ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF SUBSTITUTED N2-((1R, 4R)-4-AMINOCYCLOHEXYL)-N6-(PHENYL)-9-CYCLOPENTYL-9H-PURIN-2,6-DIAMINE DERIVATIVES

Dhanraj Kamble[1], Anil Shankarwar[1], Radhakrishna Tigote[2], Yuvaraj Sarnikar[3], Mubarak Shaikh[4], Pravin Chavan[5], Balaji Madje*[6]

[1]*Department of Chemistry, S.B.E.S. College of Science, Aurangabad, Maharashtra, India*
[2]*Department of Chemistry Dr. B. A. M. University Sub campus, Osmanabad, Maharashtra, India*
[3]*Department of Chemistry Dayanand Science College, Latur, Dist. Latur, Maharashtra, India*
[4]*Department of Chemistry, Radhabai kale Mahila Mahavidyalaya, Ahemadnagar, Maharashtra, India*
[5]*Doshi Vakil Arts College and G.C.U.B. Science & Commerce College, Goregaon-Raigad, Maharashtra, India*
[6]*Department of Chemistry Vasantrao Naik Mahavidyalaya, Aurangabad, Maharashtra, India*
**Corresponding author: drmadjebr@gmail.com*

ABSTRACT

In this study, we have synthesized N2-((1r,4r)-4-aminocyclohexyl)-N6-(substitutedphenyl)-9-cyclopentyl-9H-purin-2,6-diamine derivatives **(7a-j)** from N-(-substituted phenyl)-9-cyclopentyl-2-fluoro-9H-purin-6-amine, and cyclohexane-1,4 diamine under microwave irradiation condition and examined for their antibacterial activity against *Escherichia coli*, *Streptococcus aureus* and *Bacillus subtilis* strains. All compounds **(7a-j)** displayed moderate to good antibacterial activity in Minimum Inhibition Concentration (MIC).

Keywords: Purine, Purine fluoro-9H-purin-6-amine, N-9 Substituted-6-chloropurine, 9H-purin-2,6-diamine, Cyclohexane-1,4-diamine.

1. INTRODUCTION

The Purine antimetabolites have been used in the development of many potent medicinal agents, which exhibited antineoplastic, antileukemic, antiviral, anti-bacterial and antifungal activities [1, 2]. The purine nucleoside analogs are also used in the treatment of autoimmune diseases [3]. 6-Mercapto purine is used therapeutically as an immunosuppressive agent [4] and inhibits the growth of bacterial and mammalian cells [5]. Other 6-mercapto purine, mercapto-pyridine and mercapto-pyrimidine derivatives also exhibit antibacterial activity and have been studied as agents for targeting melanoma [6], reducing cholesterol and as vasodilators [7]. Purines and pyrimidines make up the two groups of nitrogenous bases, including the two groups of nucleotide bases. Two of the four deoxyribonucleotides and two of the four ribonucleotides, the respective building blocks of DNA and RNA, are purines. Purines are found in high concentration in meat and meat products, especially internal organs such as liver and kidney. Plant based diets are generally low in purines [8, 9]. Examples of high-purine sources include: sweetbreads, anchovies, sardines, liver, beef kidneys, brains, meat extracts (e.g., Oxo, Bovril), herring, mackerel, scallops, game meats and gravy. A moderate amount of purine is also contained in beef, pork, poultry, fish and seafood, asparagus, cauliflower, spinach, mushrooms, green peas, lentils, dried peas, beans, oatmeal, wheat bran, wheat germ, and hawthorn. Virus-infected cells have an increased demand for purine nucleotides which are needed for viral RNA or DNA synthesis and this renders the enzyme, IMPDH, as a sensitive target for antiviral chemotherapy. Synthesized 2-functionalized purine nucleosides were tested as antiviral against vaccinia virus (*in vitro*) [9]. A series of dialkyl esters of purine *N*-[2-(phosphonomethoxy) ethyl] derivatives substituted at position 2, 6, or 8 of the purine bases have been evaluated for antiviral activity [10]. N-9 Substituted-6-chloropurine derivative was highly inhibitory of *in vitro* multiplication of American Leishmania and *T. rangeli* but had no effect on *T. cruzi* epimastigotes and on mice that were acutely infected with *T. cruzi* [11]. Purine derivatives of L-Ascorbic acid were evaluated for their antitumour activity against malignant tumor cell lines: pancreatic carcinoma (Mia PaCa2), breast carcinoma (MCF7), cervical carcinoma

(HeLa), laryngeal carcinoma (Hep2), murine leukemia (L1210/0), murine mammary carcinoma (FM3A), human T-lymphocytes (Molt4/C8 and CEM/0) [12].

2. EXPERIMENTAL

2.1. Material and Methods

From the commercial sources the reagents and solvent were purchased and have been used without further purification. The melting points were taken in open capillary tubes and are uncorrected. During the course of reaction, the formation of compounds was checked by TLC on silica- Gel plates of 0.5 mm thickness and checked the location of spots by iodine and UV light. By using suitable organic solvents, all the compounds were purified by recrystallization/silica gel (100-200 mesh) gravity column. The Mass spectra of the compound were carried out by using Shimadzu GC-MS-QP-2010 model using direct inlet probe technique. ^{1}H NMR, ^{13}C NMR were recorded in $CDCl_3$ and DMSO-d6 solution on a Bruker Ac 200 0r 400 MHz spectrometer.

2.1.1. Synthesis of N-(-substituted phenyl)-9-cyclopentyl-2-fluoro-purin-6-amine (6)

N-(-substituted phenyl)-9-cyclopentyl-2-fluoro-purin-6-amine (6) was synthesized by 6-chloro-9-cyclopentyl-2-fluoro-9H-purine (1.2 gm.), dry DMF (5 mL) and corresponding substituted amine Cpd -5, (1.2 eq.), 55% NaH (2 eq.) were added, under nitrogen, and the mixture was heated at 45°Cfor 8 hrs. The resultant reaction mixture was cooled to room temperature. The product was extracted with ethyl acetate (3 x 50 mL) and the combined organic fractions were washed with brine and dried over Na_2SO_4. After concentrating, the crude product was purified by column chromatography on silica (ethyl acetate: hexane =4:10) to yield N-(-substituted phenyl)-9-clclopentyl-2-fluoro-9H-purin-6-amine as a pale yellow or off white yellow solid compound (1.25 gm, yield 78 %).

2.1.2. General procedure for the synthesis of N2-((1r,4r)-4-aminocyclohexyl)-N6-(substitute-dphenyl)-9-cyclopentyl-9H-purin-2,6-diamine (7a-j)

In a sealed tube, N-(-substituted phenyl)-9-cyclopentyl-2-fluoro-9H-purin-6-amine (0.7 gm.), and cyclohexane-1,4 diamine (3eq.) were added and the reaction was then carried out with microwave at 200°C for 40 to 80 min. The reaction mixture was cooled to the room temperature. The crude product was then purified by column chromatography on silica (methanol: DCM 3:10) to yield compound (7a-j). 1H NMR, 13C NMR, and ES-MS were used to confirm the product's production.

2.2. Spectral Data

2.2.1. N2-(4-aminocyclohexyl)-N6-(3-chloro-4-fluorophenyl)-9-cyclopentyl-9H-purine-2,6-diamine (7b):

^{1}H NMR 500 Hz, DMSO (D6): δ ppm 9.70 (bs, 1H), 8.38 (s, 1H), 8.19 (s, 1H), 7.94(s, 2H), 7.35 (s, 1H), 6.63-6.65(d, 1H), 4.69 (s, 1H), 3.66 (s, 1H), 2.96 (m, 1H), 2.02-2.09 (m, 9H), 1.88 (m, 2H), 1.68 (m, 2H), 1.50-1.53 (m, 2H), 1.30-1.37 (m, 2H); ^{13}C NMR 100 Hz, $CDCl_3$: δ ppm 152.2, 148.8, 144.8, 140.3, 138.3, 121.5, 118.3, 114.2, 66.6, 54.7, 50.7, 50.3, 34.3, 31.7, 28.8, 24.2; MS (ESI) m/e = 444 (M+H).

2.2.2. N2-(4-aminocyclohexyl)-N6-(3-chlorophenyl)-9-cyclopentyl-9H-purine-2,6-diamine (7i):

^{1}H NMR 400 Hz, $CDCl_3$: δ ppm 8.49 (bs, 2H), 8.15 (s, 1H), 7.79 (s, 1H), 7.58(s, 1H), 7.37-7.39 (m, 1H), 7.19-7.23 (m, 1H), 6.96-6.98 (m, 1H), 4.74-4.82 (m, 2H), 3.92-3.94 (m, 1H), 3.22-3.28 (t, 1H), 2.22-2.35 (m, 5H), 1.82-1.96 (m, 11H); ^{13}C NMR 100 Hz, $CDCl_3$: δ ppm 181.7, 174.2, 158.2, 151.7, 147.8, 140.5, 136.1, 134.5, 129.8, 122.5, 119.5, 117.3, 92.4, 55.3, 50.5, 32.7, 30.9, 30.0, 24.1; MS (ESI) m/e = 426 (M+H).

2.3. Biological evaluation

All the N2-((1r,4r)-4-aminocyclohexyl)-N6-(substitute-dphenyl)-9-cyclopentyl-9H-purin-2,6-diamine (**7a-j**) derivatives were checked for *in vitro* antimicrobial activity against *E. coli, Bacillus subtilis* and *Streptococcus aureus* using time dose dependent growth inhibition assay. The results of antimicrobial activity of tested compounds (**7a-j**), using tetracycline as reference standard, are shown in table 2.

2.3.1. Procedure for Antibacterial activity

Clinical isolates were grown in Luria Bertini medium (pH 6.8) for 24 hours for activation of cultures. The colony forming units (CFUs) were calculated from the broth. A 100 uL ($100x10^2$ CFUs/mL) of the medium were inoculated into fresh Luria Bertini broth (5 ml) and kept for 16 hours to 18 hours for log phase culture. This log phase culture was used for the antimicrobial assay.

2.3.2. Preparation of compounds

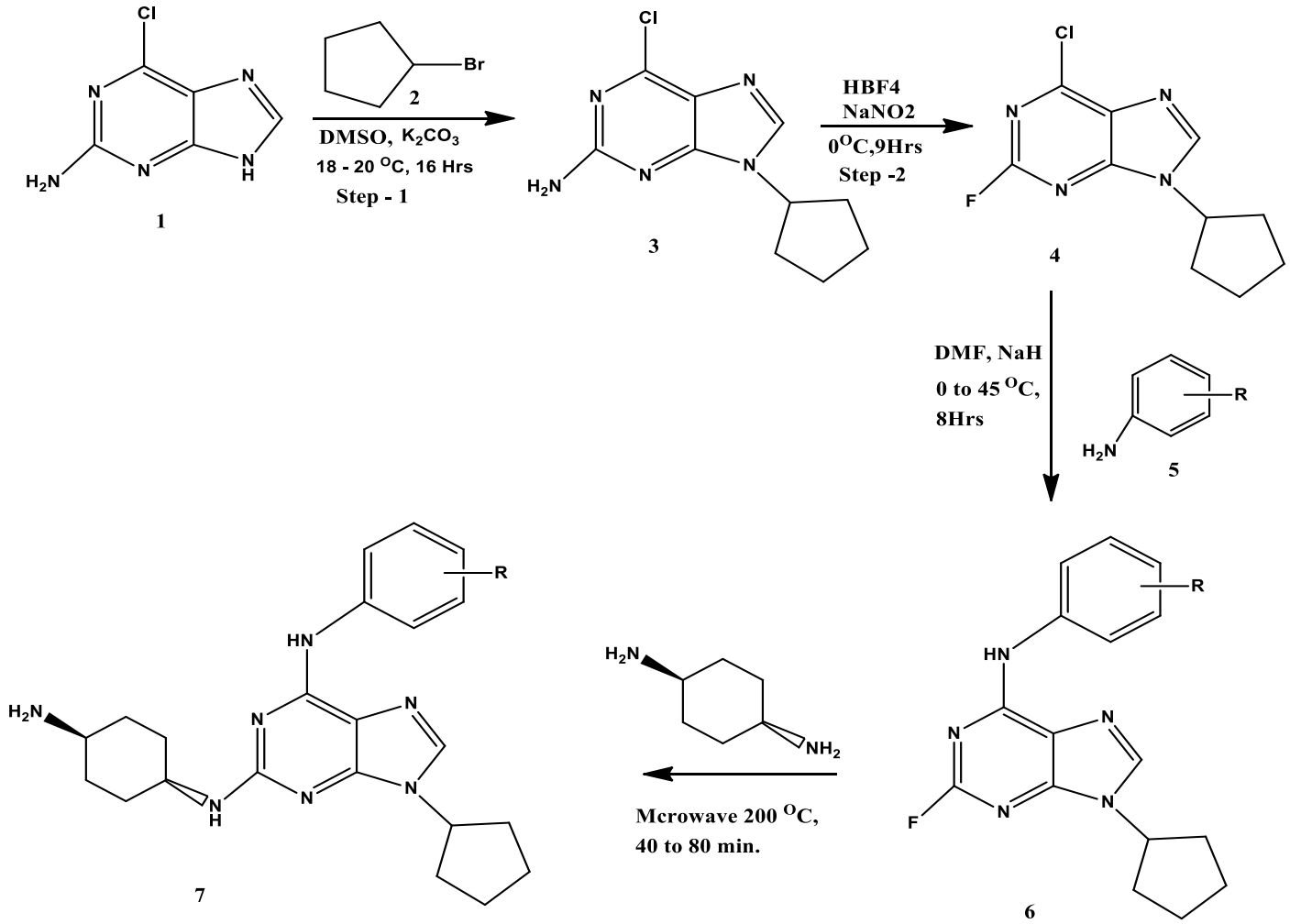
The stock solution was prepared in DMSO and diluted further for antimicrobial action.

2.3.3. Time and dose dependent effect of compounds on the growth of the pathogenic micro-organisms

The culture of MDR strain of *Escherichia coli, Streptococcus aureus* and *Bacillus subtilis* were inoculated separately into LB medium and incubated at 37°Cfor 16-18 hours. After 16 to 18 hours, the cultured tubes were exposed to the compounds at concentration of 10, 25 and 50 µg/mL. The O.D. was recorded at 660 nm after fixed interval of time. By using Graph pad prism 7, the time dependent growth of microorganism was analyzed.

3. RESULTS AND DISCUSSION

The following procedure was used for the synthesis of N2-((1r,4r)-4-aminocyclohexyl)-N6-(substitutedphenyl)-9-cyclopentyl-9H-purin-2,6-diamine (**7a-j**). The starting material (4) has been prepared by reaction with fluroboric acid, bromocyclopentane with commercially available compound (1). The compound 4 (1.2 g) was reacted with corresponding substituted amine 5 (1.2 eq) in presence of dry DMF and 55% NaH (2 eq.) under nitrogen and reaction was heated at 45°Cfor 8 hrs. The resultant compound 6 was extracted with ethyl acetate and the combine organic fraction was washed with brine and dried over Na_2SO_4. After concentration, the crude product was purified by column chromatography to yield off white solid compound 6. The compound (**7a-j**) was prepared by taking compound 6 in a sealed tube and cyclohexane 1, 4 diamine (3 eq) was added and the reaction was then carried out with microwave at 200°C for 40 to 80 min. The product was purified by column chromatography on silica (methanol: DCM 3:10) to yield compound (**7a-j**) in very good yield as shown in table 2. The formation of derivatives **(7a-j)** was justify by 1H NMR, 13C NMR and MS analysis.



Scheme 1: Synthesis of N2-((1r,4r)-4-aminocyclohexyl)-N6-(substitutedphenyl)-9-cyclopentyl-9H-purin-2,6-diamine (7a-j)

Table 1: Synthesis of N2-((1r,4r)-4-amino-cyclohexyl)-N6-(substitutedphenyl)-9-cyclo-pentyl-9H-purin-2,6-diamine (7a-j)

Entry	(R)	Product	Time (Hrs)	% Yield
1.	2-Me	(7a)	35	65
2.	3-Cl, 4-F	(7b)	45	65
3.	4-Br	(7c)	45	68
4.	2-Me, 4-Br	(7d)	50	75
5.	4-Me	(7e)	70	74
6.	4-Cl	(7f)	65	66
7.	2-F, 3-Cl	(7g)	65	73
8.	3-Br	(7h)	80	72
9.	3-Cl	(7i)	45	80
10.	4-OMe	(7j)	70	77

Reaction Condition: N-(-substituted phenyl)-9-cyclopentyl-2-fluoro-9H-purin-6-amine (0.7 gm.), and cyclohexane-1,4 diamine (3eq.) were added and then the reaction was carried out with microwave at 200°C.

3.1. Antimicrobial effect of compounds (7a-j) on pathogenic microorganism by using Time Dose method

According to the time dose method, compound **7a** was found active at 25 and 50 µg/ml concentration at 10 hrs. The growth of *E. coli* was found at MIC 14.5 µg/ml. It was not found active against *Bacillus subtilis* and *S. aureus* at any concentration. The compound **7b** was found very active against all the three bacteria. The efficiecy in controlling growth of pathogenic microorganism is carried out at very low concentration at 10 hrs. of exposure, the MIC was found at 6.3 µg/ml against *E. coli,* 7.8 µg/ml against *S. aureus* and 7.40 µg/ml against *Bacillus subtilis*. The compound **7c** was added to the 16 hrs. grown culture of the *E. coli*, the MIC was found at 12.4 µg/ml. The long phase *S. aureus* culture was used for determination of MIC of compound **7c**. A concentration of 0, 10, 25 and 50 µg/ml of compound was used against *S. aureus*, the MIC was found 10.6 µg/ml at 24 hrs. of incubation period. The compound **7c** was found more active against the *E. coli* than *S. aureus*. The compound **7d** was found active against *E. coli* and *Bacillus subtilis*. The growth of pathogenic *E. coli* and *Bacillus subtilis* was found at 10, 25 and 50 µg/ml at 10 hrs. of incubation, the MIC was found at 8.3 µg/ml against *E. coli* and 9.25 against *Bacillus subtilis*. The compound **7e** was found active against *E. coli* and inactive against *S. aureus* and *Bacillus subtilis* at 10 µL, 25 µL, 50 µL and 100 µL at 10, 16, 24 hrs. of incubation period. The compound **7f** showed activity at 10 µg/ml concentrations for 10 exposures with MIC 4.6 µg/ml against *E. coli* and 9.1ug/ml against *S. aureus*. The compound **7g** was found active at 10 µg/ml concentration at 10 hrs. incubation with MIC 7.25 µg/ml against *E. coli* and MIC 6.8 µg/ml against *S. aureus*. The compound **7h** gives activity for 10 hrs. incubation at 10, 25, and 50 µg/ml concentration with MIC 16.1 ug/ml against *E. coli* and 12.6 µg/ml against *Bacillus subtilis*. The compound **7i** was found active against all the three pathogenic bacteria. It has effectively killed bacterial cell for 10 hrs. of incubation with MIC 5.80 µg/ml against *E. coli*, 8.4 µg/ml against *S. aureus* and 4.5 µg/ml against *Bacillus subtilis*. The compound **7j** was active against the *Bacillus subtilis* and the growth was found to be inhibited at very low concentration 10µg/ml and MIC 7.01 µg/ml.

Table 2: Time and dose dependent growth inhibition assay compounds (7a-j) on pathogenic microorganism

Entry	Compound	MIC (µM)		
		E. coli	*S. aureus*	*Bacillus subtilis*
1.	(7a)	14.5	-	-
2.	(7b)	6.3	7.8	7.4
3.	(7c)	12.4	10.6	-
4.	(7d)	8.3	-	9.20
5.	(7e)	12.2	-	-
6.	(7f)	4.6	9.1	-
7.	(7g)	7.25	6.8	-
8.	(7h)	-	15.9	12.4
9.	(7i)	5.8	8.4	4.5
10.	(7j)	-	-	7.01

4. CONCLUSION

In summary, we synthesized N2-((1r,4r)-4-amino-cyclohexyl)-N6-(substitutedphenyl)-9-cyclopentyl-9H-purin-2,6-diamine derivatives (**7a-j**). Synthesized derivatives showed moderate to good inhibition of *antimicrobial activity*. These analogues are chemically tractable and hence provide ample opportunities for further modification to obtain potent anti-microbial agents. The isolated yield of the N2-((1r,4r)-4-aminocyclohexyl)-N6-(substitutedphenyl)-9-cyclo-pentyl-9H-purin-2,6-diamine derivatives (**7a-j**) are excellent.

5. ACKNOWLEDGMENT

All authors are grateful to the Department of Postgraduate Studies and Research in Chemistry, S. B. E. S. College of Science, Aurangabad, Vasantrao Naik Mahavidyalaya, Aurangabad for providing laboratory facilities and Indian Institute of Chemical Technology, Hyderabad for spectral data.

6. REFERENCES

1. Rida SM, Ashour FA, El-Hawash SA, El-Semary MM, Badr MH. *Arch Pharm (Weinheim),* 2007; **340:** 185-194.
2. Zaza G, Yang W, Kager L, Cheok M, Downing J. *Blood*, 2004, **104:**1435-144.
3. Robak T, Lech-Maranda E, Korycka A, Robak E. *Curr. Med. Chem.,* 2006, **13:**3165-3189.
4. Schwartz RS. *World J Surg*, 2006, **24**:783-786.
5. Carey NH, Mandel HG. *J. Biol. Chem.*, 1961, **236**:520-524.
6. Mars U, Larsson BS. *Pigment Cell Res*, 1995, **8**:194-201.
7. Rubina K, Abele E, Arsenyan P, Abele R, Veveris M. *Metal Based Drugs*, 2001, **8**:85-93.
8. Rosemeyer H. *Chemistry & Biodiversity*, 2004, **1**:361-401.
9. Nair, V, Bera B, Earl RK. *Nucleosides, nucleotides & nucleic acids*, 2003, **22**:115-127.
10. Holy A, Gunter J, Dvorakova H, Masojidkova M, Andrei G, Snoeck R, et al. *J. Med. Chem.*, 1999, **42:**2064-2086.
11. Avila JL, Rojas T, Avila A, Polegre MA, Robins RK. *Antimicrob. Agents Chem.*, 1987, **31**:447-451.
12. Raic-Malic S, Hergold-Brundic A, Nagl A, Grdisa M, Pavelic K, Clercq ED. *J. Med. Chem.*, 1999, **42**:2673-2678.