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Sulfonamide and carbamate derivatives of 6-chloropurine: synthesis, characterization and antimicrobial activity evaluation

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Abstract: A series of new sulfonamide derivatives, 9-(substitutedbenzenesulfonyl)-6-chloro-9*H*-purines **7(a-e)** and carbamate derivatives, 6-chloro-purine-9-carboxylic acid substituted alkyl/arylester **9(a-d)**, have been synthesized through an intermediate, sodium salt of 6-chloro-9(*H*)-purine (**6**) which was prepared by the treatment of 6-chloro-9(*H*)-purine (**4**) with sodium hydride. Structures of the newly synthesized compounds were elucidated by IR, NMR (^{1}H and^{13}C), mass spectra and elemental analysis. Antimicrobial activity against three bacterial strains and three fungal strains at two different concentrations, 100 and 200 µg/mL including MIC values was investigated. Bio-screening data disclosed that most of the sulfonamide derivatives, **7a**, **7c** and **7d**, and one carbamate derivative **9a** showed promising antimicrobial activity having MIC values in the range of 18.0-25.0 µg/mL.

Keywords: 6-Chloro-9(*H*)-purine; sulfonamides; carbamates; antimicrobial activity; minimum inhibitory concentration.

1. Introduction

It is well known that nitrogen heterocyclic compounds, particularly, purine derivatives are important targeting scaffolds for many biosynthetic, regulatory and signal transduction proteins including cellular kinases, G proteins and polymerases.[1-6] Also, they are basic structures in nucleic acids like adenine and guanine which are essential for genetic and many metabolic processes in living organisms. Purine derivatives have been playing indispensible role in different phases of the cell cycles, in cell signaling and other fundamental reactions in the biological system due to a great number of enzymes and receptors are associated with them.[7,8] Some purine derivatives like N^6-[(3-methylbut-2-en-1-yl)amino]-purine (**1**) showed cytokinin activity,[9] isoprenoid cytokinins are able to influence plant growth development and cell differentiation,[10] and agelasimines[11] (**2** and **3**) (**Figure 1**)

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exhibited promising biological activities such as antimicrobial and cytotoxic effects. Purine nucleosides and their substituted analogues also exhibited numerous biological activities such as enzyme inhibitors,[12] cytotoxic,[13] antihypergylcemic,[14] antiviral,[15] immunostimulant[16] antifungal and antibacterial agents.[17]

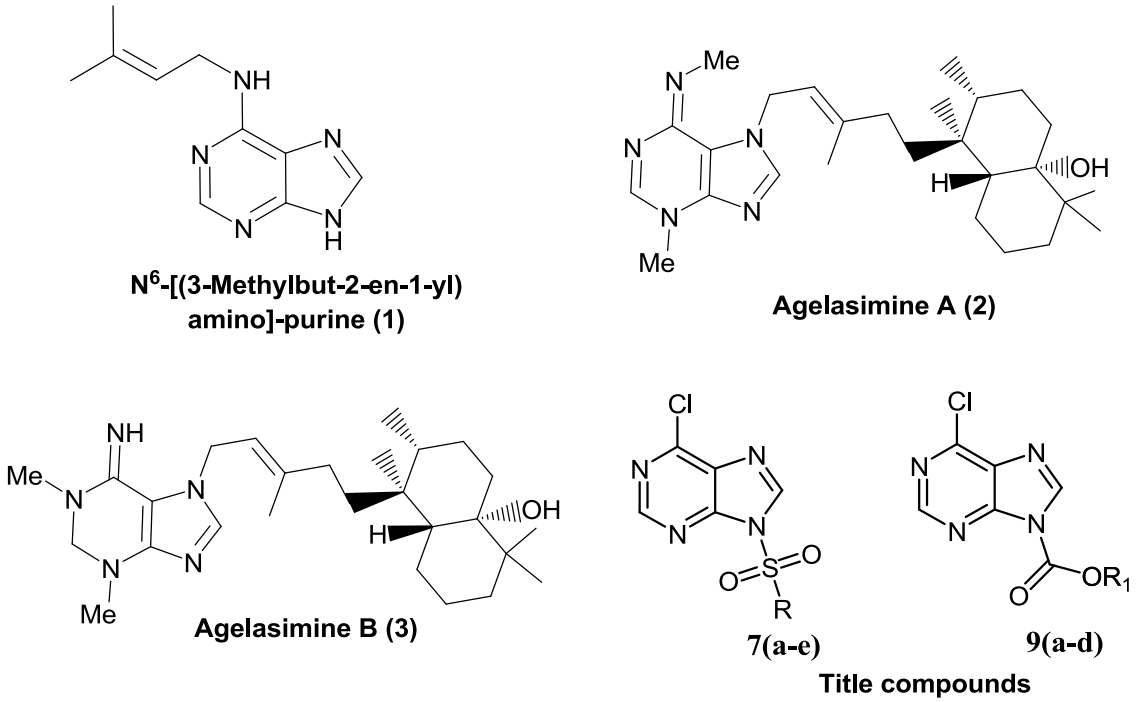


Figure 1. Some biologically active purine derivatives

Active research has been going on sulfonamide derivatives from the last five decades due to their unique importance in both chemical and biological aspects. Generally, the preparation of sulfonamide and carbamate derivatives of 6-chloropurine requires as harsh conditions, however, 9-arylsulfonyl derivatives of 6-chloropurine were synthesized using KOH (aq) as a base in acetone at 0 °C.[18] They exhibited high antimycobacterial activity (MIC 0.39 µg/mL against M. tuberculosis). Primarily, sulfonamides (-SO_2-N-) are bacteriostatic agents or an acetylated metabolite finds use in both human therapy and animal husbandry.[19] The sulfonamide derivatives have been found to be used in various biological application such as antitumor,[20] hypoglycemic,[21] anti-thyroid,[22] anti-carbonic anhydrase,[23] anti-inflammatory,[24] diuretic,[25] COX-inhibitors, the enzyme dihydropteroatesynthetase (DHPS)-the key enzyme involved in folate synthesis, anti-impotent drugs.[26] Organic carbamates are valuable synthetic intermediates and found in a variety of biologically active compounds.[27-28] For example, *O*-alkyl and *O*-aryl carbamate derivatives of the antimalarial drug, primaquine is a potential pro-drug that prevent oxidative deamination to the inactive metabolite carboxyprimaquine.[29] Recently, our group have been synthesized sulfonamide and carbamate derivatives of (2'-(1*H*-tetrazol-5-yl)-biphenyl-4-yl)methanamine and carvedilol and evaluated their antimicrobial activity, a few of them exhibited potent antimicrobial activity.[30,31]

By considering an overview facts and our continuing research in the synthesis of biologically active sulfonamide/carbamate derivatives, herein, we report the synthesis of a series of new sulfonamide and carabamate derivatives of 6-chloropurine for biological interest and their antimicrobial activity was screened.

2. Results and Discussion

Recently, our group synthesized phosphoramidate derivatives of 6-chloropurine showed potent antimicrobial activity[32] and these consequences encouraged us to design and synthesize a new series of 6-chloropurine derivatives. As in the continuation of our medicinal chemistry programme and

considering the biological potency of sulfonamide and carbamate derivatives, we synthesized a series of new sulfonamide and carbamate derivatives of 6-chloropurine and the schematic representation is depicted in **Figure 2**.

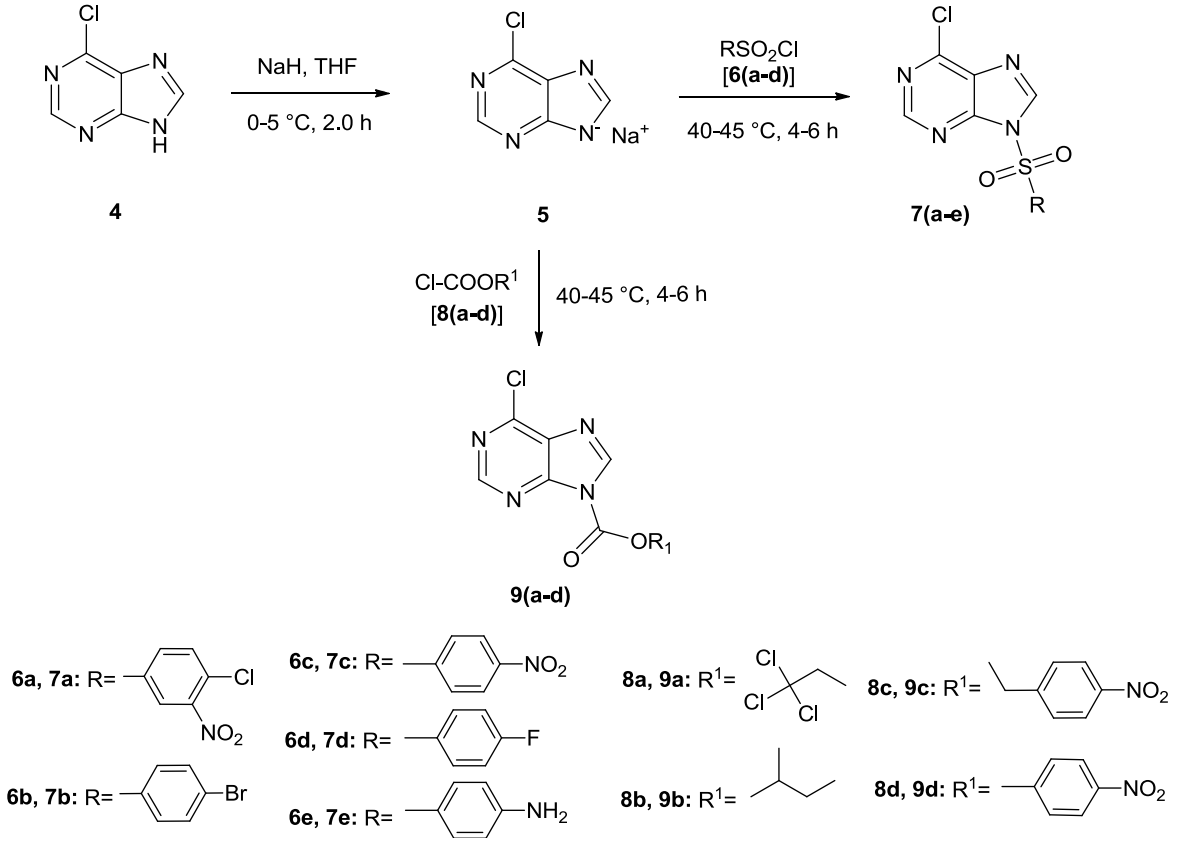


Figure 2. Synthesis of sulfonamide and carbamate derivatives of 6-chloropurine.

The title compounds were synthesized by *in situ* in two steps. Initially, 6-chloropurine **(4)** was treated with a base, sodium hydride at 5-20 °C in THF to get an intermediate, sodium salt of 6-chloropurine **(5)**. Subsequently, the intermediate **5** was directly reacted with substituted phenyl sulfonyl chlorides **6(a-e)** and substituted alkyl/phenyl chloroformates **8(a-d)** at 10-40 °C to obtain the title sulfonamides **7(a-e)** and carbamates **9(a-d)** of 6-chloropurine, respectively. After completion of the reaction, NaCl salt was filtered and the filtrate was concentrated under vacuum to get the crude products. Column chromatography was adopted to purify the crude compounds using 15-25% of methanol and chloroform as mobile phase (**Table 1**).

Structures of the newly synthesized pure compounds were elucidated by spectroscopic (IR, ^{1}H-, ^{13}C NMR and mass) data and elemental analysis. In IR spectra, absorption bands in the range of 1360-1385 cm^{-1} (str) and 1180-1195 cm^{-1} (ben) in the sulfonamide derivatives **7(a-e)**, and 1750-1770 cm^{-1} (str) in carbamate derivatives **9(a-d)** were confirmed the presence of -SO_2 and -CO functionalities, respectively. In ^{1}H NMR, two protons as singlet in the region of 8.65-8.75 ppm and 8.85-8.97 ppm were assigned to 6-chloropurine moiety and other protons of the corresponding structures were observed in their expected region. The carbon chemical shift values in the range of 158-163 ppm in the carbamate derivatives **9(a-d)** are confirmed -C(O)-. The aromatic and aliphatic carbon signals are recognized in their corresponding region. In addition, the molecular ion peaks of the corresponding mass of the compounds in their mass spectra and the relative CHN composition of the title compounds in the elemental analysis were given further evidence to elucidate the structures of the newly synthesized compounds.

The antibacterial and antifungal activities of the synthesized sulfonamide derivatives **7(a-e)** and carbamates **9(a-d)** of 6-chloropurine were investigated using disc diffusion[33,34] and agar disc-diffusion[35-37] methods, respectively. The bacterial strains like *Streptococcus aureus* (ATCC-25923),

Pseudomonas aeruginosa (ATCC-25619) and *Escherichia coli* (ATCC-9637), and fungi such as *Aspergillus flavus* (MTCC-1884), *Aspergillus niger* (MTCC- 1881) and *Candida albicans* (ATCC-2091) were chosen to screen the antimicrobial activity at two different concentrations, 100 and 200 µg/mL. Ciprofloxacin and Fluconazole were used as the standards in the antibacterial activity and antifungal activity, respectively and the samples of the title compounds were prepared in DMSO. The microbial growth of zone of inhibition data are tabulated in **Table 2** (antibacterial activity) and **Table 3** (antifungal activity).

Table 1. Physical data of the synthesized sulfonamides **7(a-e)** and carbamates **9(a-d)**.

Compd.	Time (h)	Yield (%)	M.p. ^{O}C	Compd.	Time (h)	Yield (%)	M.p. ^{O}C
7a	4.5	80	157-159	**9a**	5.5	70	88-90
7b	5.5	72	178-181	**9b**	6.0	65	74-76
7c	4.0	82	>300	**9c**	5.0	68	172-175
7d	5.5	79	210-213	**9d**	5.0	79	189-193
7e	6.0	70	206-208				

Table 2. Bacterial growth of inhibition of the synthesized compounds **7(a-e)** and **9(a-e)**.

Compd.	Bacterial zone of inhibition in mm					
	Streptococcus aureus (ATCC-25923)		*Pseudomonas aeruginosa* (ATCC-25619)		*Escherichia coli* (ATCC-9637)	
	100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL
7a	13.4	20.1	12.8	18.5	14.2	18.9
7b	6.5	12.7	7.5	14.3	14.7	20.2
7c	12.3	19.7	11.4	19.2	13.5	21.8
7d	13.0	19.0	12.6	19.1	15.7	21.2
7e	8.8	14.6	6.2	10.3	9.4	12.5
9a	13.1	20.4	11.9	18.5	14.9	22.4
9b	4.4	11.3	7.2	13.4	8.0	15.2
9c	5.9	9.7	3.4	8.6	3.9	11.3
9d	5.0	13.6	9.1	15.9	7.1	12.7
Std.	18.4	23.5	16.9	22.8	19.4	25.3

Std. – Standard: Ciprofloxacin was used as standard.

Table 3. Fungal growth of inhibition of the synthesized compounds **7(a-e)** and **9(a-e)**.

Compd.	Fungal zone of inhibition in mm					
	Aspergillus flavus (MTCC-1884)		*Aspergillus niger* (MTCC- 1881)		*Candida albicans* (ATCC- 2091)	
	100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL	100 µg/mL	200 µg/mL
7a	13.5	21.2	12.3	20.9	13.0	19.5
7b	3.7	9.3	10.5	15.2	7.4	10.9
7c	14.3	21.8	12.4	19.7	14.0	20.5
7d	12.6	22.1	10.9	20.2	11.3	19.6
7e	7.7	11.3	5.2	12.1	10.1	18.4
9a	9.7	20.5	13.1	19.4	12.8	19.0
9b	7.2	13.0	10.5	17.5	8.3	10.7
9c	9.6	13.9	4.8	10.3	9.0	18.5
9d	9.5	17.3	8.0	12.7	10.2	17.6
Std.	18.1	24.6	16.3	23.5	16.9	22.8

Std. – Standard – Fluconazole was used as standard.

Table 4. Minimum inhibitory concentrations of the active synthesized compounds.

Compd.	Minimum inhibitory concentrations in µg/mL					
	S. aureus	*P. aeruginosa*	*E. coli*	*A. flavus*	*A. niger*	*C. albicans*
7a	17.5	23.5	18.0	22.0	21.5	23.5
7b	NT	NT	19.5	NT	NT	NT
7c	24.5	18.0	20.0	19.0	24.5	22.0
7d	21.0	19.5	23.5	25.0	19.0	24.5
7e	NT	NT	NT	NT	NT	25.0
9a	24.0	23.5	25.0	19.5	23.5	26.5
9b	NT	NT	NT	NT	NT	NT
9c	NT	NT	NT	NT	NT	23.5
9d	NT	NT	NT	30.0	NT	27.0
Std[a].	7.5	9.0	7.0	NT	NT	NT
Std[b].	NT	NT	NT	6.0	9.5	8.5

NT – Not Tested; Std[a]. – Ciprofloxacin; Std[b]. – Fluconazole; *S. aureus* - *Streptococcus aureus* (ATCC-25923)*; P. aeruginosa* - *Pseudomonas aeruginosa* (ATCC-25619)*; E. coli* - *Escherichia coli* (ATCC-9637)*; A. flavus* - *Aspergillus flavus* (MTCC-1884)*; A. niger* - *Aspergillus niger* (MTCC- 1881)*; C. albicans* - *Candida albicans* (ATCC- 2091).

Minimum inhibitory concentration (MIC) was also examined to the active synthesized compounds using micro broth dilution technique[35] and summarized the results in **Table 4**. The biological data disclosed that some of the compounds exhibited potential antimicrobial activity. Whereas, the sulfonamide derivatives, **7a** bonded with 4-chloro-3-nitro phenyl ring, **7c** bearing with 4-nitro phenyl ring and **7d** connected with 4-fluoro phenyl ring, and carbamate derivative, **9a** bonded with trichloro ethyl group were exhibited promising bacterial or fungal growth of inhibition and which are closer to the standards. Also, the derivatives **7b** against *E. coli*, **7e** and **9c** against *C. albicans*, **9b** against *A. niger*, and **9d** against *A. flavus* and *C. albicans* showed promising activity. For these active compounds, the MIC values were determined to know their potency. The data revealed that the active compounds displayed minimum inhibitory concentration values in the range of 18.0-30.0 µg/mL as compared with the standards (6-10 µg/mL). In the whole observation, the sulfonamide derivatives of 6-chloropurine exhibited promising antimicrobial activity as compared with carbamate derivatives.

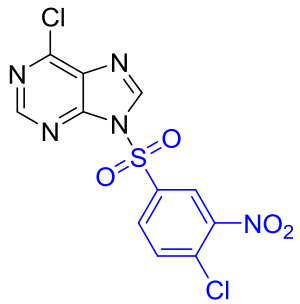
3. Experimental

All chemicals and reagents were purchased from *Sigma-Aldrich* and *Merck* and used without further purification. Silica gel 60-120 mesh was used as solid phase in the column chromatography to purify the compounds. Melting points were determined in an open capillary tube by *GUNA* digital melting point apparatus and are uncorrected. IR spectroscopic data were recorded on *Bruker ALPHA* interferometer FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectroscopic data were recorded on a *Bruker AV* 400 spectrometer in $CDCl_3$, DMSO-d_6 solvents, 400 MHz and 100 MHz were used for recording ^{1}H NMR and ^{13}C NMR respectively, and tetramethylsilane (TMS) was used as internal standard. E.S.I mass spectra were recorded on *Agilent* 1000 mass spectrometer. Elemental analysis was performed on *Thermo Finnigan FLASH EA* 1112 instrument. Chemical shifts were recorded in parts per million (ppm) and multiplicities are represented as abbreviations: s (singlet), brs (broad singlet), d (doublet), triplet (t) and m (multiplet).

*3.1. General procedure for synthesis of compounds **(7a-e)** and **9(a-d)***

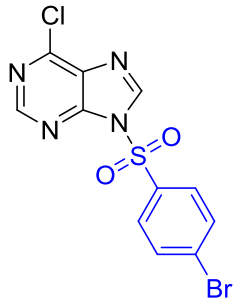
6-Chloropurine (1.5 mmol, 231 mg) **(4)** was taken into a round-bottomed flask (50 mL) containing 15 mL of THF: pyridine (2:1). The reaction mass was cooled to 5-10 ºC using ice bath and then sodium hydride (2.0 mmol, 48 mg) was added. The reaction mixture was stirred for 1.5 h at 10-20 ºC to obtain sodium salt of 6-chloropurine **(5)**. The completion of the reaction was confirmed by disappearance of starting material in the TLC. The phenyl sulfonyl chlorides and substituted alkyl/phenyl chloro formates (1.5 mmol) was added to a solution of compound **6(a-e)** (for **7a-e**) and compound **8(a-d)** (1.5 mmol) (for **9a-d**) in dry tetrahydrofuran (5.0 mL). The mixture was stirred at 10 ºC. Later slowly increased the reaction temperature to 40 ºC and stirred for 4-6 h. After completion of the reaction as checked by TLC using 25% methanol in chloroform, the reaction mass was cooled to 25 ºC and filtered to remove the salt, NaCl. The bed was washed with THF (5 mL). Column chromatography was adopted to purify the crude compounds using 15-25% of methanol and chloroform as mobile phase (**Table 1**).

3.1.1. 6-Chloro-9-(4-chloro-3-nitro-benzenesulfonyl)-9H-purine (7a):



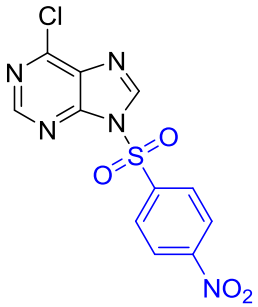
Yield: 80% , pale yellow solid, m.p. 157-159 ºC; ^{1}H NMR (DMSO-d_6) (δ/ppm) : 7.85 (d, 1H, J = 8.0 Hz, Ar-H), 8.21(d, 1H, J = 8.0 Hz, Ar-H), 8.73 (s, 1H, Ar-H), 8.83 (s, 1H, -N-CH-N-), 9.21(s, 1H, Cl-C-N-CH-N); ^{13}C NMR (DMSO-d_6) (δ/ppm) : 124.5, 129.5, 131.2, 132.9, 134.4, 138.0, 146.1, 147.9, 149.8, 150.6, 154.2; IR(ν/cm^{-1}): 1442.5 (NO_2), 1400.3 (SO_2), 1188.9 (SO_2), 690.1 (C-Cl); ESI-Ms *m/z* (%): 374 $(M+H)^+$ (100), 376 $(M+H^++2)$ (67); Analysis (% Calculated/found) for $C_{11}H_5Cl_2N_5O_4S$ C:35.31/35.26, H:1.35/1.32, N:18.72/18.67.

3.1.2. 9-(4-Bromo-benzenesulfonyl)-6-chloro-9H-purine (7b):



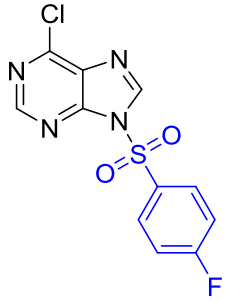
Yield: 72%, light brown solid, m.p. 178-181 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm) : 7.77 (d, 2H, J = 8.0 Hz, Ar-H), 8.18 (d, 2H, J = 8.0 Hz, Ar-H), 8.56 (s, 1H, -N-CH-N-), 8.85 (s, 1H, Cl-C-N-CH-N); ^{13}C NMR ($CDCl_3$) (δ/ppm): 128.2, 130.8, 131.0, 131.2, 133.6, 144.8, 149.5, 150.9, 153.8; IR (ν/cm^{-1}): 1367.0 (SO_2), 1189.7 (SO_2, bending), 688.5 (C-Cl, str), 569.2 (C-Br, str); ESI-Ms *m/z* (%): 373 $(M+H)^+$ (77.9), 375 $(M+H^++2)$ (100); Analysis (% Calculated/found) for $C_{11}H_6BrClN_4O_2S$ C:35.36/35.31, H:1.62/1.56, N:15.00/14.94.

3.1.3. 6-Chloro-9-(4-nitro-benzenesulfonyl)-9H-purine (7c):



Yield:82%, yellow solid, m.p. 300 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm): 8.47 (d, 2H, J = 8.0 Hz, Ar-H), 8.53 (d, 2H, J = 8.0 Hz, Ar-H), 8.92 (s, 1H, -N-CH-N-), 9.20 (s, 1H, Cl-C-N-CH-N); ^{13}C NMR ($CDCl_3$) (δ/ppm) : 125.2, 130.9, 131.6, 143.1, 148.5, 149.2, 151.3, 152.6, 153.5; IR (ν/cm^{-1}): 1442.5 (NO_2), 1366.4 (SO_2), 1187.7 (SO_2), 629.8 (C-Cl); ESI-Ms *m/z* (%): 340 $(M+H)^+$ (100), 342 $(M+H^++2)$ (33); Analysis (% Calculated/found) for $C_{11}H_6ClN_5O_4S$ C:38.89/38.83, H:1.78/1.76, N:20.62/20.59.

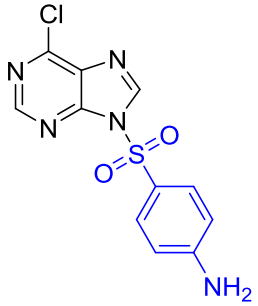
3.1.4. 6-Chloro-9-(4-fluoro-benzenesulfonyl)-9H-purine (7d):



Yield: 79%, pale brown solid, m.p. 210-213 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm): 7.33 (d, 2H, J = 8.0 Hz, Ar-H), 8.17 (d, 2H, J = 8.0 Hz, Ar-H), 8.69 (s, 1H, N-CH-N-), 9.03 (s, 1H, Cl-C-N-CH-N-); ^{13}C NMR ($CDCl_3$) (δ/ppm) :119.5, 131.2, 132.1, 134.9, 148.5, 152.2, 152.9, 154.1,168.3 (d, J =184.5 Hz); IR

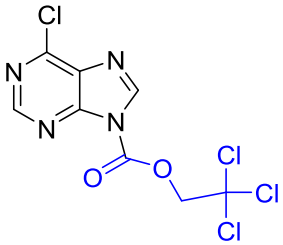
(ν/cm^{-1}): 1366.0 (SO_2),1188.5 (SO_2), 1091.4 (C-F), 626.4 (C-Cl); ESI-Ms *m/z* (%): 313.2 $(M+H)^+$ (32.1), 315 ($M+H^+$+2) (33), 288.3 [$M+H^+$-25] (30.9), 274.4 [$M+H^+$-39] (100); Analysis (% Calculated/found) for $C_{11}H_6ClFN_4O_2S$ C:42.25/42.19, H:1.93/1.92, N:17.92/17.89.

3.1.5. 4-(6-Chloro-purine-9-sulfonyl)-phenylamine (7e):



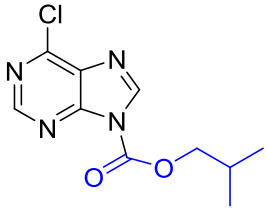
Yield: 70%, brown solid, m.p. 206-208 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm): 5.62 (brs, 2H, Ar-NH_2), 6.93 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.76 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.78 (s, 1H, -N-CH-N-), 8.96 (s, 1H, Cl-C-N-CH-N-); ^{13}C NMR ($CDCl_3$) (δ/ppm): 115.1, 126.8, 130.4, 132.9, 148.5, 152.0, 151.8, 153.7, 155.1 ppm; IR (ν/cm^{-1}): 3368.4 (NH_2), 1531.1 (NH_2), 1366.4 (SO_2), 1187.7 (SO_2), 629.8 (C-Cl); ESI-Ms *m/z* (%): 310.3 $(M+H)^+$ (31.3), 312.4 ($M+H^+$+2) (9.7), 274.4 [$M+H^+$-36] (100); Analysis (% Calculated/found) for $C_{11}H_8ClN_5O_2S$ C:42.66/42.61, H:2.60/2.57, N:22.61/22.56.

3.1.6. 6-Chloro-purine-9-carboxylic acid 2, 2, 2-trichloro-ethyl ester (9a):



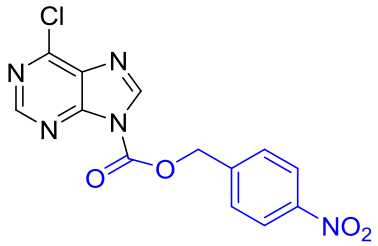
Yield:70%, off-white solid, m.p. 88-90 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm): 4.87 (s, 2H, CH_2, aliphatic), 8.64 (1H, s, -NCHN-), 8.95 (1H, s, Cl-C-N-CH-N-); ^{13}C NMR ($CDCl_3$) (δ/ppm): 76.3, 98.0, 138.2, 143.4, 148.3, 151.8, 152.6, 154.6; IR (ν/cm^{-1}): 1764.4 (C=O), 1367.5 (C-N), 1146.7 (C-O), 685.7 (C-Cl); ESI-Ms *m/z* (%) : 330.5 $[M+H]^+$ (11.6), 353.5 [M+Na] (100), 302.5 [$M+H^+$-28] (23), 288.3 [$M+H^+$-41] (35.6), 274.4 [$M+H^+$-55] (40); Analysis (% Calculated/found) for $C_8H_4Cl_4N_4O_2$ C:29.12/29.09, H:1.22/1.20, N:16.98/16.96.

3.1.7. 6-Chloro-purine-9-carboxylic acid isobutyl ester (9b):



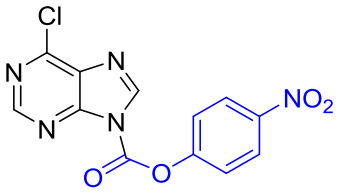
Yield:65%, pale brown solid, m.p. 74-76 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm): 1.10 (d, 6H, *J* = 6.7 Hz, $(CH_3)_2$-CH-CH_2-O), 2.25-2.18 (m, 1H, $(CH_3)_2$-CH-CH_2-O), 4.38 (d, 2H, *J* = 8.0 Hz, $(CH_3)_2$-CH-CH_2-O), 8.65 (1H, s, -N-CH-N-), 8.93 (1H, s, Cl-C-N-CH-N-); ^{13}C NMR ($CDCl_3$) (δ/ppm): 18.9 $(CH_3)_2$-CH-CH_2-O), 27.8 $(CH_3)_2$-CH-CH_2-O), 75.3 $(CH_3)_2$-CH-CH_2-O), 143.7, 146.9, 151.9, 154.2, 159.9; IR (ν/cm^{-1}): 1758.7 (C=O), 1288.5 (C-N), 1141.9 (C-O), 634.5 (C-Cl); ESI-Ms *m/z* (%): 255.2 $(M+H)^+$ (100), 256.1 ($M+H^+$+2) (33); Analysis (% Calculated/found) for $C_{10}H_{11}ClN_4O_2$ C:47.16/47.19, H:4.35/4.35, N:22.00/22.03.

3.1.8. 6-Chloro-purine-9-carboxylic acid 4-nitro-benzyl ester (9c):



Yield: 68%, pale yellow solid; m.p. 172-175 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm): 5.53 (s, 2H, -CH_2-C_6H_5), 7.58 (d, 2H, *J* = 8.0 Hz, Ar-H), 7.96-7 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.67 (s, 1H, -N-CH-N-), 9.05 (s, 1H, Cl-C-N-CH-N-); IR (ν/cm^{-1}): 1758.1 (C=O), 1448.1 (NO_2), 1140.3 (C-O), 1281.9 (C-N), 681.4 (C-Cl); ESI-Ms *m/z* (%): 334.4 $(M+H)^+$ (100), 336.2 ($M+H^+$+2) (33); Analysis (% Calculated/found) for $C_{13}H_8ClN_5O_4$ C:46.79/46.74, H:2.42/2.40, N:20.99/21.00.

3.1.9. 6-Chloro-purine-9-carboxylic acid 4-nitro-phenyl ester (9d):



Yield:79%, off-white solid; m.p. 189-193 ºC, ^{1}H NMR ($CDCl_3$) (δ/ppm) : 7.66 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.21 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.62 (s, 1H, -N-CH-N-), 8.96 (s, 1H, Cl-C-N-CH-N-); ^{13}C NMR ($CDCl_3$) (δ/ppm): 123.0, 125.4, 136.2, 138.9, 143.2, 145.5, 149.1, 152.3, 152.8, 156.4; IR (ν/cm^{-1}): 1614.6 (C=O), 1447.1 (NO_2), 1288.5 (C-N), 1166.4 (C-O), 692.5 (C-Cl); ESI-Ms *m/z* (%): 320.4 $(M+H)^+$ (100), 322.0 [$M+H^+$+2] (32.8); Analysis (% Calculated/found) for $C_{12}H_6ClN_5O_4$ C:45.09/45.03, H:1.89/1.85, N:21.91/21.89.

3.2. Antimicrobial activity

3.2.1. Antibacterial activity

The newly synthesized sulfonamides **7(a-e)** and carbamates **9(a-e)** of 6-chloro-9*H*-purine were evaluated for their antibacterial activity using disc diffusion method.[34] The bacterial strains such as *Streptococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-25619) and *Escherichia coli* (ATCC-9637) were selected to investigate the activity and Ciprofloxacin was used as a standard drug. The title compounds (1 mg) were dissolved in methanol and adjusted their concentration to 100 and 200 µg/mL to examine the activity. A standard inoculum of 1-2 x 10^{-7}CFU/mL (0.5 McFarland standards) was introduced onto the surface of sterile agar plates and made even distribution of the inoculums. The dry sterilized discs of 6 mm were soaked in the test solutions (100 and 200 µg/mL) and placed in nutrient agar medium. Blank test showed that, methanol used in the preparation of the test solutions does not affect the bacteria. The inoculated plates were inverted and incubated for 24 h at 37±3 °C. The bacterial growth of inhibition as zone of inhibition around the disc was measured in millimeters. The experiments were repeated three times and average data was taken as final result.

3.2.2. Antifungal activity

The synthesized sulfonamides **7(a-e)** and carbamates **9(a-e)** were evaluated for their antifungal activity against fungal strains such as *Aspergillus flavus* (MTCC-1884), *Aspergillus niger* (MTCC-

1881) and *Candida albicans* (ATCC- 2091) using agar disc-diffusion method.[36] Fluconazole was used as a standard drug for antifungal study. The fungal strains were maintained on Potato Dextrose Agar (PDA) medium (Hi-Media). The culture from the slant was inoculated into the Potato Dextrose broth and incubated at 37 ˚C for 48-72 h. This culture (0.1 mL) was spread on the potato dextrose agar plate. The compounds (1 mg) were dissolved in methanol and adjusted their concentration to 100 and 200 µg/mL. Sterile discs of 6 mm diameter soaked into the test solutions and these are impregnated on the surface of the media and incubated for 48-72 h at 37±3 ˚C. The zone of inhibition around the disc was measured in millimeters. The tests were repeated three times and average value was taken.

Minimum inhibitory concentrations (MICs) were examined using micro broth dilution technique.[35] In different test tubes 0.1 mL of standardized inoculum (1-2 x 10^7 CFU/mL) was added and, incubated 24 h for bacterial inoculums and 48-72 h for fungal inoculum at 37±3 ^oC. Two controls were maintained for each test sample. The growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required arresting the growth of bacteria or fungi was regarded as minimum inhibitory concentration (MIC).

4. Conclusion

In the present study as in the part of medicinal chemistry programme, we have been synthesized a series of new sulfonamide derivatives, 9-(substitutedbenzenesulfonyl)-6-chloro-9*H*-purines and carbamate derivatives, 6-chloro-purine-9-carboxylicacid substituted alkyl/arylester by in situ fashion. Antimicrobial activity against three bacterial strains and three fungal strains at two different concentrations, 100 and 200 µg/mL including MIC values was investigated. Bio-screening data disclosed that all the title compounds exhibited promising antimicrobial activity at both the concentrations. Sulfonamide derivatives **7a**, **7c** and **7d**, and one carbamate derivative **9a** showed promising growth of inhibition of selected bacterial and fungal strains and these compounds showed MIC values in the range of 18.0-25.0 µg/mL as compared with other title compounds and near activity of the standards. In whole comparison, the sulfonamide derivatives of 6-chloropurine are acted as potential antimicrobial agents than that of carbamate derivatives. The study of results encouraged us to design new library of purine derivatives as antimicrobial agents in future endeavors and might be worthy to medicinal chemistry.

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