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Derivatives of 6-Mercaptopurine: Synthesis and Biological evaluation

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ABSTRACT

Novel 7H-purine-6-thiol derivatives were synthesized by condensation of 6-mercaptopurine with different aliphatic as well as aromatic halides in presence of base in good to excellent yield. Structures of all the prepared compounds were deduced from the spectrometric studies. The compounds were also evaluated for their antimicrobial activities.

KEY WORDS: Purine, 6-Mercaptopurine

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INTRODUCTION

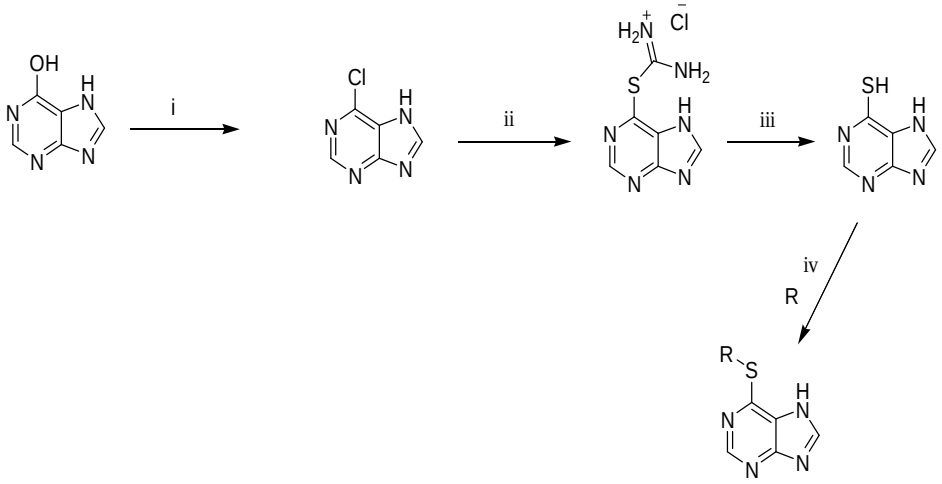
The purine ring has attracted much attention owing to its omnipresence in nature as the core structure of adenine and guanine in nucleic acids that exist at relatively high concentrations in living organisms. In addition, purines[1] are involved in many metabolic processes as cofactors associated with a great number of enzymes and receptors which play key roles at different phases of the cell cycle. For this reason purine derivatives might be expected to have a high probability of yielding bioactive compounds. As a consequence, in the past few decades, enormous efforts have been made for the construction, modification and decoration of this privileged structure. As a result, two main methods for building up purine ring system are developed. The first way includes synthesis of pyrimidine nuclei with subsequent introduction of appropriate substituent for formation of purine ring[2] . The second route begins with the synthesis of imidazole ring, followed by closure of pyrimidine ring.

More particularly, sulfur-containing purines i.e. 6-mercaptopurine, 6-thioguanine, and azathioprine, have been considered as prominent molecular motif that are frequently encountered in drugs used for cancer chemotherapy (Fig. 1). For example,6-Mercaptopurine is widely used as an ant leukemic agent in the lymph proliferative disorders, including lymphoma, childhood acute lymphoblastic leukemia, and other neoplastic conditions, whereas, Azathioprine[3] have been found to inhibit the growth of certain human malignant tumor cells. Various pharmacological effects of synthetic thiopurines including antiviral, antibacterial, antitumor, and antifungal activity were developed for treatment of patients with infections of human immunodeficiency virus (HIV) and infections caused by herpes and hepatitis virus. However, the use of thiopurines is limited by their toxicities, which include hepatotoxi city, my elosuppression, pancreatitis, and allergic reactions. For this reason, synthesis of new thiopurine[4] derivatives for improved biological activities and reduced toxicity is a never ending interest for medicinal chemists. Based on the above facts and our ongoing interest for the synthesis and biological evaluation of heterocyclic compound we report herein the synthesis of a new series of antimicrobial agents based on purine derivatives.

RESULT AND DISCUSSION

Synthesis begun with chlorination of hypoxanthine[5] using $POCl_3$ in toluene to gave 6-chloropurine, which upon coupling with thiourea in acetonitrile, followed by treatment with boiling ethanol delivered 6-mercaptopurine. Having synthesized the 6-mercaptopurine, next we focused on generation of 6-mercaptopurine derivative library by coupling of 6-mercaptopurine with different halides in the presence of base in suitable solvent. The compounds were further purified by crystallization using suitable solvents (as listed in Table-1). To our delight aliphatic

as well as aromatic halides were proved to be successful coupling partner to give good to excellent yield. The structures of all the prepared compounds including intermediates were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectroscopic studies. Motivated by these results we became interested in further evaluation of biological activities.

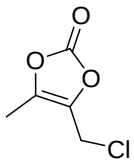
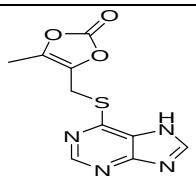
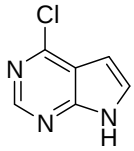
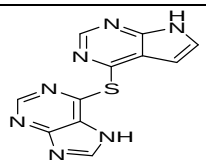
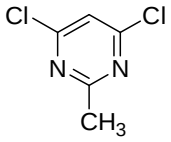
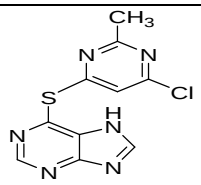
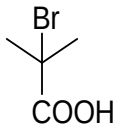
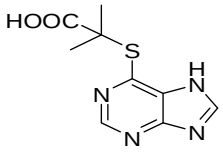
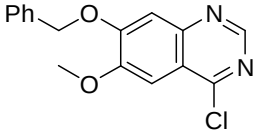
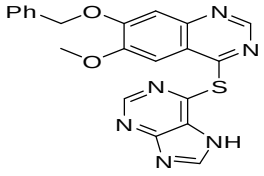
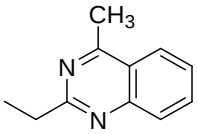
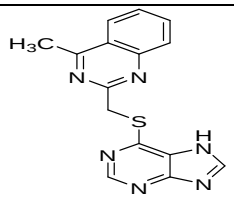
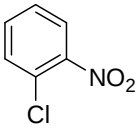
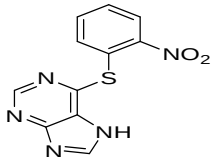


Mercaptopurine derivaties (Entry 1-9)

Scheme 1: Synthesis of mercaptopurine derivatives (1-9)

Reagents and conditions: (i) $POCl_3$, Toluene, 110°C, 4 h, (ii) Thiourea, Acetonitrile, reflux, 12 h, (iii) EtOH, , reflux, 2-3 h. (iv) base and solvent.

Table 1: Chemical structure and reaction conditions of purine derivatives.

Entry	R	Structure	Solvent	Base	%Yield
1			Acetone and water mixture	Liq Ammonia	63.4
2			Dimethyl formamide	Potassium carbonate	67.79
3			Chloroform	Triethylamine	85
4			Dichloromethane	Triethylamine	25.6
5			Dimethyl acetamide	potassium carbonate	46.2
6			Dimethyl formamide	potassium carbonate	80
7			Dimethyl sulphoxide	Caesium carbonate	85

Biological activities of the synthesized Purine derivatives

An antibacterial is a substance that either kills bacteria or inhibits their growth or both. An antifungal drug is a medication used to treat fungal infection such as athlete's foot, ring worm, and other. A number of purine derivatives were synthesized (Table 1). The minimum inhibition

concentration (MIC) values of the compounds were determined by the Microdilution method using Gram-positive bacteria *Bacillus subtilis* and Gram-negative bacteria *Escherichia coli* and Fungi *Aspergillusniger*. The microbiological assay is based upon a comparison of inhibition of growth of micro-organisms by measured concentrations of test compounds with that produced by known concentration of a standard antibiotic. Two methods generally employed are turbidometric (tubedilution) method and cup-plate method. In the turbidometric method, inhibition of growth of microbial culture in a uniform solution of antibiotic in a fluid medium is measured and is compared with the synthesized compounds. Here, the presence or absence of growth is investigated. The cup plate method depends upon diffusion of antibiotic from a well through a solidified agar layer in a Petridish or plate to an extent such that growth of added microorganisms is prevented entirely in a zone around the well containing solution of the antibiotics. The cup-plate method is simple and measurement of inhibition of microorganisms is also easy. Here, we have use this method for antibacterial screening of the test compounds. The media was prepared from nutrient agar 3%, peptone 1%, beef extract 0.5%, sodium chloride 0.5%. All the ingredients were weighed and added to water. This solution was heated on water bath for about one and half-hour till it became clear. This nutrient media was sterilized by autoclave. The antibacterial and antifungal activity was measured against *Bacillus subtillis* was used as Gram-positive bacteria, and *Escherichia coli* was used as Gram-negative bacteria and Aspergillusniger was used as fungi for this study. The master culture was prepared on agar slant of the above nutrient media and kept in refrigerator. The working culture was prepared from it by weekly transferred in nutrient agar medium . Preparation of inoculums In the aseptic condition from the working culture, small amount of culture was transferred to about 10–15 mL of sterile normal saline (0.9% NaCl solution). This solution was gently mixed and used for the antibacterial activity. About 0.1 mL of inoculum was added to the sterilized Petridish and melted agar cooled to 45°C was added, mixed gently, and allowed to solidify. Then, Watmann filter paper disk was kept in each plate which was soaked in test drug solutions. The solution was allowed to diffuse for a period of 90 min. The Petri dishes were then incubated at 37°C for 24 h after which zone of inhibition was measured. Preparation of test solution Specified quantity of the compound was weighed and dissolved in 5 mL of DMSO and further dilution was made to get the concentration of 250 and 500 µg/mL. Similarly, the standard drugs Ampicillin, Cephalexin, and Miconazole were dissolved in appropriate quantity of water to obtain the concentration of 250 and 500 µg/mL each. The images of zone of inhibition are given in Figures 1and the results are shown in Table 2. The compound which having the antibacterial effect show the zone of inhibition around the well. The zone of inhibition is measured in mm. If the zone diameter is high it indicates more antibacterial affect against respective organism

Figure 1 Zone of inhibition for Entry-5

Table 2: Biological screening of the synthesized compounds

Entry	Concentration (mic/ml)	Zone of inhibition (mm)		
		E.coli	Bacillus subtilis	Aspergillus niger
I.	250	16	12	0
	500	16	18	0
II.	250	0	12	10
	500	0	12	15
III.	250	11	0	15
	500	13	0	20
IV.	250	0	0	10
	500	0	0	10
V.	250	16	11	20
	500	16	13	25
VI.	250	0	0	10
	500	0	0	10
VII.	250	0	14	10
	500	0	15	10
VIII.	250	0	0	20
	500	0	0	10
IX.	250	0	0	0
	500	0	0	0

EXPERIMENTAL SECTION

Synthesis of compound-1

Acetone (20 ml) and Water (10 ml) was added to flask containing 7H-purine-6-thiol (2 g, 13.14 mmol). To this 4-(chloromethyl)-5-methyl-1,3-dioxol-2-one (2.1 g, 14.14 mmol) followed by 20% Liq Ammonia solution (1 ml) was added. The solution was stirred for 15 hours and cooled to 5°C by using ice. Acetic acid (2 ml) was added to the reaction mixture. The reaction mixture was stirred for 2h at 5°C. The solvent was removed by vacuum resulting yellow powder purified by acid-base treatment in Acetone and water mixture to yield 2.2 gm (63.4%) 4-((7H-purin-6-ylthio)methyl)-5-methyl-1,3-dioxol-2-one (1) HPLC Purity-92.44%; melting point-180-182^{0}C; ^{1}H NMR (DMSO, 500 MHz) δ ppm 13.61 (S, 2H), 8.75 (S, 1H), 8.472 (S,1H), 4.55 (S, 2H), 2.079 (S,3H); ^{13}C NMR (DMSO, 100 MHz) δ ppm 156.91, 152.41, 151.72, 149.99, 143.86, 137.77, 135.04, 130.79, 20.82, 9.59; MS (m/z): 264.65 [M^+., $C_{10}H_8N_4O_3S$]

Synthesis of compound-2

Dimethyl form amide (5 ml) was added to flask containing 7H-purine-6-thiol (1 g, 6.57 mmol). Added 4-chloro-7H-pyrrolo[2,3-d] pyrimidine (1.51 g, 9.83 mmol). To this potassium carbonate (3.63g, 26.26 mmol) was added. The solution was stirred for 8 hours at 55 to 60°C and cooled to 25°C by using ice. Filtered the reaction mixture. Water (20 ml) and Acetic acid (2 ml) was added to the reaction mixture. The reaction mixture was stirred for 2h at 5°C. The solvent was removed by vacuum resulting off white powder.

Obtained solid was purified by Acetone to yield 1.2 gm (67.79%) 6-(7H-pyrrolo[2,3-d]pyrimidin-4-ylthio)-7H-purine (2); MS (m/z): 269.65 [M^+., $C_{11}H_7N_7S$]

Synthesis of compound-3

Chloroform (30 ml) was added to flask containing 7H-purine-6-thiol (2 g, 13.14 mmol). To this 4,6-dichloro-2-methylpyrimidine (3.21 g, 19.71 mmol) followed by Triethylamine (6.2 g,52.56mmol) was added. The solution was stirred for 8 hours at reflux and cooled to 25°C. Water (50 ml) followed by con Hydrochloric acid (5 ml) was added to the reaction mixture. The reaction mixture was stirred for 30 min at 25°C. Separated the layers, concentrated the organic layer completely under vacuum. Acetone (20 ml) was added to the residue. Stirred the reaction mixture for 2h at 25°C, further cooled the reaction mixture to 5°C. The reaction mixture was stirred for 2h. The solvent was removed by vacuum resulting off white powder.Obtained solid was taken in methanol (20ml) heat to 55 to 60°C to get clear solution. Cool to 0 to 5°C. Reaction mixture was stirred for 2h

at 0 to 5°C. The solvent was removed by vacuum resulting off white powder of 6-(6-chloro-2-methylpyrimidin-4-ylthio)-7H-purine. Yield (3.1 g, 85%); MS (m/z): 278.65 [M^+., $C_{10}H_7ClN_6S$]

Synthesis of compound-4

Dichloromethane (20 ml) was added to flask containing 7H-purine-6-thiol (2 g, 13.14 mmol). To this 2-bromo-2-methylpropanoic acid (4.39 g, 26.28 mmol) followed by Triethylamine (3.1 g, 26.28 mmol) was added. The solution was stirred for 12 hours at 25°C. Reaction mixture was concentrated completely. Water (50 ml) followed by Acetic acid (3 ml) was added to the reaction mixture. The reaction mixture was stirred for 2 hours, further cooled the reaction mixture to 5°C. The reaction mixture was stirred for 2h. The solvent was removed by vacuum resulting off white powder.

Obtained solid was taken in Acetone (30ml) heat to 50 to 55°C to get clear solution. Cool to 0 to 5°C. Reaction mixture was stirred for 2h at 0 to 5°C. The solvent was removed by vacuum resulting off white powder of 2-(7H-purin-6-ylthio)-2-methylpropanoic acid. Yield (800 mg, 25.6%) ; MS (m/z): 238.85 [M^+., $C_9H_{10}N_4O_2S$]

Synthesis of compound-5

Dimethyl acetamide (10 ml) was added to flask containing 7H-purine-6-thiol (2 g, 13.14 mmol). To this 7-(benzyloxy)-4-chloro-6-methoxyquinazoline (4.2 g, 19.71 mmol) followed by potassium carbonate (6.25 g, 26.28 mmol) was added. The solution was stirred for 12 hours at 25°C. Water (100 ml) followed by Acetic acid (5 ml) was added to the reaction mixture. The reaction mixture was stirred for 2 hours, further cooled the reaction mixture to 5°C. The reaction mixture was stirred for 2h. The solvent was removed by vacuum resulting off white powder.Obtained solid was taken in Acetone (50ml) heat to 50 to 55°C to get clear solution. Cool to 0 to 5°C. Reaction mixture was stirred for 2h at 0 to 5°C. The solvent was removed by vacuum resulting off white powder 4-(7H-purin-6-ylthio)-7-(benzyloxy)-6-methoxyquinazoline. Yield (2.5 g, 46.2%); MS (m/z): 417.10 [M^+., $C_{21}H_{16}N_6O_2S$]

Synthesis of compound-6

Dimethyl form amide (10 ml) was added to flask containing 7H-purine-6-thiol (2 g, 13.14 mmol). To this 2-(chloromethyl)-4-methylquinazoline (2.78 g, 14.45 mmol) followed by potassium carbonate (6.25 g, 26.28 mmol) was added. The solution was stirred for 4 hours at 60 to 65°C. Water (50 ml) followed by Acetic acid (5 ml) was added to the reaction mixture. The reaction mixture was stirred for 2 hours, further cooled the reaction mixture to 5°C. The reaction mixture was stirred for 2h. The solvent was removed by vacuum resulting off white powder.Obtained solid was taken in Ethyl Acetate (20ml) heat to 50 to 55°C. Cool to 0 to 5°C. Reaction mixture was stirred for 2h at 0 to

5°C. The solvent was removed by vacuum resulting off white powder 2-((7H-purin-6-ylthio) methyl)-4-methylquinazoline. Yield (2.02 g, 80%); MS (m/z): 306.95 [M^+., $C_{15}H_{12}N_6S$]

Synthesis of compound-7

Dimethyl sulphoxide (10 ml) was added to flask containing 7H-purine-6-thiol (2 g, 13.14 mmol). To this 1-chloro-2-nitrobenzene (3.1 g, 19.71 mmol) followed by Caesium carbonate (8.5 g, 26.28 mmol) was added. The solution was stirred for 4 hours at 60 to 65°C. Water (70 ml) followed by Con Hydrochloric acid (2.4 ml) was added to the reaction mixture. The reaction mixture was stirred for 3 hours. The solvent was removed by vacuum resulting off white powder.Obtained solid was taken in Methanol (40ml) heat to 50 to 55°C. Cool to 0 to 5°C. Reaction mixture was stirred for 2h at 0 to 5°C. The solvent was removed by vacuum resulting off white powder 6-(2-nitrophenylthio)-7H-purine. Yield (1.76 g, 85%); MS (m/z): 273.95 [M^+., $C_{11}H_7N_5O_2S$]

Synthesis of compound-8

Dichloromethane (15 ml) was added to flask containing 7H-purine-6-thiol (1 g, 6.57 mmol). To this 1-(2-chloroacetyl) pyrrolidine-2-carboxamide (1.5 g, 7.89 mmol) followed by Triethyl amine (1.33 g, 13.14 mmol) was added. The solution was stirred for 6 hours at 25°C. Water (15 ml) was added. Layers were separated. Organic layer was extracted by 10 ml 1N HCl. Organic layer was concentrated completely under reduce pressure. Acetone (10 ml) was charged to the residue, stirred reaction mixture for 2 hours. The solvent was removed by vacuum resulting off white powder. Obtained solid was taken in Methanol (15ml) heat to 50 to 55°C. Cool to 0 to 5°C. Reaction mixture was stirred for 2h at 0 to 5°C. The solvent was removed by vacuum resulting off white powder 1-(2-(7H-purin-6-ylthio)acetyl)pyrrolidine-2-carboxamide. Yield (1.65 g, 82%); MS (m/z): 306.85 [M^+., $C_{12}H_{14}N_6O_2S$]

Synthesis of compound-9

Tetrahydrofuran (10 ml) was added to flask containing 7H-purine-6-thiol (1 g, 6.57 mmol). To this 4-Bromomethyl-2-biphenylcarbonitrile (2.14 g, 7.86 mmol) followed by Triethyl amine (1.33 g, 13.14 mmol) was added. The solution was stirred for 15 hours at 25°C. Reaction mixture was concentrated completely under reduce pressure. Water (10 ml) was charged to the residue, stirred reaction mixture for 2 hours. The solvent was removed by vacuum resulting off white powder. Obtained solid was taken in Isopropyl alcohol (15ml) heat to 70 to 55°C. Cool to 0 to 5°C. Reaction mixture was stirred for 2h at 0 to 5°C. The solvent was removed by vacuum resulting off white powder of compound PU-9. Yield (1.69 g, 75%); MS (m/z): 344.15 [M^+. $C_{19}H_{13}N_5S$]

CONCLUSION

In summary we have synthesized 6-mercaptopurine derivatives via coupling of 6-mercaptopurine and aliphatic/aromatic halides using base in suitable solvent. The compounds were further purified by crystallization in suitable solvent to obtained desired 6-mercaptopurin derivatives in good to excellent yield having high purity. Structure of the compounds were determined by spectroscopic analysis i.e. 1H NMR, 13C NMR, Mass spectroscopy. Successful synthesis of a series of biologically active 6-substituted purines from commercially available 6-Mercaptopurine and evaluation of the antifungal activities. Nine derivatives were prepared. From the results of the antifungal screening, it can be concluded that the six new derivatives 2, 3, 4, 5, 6, 7 and 8 were found to be active against the fungal strains. Therefore they may be used as lead compounds for further development.

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