spiro[thiadiazoline-(pyrazolo[3,4-d]pyrimidine)] (Scheme 3).

The structure of compound **4b** was established by IR, ^{1}H NMR, ^{13}C NMR, and mass spectrum. Its IR spectrum showed a characteristic absorption band at 1647 cm^{-1} for the >C=N- indicating the spirocarbon formation. Its ^{1}H NMR

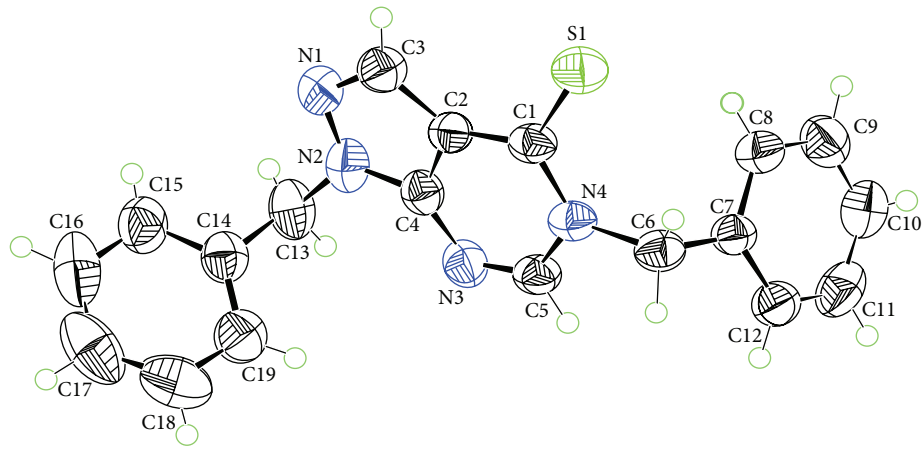
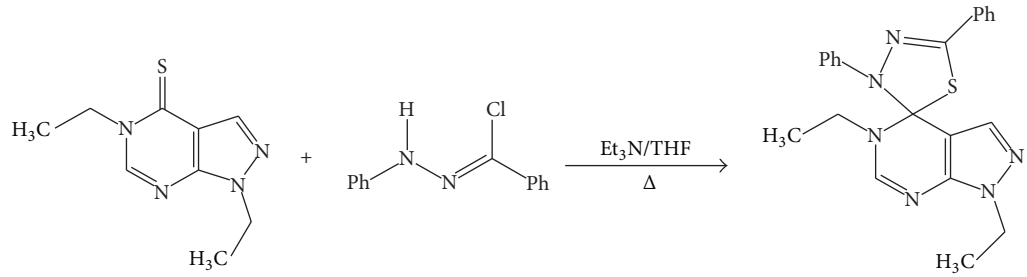
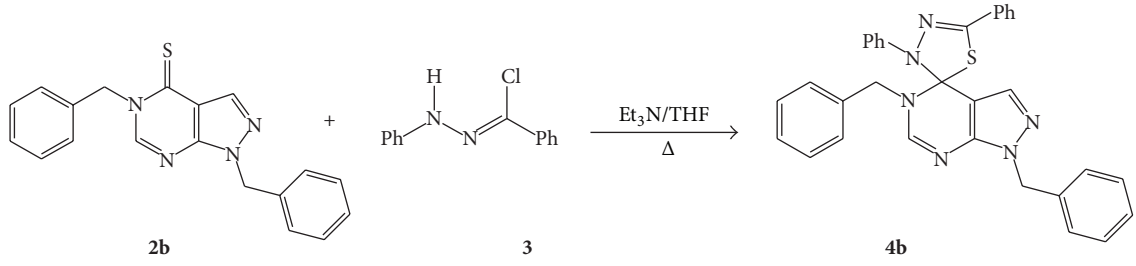


Figure 4: ORTEP presentation of compound **2b**.



2a **3** **4a**

Scheme 2: 1,3-Dipolar cycloaddition of 1,5-diethyl-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-thione **2a** and DPNI **3**.



2b **3** **4b**

Scheme 3: 1,3-Dipolar cycloaddition of 1,5-dibenzyl-1H-pyrazolo[3,4-d]pyrimidine-4(5H)-thione **2b** and DPNI.

spectrum exhibited peaks at 7.25–7.32 ppm (20H) indicating the presence of aromatic protons. The two singlets at 3.65 ppm and 4.28 ppm relating to two protons of the two CH_2 groups were also observed. ^{13}C NMR spectra of **4b** exhibited, in particular, a spiro carbon signal at 77.2 ppm. The mass spectrum shows a peak at m/z 527.39 corresponding to [M + H].

3.1. In Vitro Antibacterial Activity. We studied spiropyrazolo[3,4-d]pyrimidines newly synthesized for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Enterococcus faecalis* (ATCC-29212) strains of bacteria by the diffusion method disk [32]. The MIC values of the compound against bacteria are presented in Table 1.

We synthesized new spirocompounds with a high yield by cycloaddition reaction. They have showed moderate antibacterial activity against selected bacteria. All these compounds showed at an average concentration low antibacterial activity against the bacteria, except that compounds **1a** and **4a** showed higher activity, even at higher concentrations. Compound **4b** showed higher activity against *S. aureus* and *E. faecalis* compared to bacteria *E. coli* and *P. aeruginosa.*

4. Conclusion

In conclusion, a new class of heterocyclic spiro[thiadiazoline pyrazolopyrimidine] compounds was synthesized and their

Table 1: Antibacterial activity of the compounds: MIC in μg/mL.

Product	MIC in μg/μL			
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
1	250	125	250	250
1a	250	250	250	—
1b	125	62.5	62.5	250
2	250	125	125	250
2a	250	125	250	250
2b	250	250	250	250
4a	125	62.5	125	250
4b	31.25	31.25	125	125
Chlor	1.875	3.75	15	7.5
Amp	2.5	1.25	5	5

MIC: minimum inhibitory concentration.

structure was determined and also tested for their antibacterial activity *in vitro*. This study is expected to take the tests of anti-inflammatory drugs, antifungal, and anticancer activity because the literature gives some very interesting results on these topics.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The authors thank Pharmaceutical Laboratories PHARMA 5 for supporting this study.

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