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7-DEAZAPURINES: SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL EVALUATION OF SOME NEW PYRROLO[2,3-*d*]PYRIMIDINE-2-THIONES

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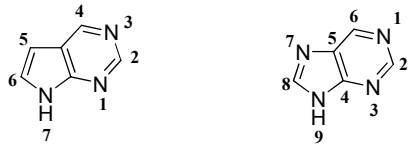
Abstract: Some new 3-aryl-4-imino-7-methyl-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-thiones have been prepared through cyclocondensation reaction of 2-amino-1-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile with aryl isothiocyanates in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) as a base catalyst in DMF under reflux. The synthesized compounds were characterized on the basis of IR, ^{1}H NMR, and ^{13}C NMR spectral and microanalytical data and evaluated for their antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*) and Gram-negative bacteria (*Escherichia coli*).

Keywords: pyrrolo[2,3-*d*]pyrimidine, aryl isothiocyanates, DABCO, antibacterial**.**

Introduction

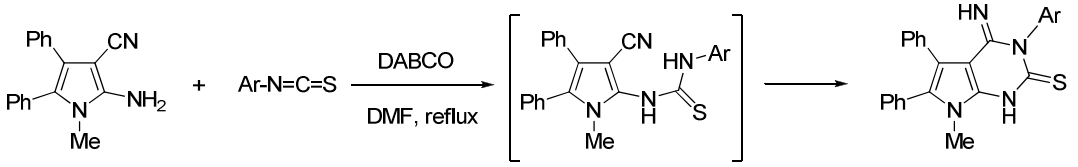
Pyrrolo[2,3-*d*]pyrimidines are a class of fused heterocycles interesting from view point of biological activities. These compounds are reported to possess significant anti-HIV,[1] antimicrobial,[2], antitumor,[3] antiviral,[4] and antiangiogenic[5] activity with potential application as enzyme inhibitors.[6,7] Furthermore, a number of these compounds have been substantially investigated as a part of the synthesis of new C-nucleosides with potential biomedical interest, since they have been found to exhibit pronounced growth inhibitory activity to several leukemic cell lines.[8-11] On the other hand, pyrrolo[2,3-*d*]pyrimidine moiety (**I**) (Fig. 1) may be regarded as an analogue of purine (**II**) in which its N-7 (purine numbering) has been replaced by a CH group and therefore can be named as 7-deazapurine. This moiety has been shown to induce neurogenesis in murine embryonic stem cells[12] and synthesized as analogues of potent A1- and A2-adenosine receptor antagonists.[13]



(I) (II)

Figure 1. Structures of pyrrolo[2,3-*d*]pyrimidine (7-deazapurine) moiety (**I**) and purine (**II**)

Inspired by these facts and due to our interest in the synthesis of heterocyclic compounds with potential biological activities,[14-25] we report here a convenient synthesis of new 3-aryl-4-imino-7-methyl-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-thiones **3a-d** by reaction of 2-amino-1-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile with aryl isothiocyanates in the presence of 1,4-diazabicyclo[2.2.2]octane (DABCO) as a base catalyst in boiling DMF (Scheme 1). Antibacterial assay was examined against two strains of Gram positive bacteria, *Staphylococcus aureus* (*S. aureus*, PTCC† 1112) and *Micrococcus luteus* (*M. luteus*, PTCC 1110), and one strain of Gram negative bacteria, *Escherichia coli* (*E. coli*, PTCC 1330) using microtiter plate technique.



Scheme 1. Synthesis of new 3-aryl-4-imino-7-methyl-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-thiones

Experimental

Equipment

Melting points were recorded on a Stuart SMP3 melting point apparatus. The IR spectra were obtained with KBr disks using a Tensor 27 Bruker spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 300 FT spectrometer at 300 and 75 MHz frequencies for ^{1}H and ^{13}C, respectively, using TMS as internal standard. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer.

Materials and Methods

All chemicals were purchased from Merck and Aldrich and used without additional purification. Bacterial strains were obtained from the Iranian Research Organization for Science and Technology (IROST) in Iran. The antibacterial activity of the synthesized compounds was examined against *S. aureus* PTCC 1112 and *M. luteus* PTCC 1110 as Gram-positive and *E. coli* PTCC 1330 as Gram-negative bacteria in 24-well microtiter plates using molten Mueller Hinton agar (QUELAB- Canada) cooled to 45-50 ^{o}C in a water bath. Synthesized compounds were solved in dimethyl sulfoxide (DMSO). These solutions with the sterile distilled water added to the wells with a final concentration of 6 mg/ml. After the mixing and solidification of the media, 0.01 mL of every bacterial suspension, equivalent to McFarland tube No. 0.5 (10^{8} CFU/mL), inoculated on the agar of every well. The culture plates were then incubated at 37 ^{o}C for 24 hours. After reporting of the results, bacteria from the surface of every well were inoculated on Mueller- Hinton agar plates without synthesized compounds to know bacteriostatic or bactericidal effect of compounds.[26,27] The Mueller Hinton agar contained DMSO was used as negative control while gentamycin was used as positive control. After re-inoculation, growth of bacteria showed the bacteriostatic effect and lack of growth showed the bactericidal effect of synthesized compounds.

General procedure for the synthesis of 3-aryl-4-imino-7-methyl-5,6-diphenyl-3,4-dihydro-1H-pyrrolo[2,3-d]pyrimidine-2(7H)-thiones ***4a-d***

† Persian Type Culture Collection

To a solution of 2-amino-1-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile **1** (1 mmol) and DABCO (0.5 mmol) in DMF (10 ml), an aryl isothiocyanate **2a-d** (1 mmol) was added. The reaction mixture was heated under reflux for 5-6 h. The reaction was monitored by TLC. Upon completion, the solvent was evaporated in *vacuo* at 70 °C, the residue was dissolved in water (20 ml), and subsequently neutralized by 1 N HCl. The crude product was collected and washed with hot ethanol and hot ethyl acetate, separately and repeatedly, to give compounds **4a-d** in 83-91 % yields.

4-Imino-7-methyl-3,5,6-triphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-thione (**4a**): Yield 85%; M.P. 277-279 °C; IR (KBr disc): υ 3445, 3399, 3055, 2952, 1608, 1555, 1493, 1437, 1339, 1262, 1206, 954, 756, 704 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.77 (s, 3H, CH_3), 6.88 (s, 1H, NH), 6.90 (t, J = 7.2 Hz, 1H, arom-H), 7.15 (t, J = 7.8 Hz, 2H, arom-H), 7.25-7.40 (m, 11H, arom-H and NH), 7.58 (d, J = 8.1 Hz, 2H, arom-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.0, 100.7, 113.0, 119.2, 122.2, 127.4, 127.5, 128.2, 128.4, 128.7, 128.8, 130.4, 130.7, 130.8, 134.4, 139.3, 151.8, 153.5, 162.2; Found, %: C 73.42; H 4.86; N 13.80; S 7.77. $C_{25}H_{20}N_4S$. Calculated, %: C 73.50; H 4.93; N 13.71; S 7.85.

4-Imino-7-methyl-3-(4-methylphenyl)-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-thione (**4b**): Yield 88%; M.P. 295-296 °C; IR (KBr disc): υ 3441, 3395, 3067, 2914, 1608, 1553, 1504, 1433, 1337, 1265, 1205, 955, 764, 705 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.19 (s, 3H, CH_3), 3.78 (s, 3H, CH_3), 6.82 (s br., 1H, NH), 6.93 (d, J = 8.1 Hz, 2H, arom-H), 7.25-7.40 (m, 11H, arom-H and NH), 7.45 (d, J = 8.1 Hz, 2H, arom-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 30.1, 100.6, 113.1, 119.3, 127.5, 128.2, 128.4, 128.6, 128.8, 129.2, 130.4, 130.8, 131.8, 134.3, 134.4, 136.7, 151.6, 153.5, 162.0; Found, %: C 73.81; H 5.33; N 13.38; S 7.42. $C_{26}H_{22}N_4S$. Calculated, %: C 73.90; H 5.25; N 13.26; S 7.59.

4-Imino-7-methyl-3-(3-methylphenyl)-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-thione (**4c**): Yield 91%; M.P. 291-294 °C; IR (KBr disc): υ 3425, 3393, 3055, 2945, 1621, 1558, 1485, 1442, 1386, 1265, 1209, 954, 767, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 3.74 (s, 3H, CH_3), 6.74 (d, J = 7.5 Hz, 1H, arom-H), 6.85 (s br., 1H, NH), 7.02 (t, J = 7.8 Hz, 1H, arom-H); 7.20-7.40 (m, 12H, arom-H and NH), 7.68 (s, 1H, arom-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.6, 30.0, 100.7, 113.0, 116.2, 119.8, 123.0, 127.5, 128.1, 128.4, 128.5, 128.8, 130.4, 130.7, 130.8, 134.3, 134.4, 136.8, 139.3, 151.7, 153.6, 162.2; Found, %: C 73.79; H 5.14; N 13.13; S 7.74. $C_{26}H_{22}N_4S$. Calculated, %: C 73.90; H 5.25; N 13.26; S 7.59.

3-(3-Chlorophenyl)-4-imino-7-methyl-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7*H*)-thione (**4d**): Yield 83%; M.P. 272-274 °C; IR (KBr disc): υ 3426, 3389, 3058, 2927, 1616, 1552, 1475, 1432, 1384, 1333, 1273, 1204, 953, 764, 702 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 3.75 (s, 3H, CH_3), 6.85-6.88 (m, 2H, arom-H and NH), 7.03 (t, J = 8.1 Hz, 1H, arom-H); 7.15-7.50 (m, 12H, arom-H and NH), 7.65 (t, J = 2.1 Hz, 1H, arom-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 30.0, 100.9, 112.8, 117.3, 119.0, 122.2, 127.7, 128.2, 128.3, 128.4, 128.9, 129.7, 130.2, 130.7, 134.3, 134.8, 136.0, 140.5, 151.9, 153.3, 162.1; Found, %: C 67.96; H 4.46; N 12.57; S 7.16. $C_{25}H_{19}ClN_4S$. Calculated, %: C 67.79; H 4.32; N 12.65; S 7.24.

Results and discussion

The starting material 2-amino-1-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile **1** was prepared according to the literature method.[28] Cyclocondensation of this compound with aryl isothiocyanates **2a-d** in the presence of DABCO as a base catalyst in DMF under reflux gave products identified as 3-aryl-4-imino-7-methyl-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7H)-thiones **4a-d**. None or only trace amounts of the products were formed in the absence of DABCO in the reaction conditions, indicating that the presence of DABCO is

necessary for the reaction. The structure of new products **4a-d** was established from their spectral and microanalytical data. For example, the ^{1}H NMR spectrum of compound **3b** in $CDCl_3$ showed two singlets at δ = 2.19, and 3.78 ppm for methyl groups, a single broad band at δ = 6.82 ppm for one of the NH groups, two doublets at δ = 6.93, and 7.45 ppm for *para*-substituted aromatic ring protons as well as the characteristic signals at δ = 7.25-7.40 ppm for two phenyl groups. The signal for another NH proton has overlapped with the aromatic signals. The IR spectrum was devoid of the CN absorption band at 2205 cm^{-1} of the precursor, which shows the inclusion of the nitrile moiety in the cyclocondensation process. Further proof came from ^{13}C NMR spectrum which showed the characteristic signals at δ 20.7 and 30.1 ppm for methyl groups as well as the other signals in the aromatic region. Also this compound gave satisfactory elemental analysis data corresponding to the molecular formula $C_{26}H_{22}N_4S$. The formation of the products **4a-d** is assumed to proceed *via* the intermediate **3a-d** prepared by initial nucleophilic attack of the amino group activated by DABCO in 2-position of pyrrole at the carbon site of isothiocyanates **2a-d**. However, under the conditions used, attempts to isolate intermediate **3a-d** failed, even after careful monitoring of the reaction (Scheme 1).

The synthesized compounds **4a-d** were screened for the antibacterial activity against reference strains of *S. aureus*, *M. luteus* and *E. coli* bacteria. All compounds inhibited the growth of tested bacteria at the concentration of 6 mg/ml. The results revealed that compounds **4b-d** have bactericidal activity against *M. luteus* and bacteriostatic activity against *S. aureus* and *E. coli*, while compound **4a** has bactericidal effect on all microorganisms tested.

In conclusion, we have reported a convenient synthesis of some new 3-aryl-4-imino-7-methyl-5,6-diphenyl-3,4-dihydro-1*H*-pyrrolo[2,3-*d*]pyrimidine-2(7H)-thiones by cyclocondensation reaction of 2-amino-1-methyl-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile with aryl isothiocyanates in the presence of DABCO as a base catalyst in refluxing DMF. Antibacterial assay of the synthesized compounds showed that most of them have good bactericidal and bacteriostatic effects on *S. aureus*, *M. luteus* and *E. coli* bacteria.

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