International Journal of Advanced Multidisciplinary Research
ISSN: 2393-8870
www.ijarm.com
DOI: 10.22192/ijamr Volume 5, Issue 5 -2018

Research Article

DOI: http://dx.doi.org/10.22192/ijamr.2018.05.05.004

Antibacterial and Antifungal Activities on Derivatives of Adenine

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Keywords

Adenine derivatives, FTIR , ^{1}H NMR, ^{13}CNMR spectra Antibacterial activity, Antifungal activity

Abstract

The Schiff bases of adenine derivatives **(I)** 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol, **(II)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol, **(III)** N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine**, (IV)** N-(furan-2-ylmethylene)-9H-purin-6-amine, **(V)** N-(pyridin-4-ylmethylene)-9H-purin-6-amine, **(VI)** Adenine + 5-Nitrosalicylaldehyde, **(VII)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxy-6-nitrophenoland **(VIII)** 2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino)phenol were prepared and characterized by physical and analytical data, FTIR , ^{1}H NMR, ^{13}CNMR spectra and were screened against gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria *Escherichia coli, Klebsiella aerogenes* for antibacterial activity and were screened against *Aspergillus niger* and *Candida albicans* for antifungal activity by disc diffusion method. Ciprofloxacin and Nystatin were used as standard for bacteria and fungi.

Introduction

The treatment of Leprosy, Typhoid, Tuberculosis, Malaria and fungi have been hampered by using presently available antibiotics because of the development of antibiotic resistance of bacteria, parasites and fungi[1-4]. The antibiotic required for the antibiotic resistance microbes produce anemia, agranulocytosis, liver damage and crystal-laurea. They may also cause pain and inflammation. In recent years[4-6], derivatives of nucleic acid base were found to have potential non-toxic and non-antibiotic resistance of antibacterial, antifungal, mosquito larvicidal, antiparasitic and anticancer properties. They have been prepared by starting from nucleic acid bases like cytosine or adenine and aldehydes or ketones. In the present study we have prepared I [N-(pyridin-4-ylmethylene)-9H-purin-6-amine], II,III (N-(pyridin-4-ylmethylene)-9H-purin-6-amine,)and have been subjected to in vitro antimicrobial activities against various pathogenic bacteria and fungi.

Materials and Methods

Materials

All the reagents used were of AR grade. Commercially available rectified spirit was dried over anhydrousquicklime for 24 hours, filtered and distilled before use (BP 78oC). Dimethylsulphoxide (sigma) and N,Ndimethylformamide (sigma) were used as such adenine, 3,5-diiodosalicylaldehyde, vaniline,

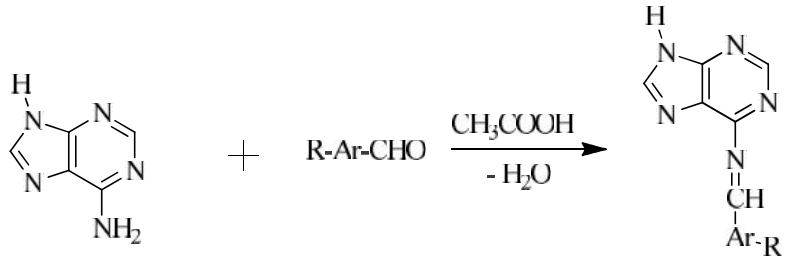
3,45-trimethoxybenzaldehyde, furfural, pyridine-4-carboxaldehyde, 5-nitrosalicylaldehyde, 5-nitrovaniline and 4-diethylaminosalicylaldehyde were purchased from Alfa Aesar.

Instruments

Melting points were determined using Elico melting point apparatus. Elemental analysis were performed using Elementar Vario EL III. IR spectra of the compounds were recorded with KBr pellets with carry630 FTIR Spectrometer in the 4000-400 cm-1range. The 1HNMR and 13CNMRspectra were recorded on a Bruker 400 MHz FT- PMR Spectrometer.

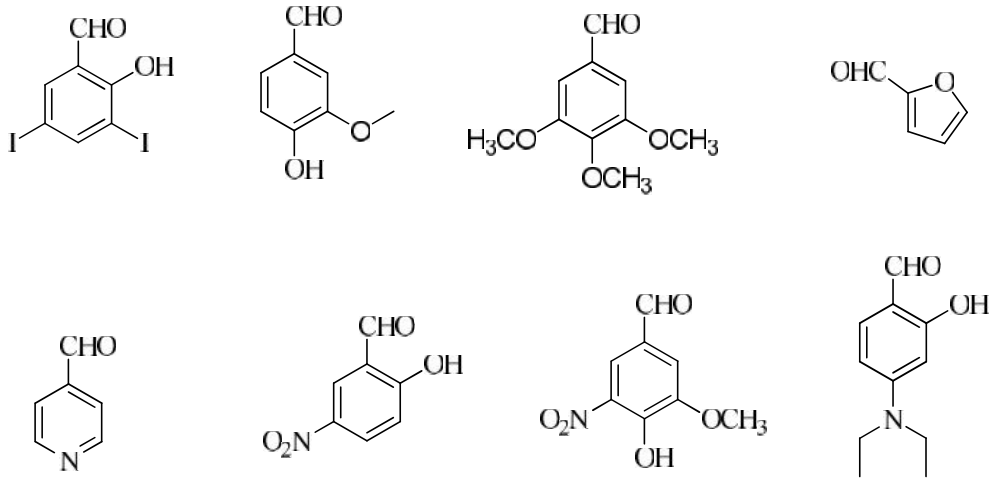
General preparation of derivatives of adenine

All the azomethine compounds of derivatives of adenine were where prepared as reported in the literature[8-13] by the following scheme – 1.



Scheme 1

Where, Ar-CHO =



Preparation of (I)2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol

Equimolar quantities of 0.01 mole of adenine (1.36 g) and 3,5-diiodosalicylaldehyde (3.73 g, 0.01mol) were dissolved in 20 ml of DMSOand 3 drop of glacial acetic acid was added andrefluxed for 3 hours. After completion of the reaction (monitored by TLC), some solvent was distilled out,the reaction mixture was poured on ice cold water andthe solid 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol came out which was filteredand then recrystallized by DMSO and then dried overvacuum desiccator.

Preparation of (II)4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol

4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol was prepared from equimolar quantity ofadenine (1.36 g, 0.01mol) and 3,5-diiodosalicylaldehyde (3.73 g, 0.01mol) in 30 ml of methanolwere heated at 70oC on water bath for 4-hrs inpresence of few drops of glacial acetic acid. The crudeproduct were obtained after removal of methanolunder reduced pressure. The products wererecrystallized from methanol and then dried overvacuum desiccator.

Preparation of (III)N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine

A mixture of 3,4,5-trimethoxybenzaldehyde 1.96 g (0.01mol) and adenine 1.36 g (0.01mol) were grained with a pestle in an open mortar at room temperature for 3minutes. To this reaction mixture sulphuric acid 2dropsand 20ml DMF were added and grained for 5minutes. On completion of reaction as monitored by TLC, the light yellow solid N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine was separated out. The obtained solid was isolated by simple Buchner filtration and was recrystallized from DMF and then dried over vacuum desiccator.

Preparation of (IV)N-(furan-2-ylmethylene)-9H-purin-6-amine

25ml of ethanolic solution of adenine(1.36 g,0.01mol) was added to 25ml of ethanolic solution of furfural (0.96 g 0.01mol).Then three drops of NH_4OH was added to the reaction mixture. The reaction mixture was heated and refluxed for about 5 hours at 90°C. The resulting solution was further concentrated by water bath. The product N-(furan-2-ylmethylene)-9H-purin-6-amine obtained was precipitated, cooled and collected after filtration. The precipitate was purified by washing with distilled water and then with ethanol. The N-(furan-2-ylmethylene)-9H-purin-6-amine was again recrystallized in ethanol and then dried over vacuum desiccator.

Preparation of (V)N-(pyridin-4-ylmethylene)-9H-purin-6-amine

Adenine (1.36 g, 0.01mol) was dissolved in 5ml of hot glacial acetic acid, 1.07 g (0.01mol) of Pyridine-4-carboxaldehyde was dissolved in 5ml of glacial acetic acid and were mixed. The reaction mixture wasrefluxed with stirring for 5 hours. The mixture wasallowed to cool, and poured onto ice. The crude solid4N-(pyridin-4-ylmethylene)-9H-purin-6-amine was filtered off and washed with distilled water, then recrystallized from acetic acidand then dried over vacuum desiccator.

Preparation of (VI)N-(furan-2-ylmethylene)-9H-purin-6-amine

The N-(furan-2-ylmethylene)-9H-purin-6-aminewas prepared by stirring a methanolic solution of adenine (1.36 g 0.01mol)with furan-2-carbaldehyde (0.96 g 0.01mol)in 1:1stoichiometric ratio at room temperature over 24hours.The precipitate obtained were filtered and washed with methanol and recrystallized from methanol and then dried over vacuum desiccator.

Preparation of (VII)4-(((9H-purin-6-yl)imino)methyl)-2-methoxy-6-nitrophenol

A mixture of 5-nitrovaniline (1.97 g,0.01mol)and adenine (1.36 g, 0.01mol) was grained in a mortar with a pestle made of porcelain for10 minutes. The mixture turned pasty after few minutes of grainding. It was grained till yellow colour product appears. The mixture was left overnight. The resultant product 4-(((9H-purin-6-yl) imino) methyl)-2-methoxy-6-nitrophenol was recrystallized using ethanol and then dried over vacuum desiccator.

Preparation of (VIII)2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino)phenol

2.7 grams of adenine (0.02mol) was mixed with 3.9 g of 4-diethylaminosalicylaldehyde (0.02mol)and was grained well in acidic acid medium at room temperature. The mixture was transferred into hundred milliliter Round Bottom flask and was refluxed for six hours in oil bath. The solid product 2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino) phenol was filtered and washed with ethanol and recrystallized in DMSO and then dried over vacuum desiccator.

Antimicrobial susceptibility test by Disc diffusion Technique

Principle

Disc impregnated with known concentration of **antibacterial** drug are placed on an agar plate that has been inoculated uniformly over the entire plate with a culture of the bacterium to be tested. The plate is incubated for 18 to 24 hours at 37^{o}C. During this period, the **antibacterial** agent diffuses through the agar and may prevent the growth of the organism. Effectiveness of susceptibility is proportional to the diameter of the inhibition zone around the disc. Organisms which grow up to the edge of the disc are resistant.

Procedure

The plate was labeled with the name of the culture, sample and standard at the bottom of the plate.

Then sterile cotton swab on a wooden applicator stick was dipped into the bacterial suspension. Excess fluid was removed by rotating the swab and rubbed gently over the plate to obtain uniform distribution of the inoculums. The sterile disc was held on the inoculated plate with the help of micropipette. The sample was leveled in the sterile disc and incubated at 37°C in an incubator. After incubation, the diameter of the zone of inhibition of growth was measured.

Observation Report

Table 1.

Inhibition zone > 15mm	**Highly active**
Inhibition zone > 10mm	**Moderatively active**
Inhibition zone > 5mm	**Slightly active**
Inhibition zone ≤ 5mm	**Inactive**

Results and Discussion

The physical and analytical data of the derivatives of adenine **(I)** 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol, **(II)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol, **(III)** N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine**, (IV)** N-(furan-2-ylmethylene)-9H-purin-6-amine, **(V)** N-(pyridin-4-ylmethylene)-9H-purin-6-amine, **(VI)** ADENINE + 5-NITROSALICYLALDEHYDE, **(VII)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxy-6-nitrophenol and **(VIII)** 2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino)phenol are given in Table 2.

[I] 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol

FTIR (cm^{-1}): 3568 & 690 cm^{-1} (—O—H), 3235 & 877 cm^{-1} (—N—H), 1147 cm^{-1} (Ar—OH), 1615 cm^{-1} (—N=C—), 1645 cm^{-1} (—N=CH), 1124 cm^{-1} (—N—C—) & 643 cm^{-1} (Ar—I)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 8.07 (s, 1H), 7.88 (s, 1H), 5.35 (s, 1H)

^{13}CNMR δ (ppm): 161.8 (s), 160.0 (s), 159.1 (s), 153.8 (s), 152.4 (s), 147.6 (s), 144.5 (s), 136.9 (s), 125.2 (s), 121.7 (s), 88.6 (s), 83.8 (s)

[II] 4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol

FTIR (cm^{-1}): 3610 & 640 cm^{-1} (—O—H), 3410 & 820 cm^{-1} (—N—H), 1650 cm^{-1} (—N=CH), 1610 cm^{-1} (—N=C—), 1250 cm^{-1} (Ar—OH), 1240 cm^{-1} (Ar—OR) & 1120 cm^{-1} (ArO—R)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.52 (s, 1H), 7.34 (d, 1H), 6.91 (d, 1H), 5.35 (s, 1H) & 3.83 (s, 3H)

^{13}CNMR δ (ppm): 160.0 (s), 158.6 (s), 153.8 (s), 152.4 (s), 151.0 (s), 149.3 (s), 144.5 (s), 130.9 (s), 125.2 (s), 122.9 (s), 117.0 (s), 112.1 (s), 56.1 (s)

[III] N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine

FTIR (cm^{-1}): 3244 & 787 cm^{-1} (—N—H), 1624cm^{-1} (—N=CH), 1597 cm^{-1} (—N=C—), 1210 cm^{-1} (Ar—OR) and 1120 cm^{-1} (ArO—R)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.14 (s, 2H) and 3.83 (s, 9H)

^{13}CNMR δ (ppm): 160.0 (s), 158.6 (s), 153.8 (s), 153.2 (d), 152.4 (s), 144.5 (s), 141.5 (s), 133.0 (s), 125.2 (s), 104.0 (s), 60.8 (s) and 56.1 (s)

[IV] N-(furan-2-ylmethylene)-9H-purin-6-amine

FTIR (cm^{-1}): 3270 & 740 cm^{-1} (—N—H), 1650 cm^{-1} (—N=CH), 1570 cm^{-1} (—N=C—) & 1230 cm^{-1} (ArO—R)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 7.75 (d, 1H), 7.50 (s, 1H), 6.93 (d, 1H) & 6.52 (t, 1H)

^{13}CNMR δ (ppm): 160.0 (s), 153.8 (s), 152.4 (s), 150.4 (s), 146.4 (s), 144.5 (s), 144.4 (s), 125.2 (s), 118.9 (s) & 112.6 (s)

[V] N-(pyridin-4-ylmethylene)-9H-purin-6-amine

FTIR (cm^{-1}): 3310 & 870 cm^{-1} (—N—H), 1670 cm^{-1} (—N=CH) & 1630 cm^{-1} (—N=C—)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 9.03 (s, 1H), 8.66 (d, 2H), 8.57 (s, 1H), 8.00 (s, 1H), 7.98 (d, 2H) & 7.50 (s, 1H)

^{13}CNMR δ (ppm): 160.0 (s), 158.6 (s), 153.8 (s), 152.4 (s), 149.4 (d), 144.5 (s), 144.3 (s), 125.2 (s) & 120.4 (s)

[VII] 4-(((9H-purin-6-yl)imino)methyl)-2-methoxy-6-nitrophenol

FTIR (cm^{-1}): 3685 & 670 cm^{-1} (—O—H), 3253 & 796 cm^{-1} (—N—H), 1660 cm^{-1} (—N=CH), 1624 cm^{-1} (—N=C—), 1597 cm^{-1} (Ar—NO$_2$), 1219 cm^{-1} (Ar—OR) & 1084 cm^{-1} (ArO—R)

^{1}HNMR δ (ppm) : 11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.91 (s, 2H), 5.35 (s, 1H) & 3.83 (s, 3H)

^{13}CNMR δ (ppm): 160.0 (s), 158.6 (s), 153.8 (s), 152.4 (d) (Ar-O- & Aromatic Heterocyclic: N-C=N), 144.5 (s), 140.3 (s), 138.1 (s), 128.2 (s), 125.2 (s), 118.2 (s), 116.5 (s) & 56.1 (s)

[VIII] 2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino)phenol

FTIR (cm^{-1}): 3307 & 859 cm^{-1} (—N—H), 3244 & 643 cm^{-1} (—O—H), 1237 cm^{-1} (R—N—), 1327 cm^{-1} (Ar—N—), 1588 cm^{-1} (—N=C—) and 1633 cm^{-1} (—N=CH)

^{1}HNMR δ (ppm): 11.0 (s, 1H), 9.03 (s, 1H), 8.57 (s, 1H), 8.48 (s, 1H), 7.48 (d, 1H), 6.37 (d, 1H), 6.30 (s, 1H), 5.35 (s, 1H), 3.41 (q, 4H) and 1.15 (t, 6H)

^{13}CNMR δ (ppm): 162.0 (s), 161.8 (s), 160.0 (s), 153.8 (s), 153.3 (s), 152.4 (s), 144.5 (s), 132.8 (s), 125.2 (s), 110.0 (s), 104.5 (s), 99.1 (s), 47.1 (d) and 12.9 (s)

Table 2.The physical and analytical data of the derivatives of adenine

Derivatives of adenine	Molecular Weight	Nature of Appearance	% of yield	Elemental Analysis (in %)				
				C	H	O	N	I
[I] $C_{12}H_7I_2N_5O$	491.02	Yellow Crystalline Solid	83	29.35	1.44	3.26	14.26	51.69
[II] $C_{13}H_{11}N_5O_2$	269.25	Yellow Crystalline Solid	88	57.99	4.12	11.88	26.01	-
[III] $C_{15}H_{15}N_5O_3$	313.33	Yellow Crystalline Solid	87	57.50	4.83	15.32	22.35	-
[IV] $C_{10}H_7N_5O$	213.19	Yellow Crystalline Solid	66	56.34	3.31	7.50	32.85	-
[V] $C_{11}H_8N_6$	224.22	Yellow Crystalline Solid	70	58.92	3.60	-	37.48	-
[VI]	284.23	Yellow Crystalline Solid	71	50.71	2.84	16.89	29.57	-
[VII] $C_{13}H_{10}N_6O_4$	316.25	Yellow Crystalline Solid	81	49.69	3.21	20.36	26.74	-
[VIII] $C_{16}H_{18}N_6O$	310.35	Yellow Crystalline Solid	69	61.92	5.85	5.16	27.08	-

Antibacterial bioassay

Antibacterial activities[14,15] of derivatives of adenine were screened against bacterial gram positive bacteria *Staphylococcus aureus*, and gram negative bacteria *Escherichia coli*, *Klebsiella aerogenes* and *Bacillus subtilis* by disc diffusion method and the results obtained were formulated in Table.3 and Fig. 1-4. The experiments were carried out in DMSO solution at a concentration of 100ppm using Muller Hinton agar media. Ciprofloxacin was used as a standard drug.

Antifungal bioassay

Antifungal[16,17] screening of derivatives of adenine were carried out against *Aspergillus niger* and *Candida albicans* by disc diffusion method and the results obtained were formulated in Table 3.and Fig.5 and 6. The test was carried out in DMSO solution at a concentration of 100 ppm. Results were compared with standard drug Nystatin at the same concentration.

Table 3. Antibacterial and antifungal activity of derivatives of adenine

Compounds	Gram negative Bacteria		Gram positive Bacteria		Fungi	
	E. coli	*K. aerogenes*	*B. subtilis*	*S. aureus*	*A. niger*	*C. albicans*
I	32	28	34	30	31	28
II	30	26	32	29	28	26
III	29	25	30	28	26	26
IV	28	24	29	26	24	24
V	27	23	27	25	23	22
VI	26	22	25	23	23	20
VI	24	20	24	22	18	19
VII	20	19	23	21	17	18
VIII	18	18	22	19	15	17
S. control	-	-	-	-	-	-
Standard	38	30	40	35	35	32

Fig.1 **Fig.2** **Fig.3** **Fig.4**

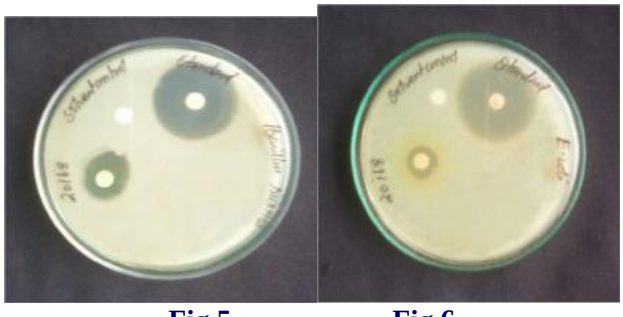


Fig.5 **Fig.6**

The antibacterial and antifungal activity of azomethine compounds I- VIII (table. 3 and figure 1-6) clearly indicate that they inhibit the growth of tested bacteria and fungi in the decreasing order I>II>III>IV>V>VI>VII>VIII. Azomethine compounds I- VIII prevent bacterial reproduction by acting as an antimetabolite to paraaminobenzoic acid (PABA), where PABA is a vital component in the biosynthesis of tetrahydrofolic acid. Competitive inhibition of PABA processing enzymes by I-VIII ultimately blocks the action of dihydrofolic acid synthetase, and therefore prevents dihydrofolic acid formation. As bacteria are unable to take up tetrahydrofolic acid from their surroundings, inhibition of dihydrofolic acid synthetase will starve the bacteria of thymidine and uridine. These two nucleosides are required for DNA replication and transcription, therefore cell growth and division is disrupted, and thus provides enough time for the body's own immune system to eliminate the bacterial threat[20].

The nature of bonding and structure of azomethine organic compounds were elucidated by the elemental analysis, FTIR, Melting Point, NMR, Chromatography and Molar ratio methods Gomathi et.al were prepared 4-(3-ethoxy-2-hydroxybenxzelideneaminno-N-(thiazole-2-yl-) benzenesulfonamide[19], Mohamed et.al and were prepared 4-(-phenyl-propylideneamino)-benzene sulfonamide[18]. In accordance with the data obtained from antibacterial activities of 4-((2-hydroxy-3,5-diiodobenzylidene)amino)benzenesulfonamide and 4-((2-hydroxy-3,5-diiodobenzylidene)amino)-N-(thiazole-2-yl-)benzenesulfonamide, were moderately inhibited the growth of tested bacteria but our derivatives of adenine **(I)** 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol, **(II)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol, **(III)** N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine**,** **(IV)** N-(furan-2-ylmethylene)-9H-purin-6-amine, **(V)** N-(pyridin-4-ylmethylene)-9H-purin-6-amine, **(VI)** ADENINE + 5-NITROSALICYLALDEHYDE, **(VII)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxy-6-nitrophenol and **(VIII)** 2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino)phenol were highly inhibited the growth of tested bacteria. In accordance with the data obtained from antifungal activities of 4-(3-ethoxy-2-hydroxybenxzelideneamino-N-(thiazole-2-yl-)benzenesulfonamide (Gomathi et.al) were moderately inhibited the growth of tested fungi but our derivatives of adenine **(I)** 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol, **(II)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol, **(III)** N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine**,** **(IV)** N-(furan-2-ylmethylene)-9H-purin-6-amine, **(V)** N-(pyridin-4-ylmethylene)-9H-purin-6-amine, **(VI)** ADENINE + 5-NITROSALICYLALDEHYDE, **(VII)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxy-6-nitrophenoland **(VIII)** 2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino)phenol were highly inhibited the growth of tested fungi. From the results and previous work, antibacterial and antifungal activity studies were indicated that iodine substituted derivatives of sulfonamides were more active against bacteria and fungi than other derivatives of adenine.

Conclusion

The derivatives of of adenine **(I)** 2-(((9H-purin-6-yl)imino)methyl)-4,6-diiodophenol, **(II)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxyphenol, **(III)** N-(3,4,5-trimethoxybenzylidene)-9H-purin-6-amine**,** **(IV)** N-(furan-2-ylmethylene)-9H-purin-6-amine, **(V)** N-(pyridin-4-ylmethylene)-9H-purin-6-amine, **(VI)** ADENINE + 5-NITROSALICYLALDEHYDE, **(VII)** 4-(((9H-purin-6-yl)imino)methyl)-2-methoxy-6-nitrophenoland **(VIII)** 2-(((9H-purin-6-yl)imino)methyl)-5-(diethylamino)phenol were prepared and bio-assay were tested against important bacteria and fungi. It was shown that the growth of bacteria and fungi were highly inhibited by the derivatives of adenine.

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DOI:10.22192/ijamr.2018.05.05.004	

How to cite this article:
S. Sowrirajan, N. Elangovan, T. Chinnamani, G. Vijayakumar and T. Kolochi. (2018). Antibacterial and Antifungal Activities on Derivatives of Adenine. Int. J. Adv. Multidiscip. Res. 5(5): 30-37.
DOI: http://dx.doi.org/10.22192/ijamr.2018.05.05.004