ejpmr, 2020,7(7), 704-711

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

MOLECULAR DOCKING, SYNTHESIS, CHARACTERISATION AND IN VITRO ANTI-FUNGAL EVALUATION OF SOME NOVEL DERIVATIVES OF 6-CHLORO-9-(3-CHLORO-4-FLUOROPHENYL)-9H-PURIN-2-AMINE

Vijaya Kumar P.*, Yadati Narasimha Spoorthy[1] and L. K. Rvindranath[2]

Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India.

***Corresponding Author: Vijaya Kumar P.**
Department of Chemistry, Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh, India.

DOI: 10.20959/ejpmr20207-8702

Article Received on 10/05/2020 **Article Revised on 01/06/2020** **Article Accepted on 22/06/2020**

ABSTRACT

4, 6-dichloropyrimidine-2,5-diamine hydro chloride on treatment with 3-chloro-4-fluoro aniline in presence of alcohol results in the formation of 6-chloro-N4-(3-chloro-4-fluorophenyl) pyrimidine-2, 4, 5-triamine (1). The latter on reaction with Tri ethyl ortho formate in presence of acid form 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine (2).A new series of derivatives were synthesized from 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine. The structures of these analogues have been established by ^{1}H NMR, IR and Mass spectral data. This study describes the anti-fungal activity of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine analogues. The docking study results disclosed that few derivatives of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine had outstanding anti-fungal activity which was superior to the activity of abacavir also.

KEYWORDS: 6-chloro purine, ethanol amine, hydrazine hydrate,6-Alcoxy purines, benzyl amines, Anti-fungal activity.

INTRODUCTION

Nitrogen containing hetero cyclic compounds are very important in the field of synthetic organic chemistry. Purine analogues are antimetabolites that mimic the structure of metabolic purines. Azathioprine is the main immunosuppressive cytotoxic substance which is used in transplantations to control rejection reactions.[1] Thiopurines such as thioguanine are used to treat acute leukemias and remissions in acute granulocytic leukemias.[2] Pentostatin and cladribine are adenosine analogues that are used primarily to treat hairy cell leukemia.[3-8] The synthesis of different derivatives of purine is an effective way for the development of new drugs and it would be a valuable addition to the present literature. Thus different purinyl derivative of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine (3-13) were synthesized. The structures of these compounds have been established by Mass, ^{1}H NMR, IR studies, and synthesis. All the new compounds were screened for their anti-fungal activity. Some of the derivatives found to have better activity.

MATERIALS AND METHODS

Melting points were determined on Mel-temp apparatus, laboratory devices, Cambridge, MA, USA and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz on a BRUKER avance-11 FT-NMR spectrometer. The electron spray ionisation MS (ESIMS) studies were performed on triple quadrupole mass spectrometer waters Quattro Micro API. The IR spectra of synthesized derivatives were recorded using KBr pellets on Perkin-Elmer SPECTRUM 100 FT-IR spectrophotometer.

Synthesis of 6-chloro-N4-(3-chloro-4-fluorophenyl) pyrimidine-2, 4, 5-triamine (1)

A solution of 2,5-Diamino4,6-dichloropyrimidine hydro chloride (0.1053 moles) and 3-chloro-4-fluoro aniline (0.1159 moles) in n-Butanol (225 mL) was refluxed for 3 hrs. After the full conversion of the starting material was detected by TLC, the reaction mass cooled to room temperature. The reaction mixture stirred at room temperature for 2 hrs. The solid is filtered off, washed with n-Butanol (25 mL) and dried under suction. Material taken in water and ethyl acetate mixture, reaction mass PH adjusted to 7.50 -8.00 (7.71), stirred for 45 min, separated organic layer and distilled out solvent under reduced pressure to get 6-chloro-N4-(3-chloro-4-fluorophenyl) pyrimidine-2, 4, 5-triamine.

Yield 28.43 g (94.00%) of compound (1). IR (KBr pellet), ν, cm^{-1}:3367, 3299, 3166, 1498, 1345, 1300, 1058. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.17 (2H, S, NH_2), 5.98 (2H, S, NH_2), 7.26-8.06 (3H, m, ArH), 8.46 (1H, S, NH), MS, m/z (%) =288(M+1,100%), m.p 203-205°C.

Synthesis of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine[2]

A solution of 6-chloro-N4-(3-chloro-4-fluorophenyl) pyrimidine-2, 4, 5-triamine (20gms) and triethylorthoformate (200 ml) taken in round bottom flask and slowly added conc. hydrochloric acid (0.1808 moles) at 25-30°C. Then the reaction mass was stirred for 8 hrs at room temperature. After the full conversion of the starting material was detected by TLC, The solid is filtered off, washed with methanol (20 mL) and dried under suction.

Yield 15.60g (75.00%) of compound (2). IR (KBr pellet), ν, cm^{-1}:3398, 3313, 2926, 1503, 1360, 1266, 1043. ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 7.09 (2H, S, NH_2), 7.61-8.15 (3H, m, ArH), 8.51 (1H, S, CH), MS, m/z (%) =298(M+1,100%), m.p 294-296^{o}C.

Synthesis of 3-(2-amino-9-(3-chloro-4-fluorophenyl)-9H-purin-6-ylamino)butan-1-ol[3]

A solution of the 6-chloro purine(1.0gms), DIPEA(0.00369 moles) and 3-Amino butanol (0.00369 moles) in n-butanol was stirred for 8 hrs at 70-80°C temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using isopropyl alcohol and dried under suction.

Yield 1.07g (91.00%) of compound (3). IR (KBr pellet), ν, cm^{-1}:3505(-OH), 3428 &3346(-NH_2), 3225(N-H), 1348(C-H), 1505(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.18-1.20 (d, 3H, CH_3),1.64-1.78 (m, 2H, CH_2), 3.46-3.48(d, 2H, CH_2), 4.42 (s, 1H, -OH), 4.47-4.50 (t, 1H, CH), 6.05 (b, 2H, NH_2),7.17 (s, 1H, NH), 7.58-8.20 (m, 3H, ArH), 8.21 (s, 1H, CH), Mass: 351(M+1), mp 137-139^{o}C.

Synthesis of 2-(2-amino-9-(3-chloro-4-fluorophenyl)-9H-purin-6-ylamino)ethanol[4]

A solution of the 6- chloro purine (1.0gms), DIPEA (0.00369 moles) and Ethanol amine (0.00369 moles) in n-butanol was stirred for 7 hrs at 70-80°C temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using isopropyl alcohol and dried under suction.

Yield 1.02g (94.00%) of compound (4). IR (KBr pellet), ν, cm^{-1}:3473(-OH) 3428&3340(-NH_2), 3223(N-H), 1349(C-H), 1532(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 3.24-3.28(t, 2H, CH_2), 3.68-3.74(t, 2H, CH_2), 4.47 (s, 1H, OH), 6.06 (s, 2H, NH_2), 7.59-8.21 (m, 4H, ArH), 7.18 (s, 1H, NH), 8.22 (s, 1H, CH), Mass: 323.2(M+1), mp 169-171^{o}C.

Synthesis of N^6-butyl-9-(3-chloro-4-fluorophenyl)-9H-purine-2,6-diamine[5]

A solution of the 6- chloro purine (1.0gms), tri ethyl amine (0.00369 moles) and n-butyl amine (0.00369 moles) in n-butanol was stirred for 7 hrs at 70-80°C temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using iso propyl alcohol and dried under suction.

Yield 0.96g (86.00%) of compound (5). IR (KBr pellet), ν, cm^{-1}:3419&3327(-NH_2), 3266(N-H), 1349(C-H), 1540(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 0.88-.93 (t, 3H, CH_3), 1.27-1.40 (dt, 2H, CH_2), 1.52-1.61 (m, 2H, CH_2), 3.41-3.42(t, 2H,CH_2), 6.02 (s, 2H, NH_2), 7.37 (b, 1H, NH), 7.58-8.21 (m, 3H, ArH), 8.22 (s, 1H, CH), Mass: 335.2(M+1), mp 143-147^{o}C.

Synthesis of 9-(3-chloro-4-fluorophenyl)-N^6-propyl-9H-purine-2,6-diamine[6]

A solution of the 6-chloro purine(1.0gms), tri ethyl amine (0.00369 moles) and n-propyl amine (0.00369 moles) in n-butanol was stirred for 6 hrs at 70-80°C temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using iso propyl alcohol and dried under suction.

Yield 0.91g (85.00%) of compound (6). IR (KBr pellet), ν, cm^{-1}:3340&3308(-NH_2), 3219(N-H), 1366(C-H), 1528(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 0.87-.92 (t, 3H, CH_3), 1.53-1.66 (dt, 2H, CH_2), 3.37-3.41(t, 2H, CH_2), 6.03 (s, 2H, NH_2), 7.39 (b, 1H, NH), 7.58-8.21 (m, 3H, ArH), 8.22 (s, 1H, CH), Mass: 321.2(M+1), mp 181-183^{o}C.

Synthesis of N^6-allyl-9-(3-chloro-4-fluorophenyl)-9H-purine-2,6-diamine[7]

A solution of the 6- chloro purine (1.0gms),tri ethyl amine (0.00369 moles)and Allyl amine (0.00369 moles) in n-butanol was stirred for 9 hrs at 70-80°C temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using isopropyl alcohol and dried under suction.

Yield 0.99g (93.00%) of compound (7). IR (KBr pellet), ν, cm^{-1}:3419&3282(-NH_2), 3212(N-H), 1348(C-H), 1535(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.09 (s, 2H, CH_2), 5.04-5.19 (m, 2H, CH_2),5.89-6.02 (m, 1H, CH), 6.07 (s, 2H, NH_2), 7.55 (b, 1H, NH), 7.59-8.21 (m, 3H, ArH), 8.22 (s, 1H, CH), Mass: 319.2 (M+1), mp 166-168^{o}C.

Synthesis of N^6-benzyl-9-(3-chloro-4-fluorophenyl)-9H-purine-2,6-diamine[8]

A solution of the 6- chloro purine (1.0gms), tri ethyl amine (0.00369 moles) and benzyl amine (0.00369 moles) in n-butanol was stirred for 12 hrs at reflux temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using ethanol and dried under suction.

Yield 1.10g (89.00%) of compound (8). IR (KBr pellet), ν, cm^{-1}:3497&3347(-NH_2), 3210(N-H), 1359(C-H), 1530(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.67 (s, 2H, CH_2), 6.07 (s, 2H, NH_2), 7.19-8.19 (m, 8H, ArH), 8.20 (s, 1H, NH), 8.21 (s, 1H, CH), Mass: 369.2(M+1), mp 183-186^{o}C.

Synthesis of N^6-(4-fluorobenzyl)-9-(3-chloro-4-fluorophenyl)-9H-purine-2,6-diamine[9]

A solution of the 6- chloro purine (1.0gms) tri ethyl amine (0.00369 moles) and 4-Fluoro benzyl amine (0.00369 moles) in n-butanol was stirred for 12 hrs at reflux temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using ethanol and dried under suction.

Yield 1.19 g (92.00%) of compound (9). IR (KBr pellet), ν, cm^{-1}:3347&3308(-NH_2), 3219(N-H), 1344(C-H), 1506(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.86 (s, 2H, NH_2), 6.20 (s, 2H, NH_2), 7.29-7.97 (m, 4H, ArH), 8.43 (s, 1H, NH), 8.72 (s, 1H, CH), Mass: 387.20(M+1), mp 308-310°C.

Synthesis of 9-(3-chloro-4-fluorophenyl)-N6-(1-phenylethyl)-9H-purine-2,6-diamine[10]

A solution of the 6- chloro purine (1.0gms), tri ethyl amine (0.00369 moles) and 1-phenyl ethyl amine (0.0369 moles) in n-butanol was stirred for 13 hrs at reflux temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using ethanol and dried under suction.

Yield 1.11g (87.00%) of compound (10). IR (KBr pellet), ν, cm^{-1}:3400&3340(-NH_2), 3219(N-H), 1349(C-H), 1504(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.52-1.54 (d, 3H, CH_3), 5.52 (b, 1H, CH), 6.02 (s, 2H, NH_2), 7.17-7.92 (m, 8H, ArH), 8.18 (s, 1H, NH), 8.20 (s, 1H, CH), Mass: 383.2(M+1), mp 139-140°C.

Synthesis of 9-(3-chloro-4-fluorophenyl)-6-hydrazinyl-9H-purin-2-amine[11]

A solution of 6- chloro purine (1.0 gm), hydrazine hydrate (0.01 moles) and triethyl amine (0.00369 moles) in 10 ml of ethanol was refluxed for 4 hrs. After the full conversion of the starting material was detected by TLC, the reaction mass cooled to room temperature. The reaction mixture stirred at room temperature for 1hr. The solid is filtered off, washed with ethanol (5 mL) followed by water and dried under suction.

Yield 0.89g (91.00%) of compound (11). IR (KBr pellet), ν, cm^{-1}:3407&3311(-NH_2), 3195(N-H), 1361(C-H), 1531(N-H). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.46 (s, 2H, NH_2), 6.13 (s, 2H, NH_2), 7.58-8.22 (m, 3H, ArH), 8.23 (s, 1H, CH), 8.63 (s, 1H, NH), Mass: 294.1(M+1), mp 249-251°C.

Synthesis of 9-(3-chloro-4-fluorophenyl)-6-ethoxy-9H-purin-2-amine[12]

A solution of the 6- chloro purine (1.0gms) and sodium ethoxide (0.0067 moles) in ethanol was stirred for 3 hrs at reflux temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using ethanol and dried under suction.

Yield 0.80g (78.00%) of compound (12). IR (KBr pellet), ν, cm^{-1}: 3395&3342(-NH_2), 2988(Aliphatic C-H), 1612(C=N), 1350(C-H), 1298(O-CH_2). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 1.36-1.40 (t, 3H, CH_3), 4.45-4.52(q, 2H, CH2), 6.61 (s, 2H, NH_2), 7.61-8.20 (m, 3H, ArH), 8.31 (s, 1H, CH), Mass: 308.1(M+1), m.p 209-211 °C.

Synthesis of 9-(3-chloro-4-fluorophenyl)-6-methoxy-9H-purin-2-amine[13]

A solution of the 6- chloro purine (1.0gms) and sodium methoxide (0.0067 moles) in alcohol was stirred for 4 hrs at reflux temperature. After the full conversion of the starting material was detected by TLC, distilled out solvent under reduced pressure and the solid was isolated using methanol and dried under suction.

Yield 0.79g (81.00%) of compound (13). IR (KBr pellet), ν, cm^{-1}: 3510&3360(-NH_2), 2966(Aliphatic C-H), 1603(C=N), 1343(C-H), 1283 (O-CH_3). ^{1}HNMR (DMSO-d_6), δ, ppm (J, Hz): 4.00 (s, 3H, CH3), 6.61 (s, 2H, NH_2), 7.64-8.19 (m, 4H, ArH), 8.30 (s, 1H, CH), Mass: 294.1(M+1), mp 233-236 °C.

ANTI-FUNGAL ACTIVITY

Methodology of Docking study

The structure of Phytase of Staphylococcus (PDB ID: 1T2P) was obtained from PDB database and given in figure 1. After removal of the unnecessary chains and hetero atoms using SPDBV software, hydrogens were added to the protein and used for active site identification.

Active site of Phytase was identified using CASTp server. This program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, also includes alpha shape and discrete flow theory. CASTp identifies, specifies and measures pockets and pocket mouth openings, as well as cavities and it provides geometrical information of pockets, mouth openings and cavities.

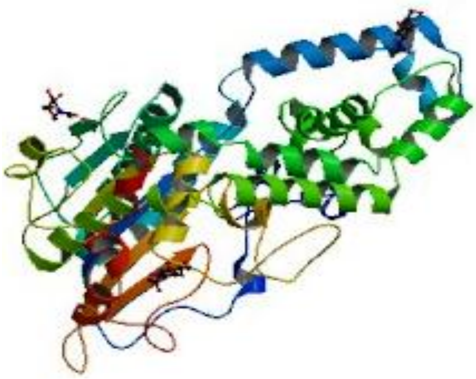


Fig 1: structure of Phytase.

Docking was carried out using GOLD (Genetic Optimization of Ligand Docking) software which is based on genetic algorithm (GA) which allows as partial flexibility of protein and full flexibility of ligand. The synthesized compounds are docked to the active site of the Phytase. The interaction of these synthesized compounds with the active site residues are thoroughly studied using molecular mechanics calculations with predefined parameters. After docking, the individual binding poses of each ligand were observed and their interactions with the protein were studied. The best and most energetically favourable conformation of each ligand was selected and used for calculation of binding energy. The binding energy of most favourable conformation was calculated using gold score fitness function. This function is made up of four components which includes Protein-ligand hydrogen bond energy [S (hb_ext)], Protein-ligand vander Waals energy (score is multiplied by a factor of 1.375 when the total fitness score is computed) [S (vdw_ext)], Ligand internal vander Waals energy [S (hb_int)], Ligand intramolecular hydrogen bond energy [S (vdw_int)].

The fitness function has been optimized for the prediction of ligand binding positions and is given below.

GoldScore = S (hb_ext) + S (vdw_ext) + S (hb_int) + S (vdw_int)

RESULTS AND DISCUSSION

The reaction of N-(2-amino-4,6-dichloropyrimidin-5-yl)formamide with ethanolic HCl in presence of ethanol at 50-55°C results in the formation of 4,6-dichloropyrimidine-2,5-diamine Hydro chloride.

6-chloro-N4-(3-chloro-4-fluorophenyl) pyrimidine-2, 4, 5-triamine (1) was prepared by reaction of 3-Chloro-4-Fluoro aniline with 4,6-dichloropyrimidine-2,5-diamine Hydro chloride in refluxing n-Butanol for 3 hrs. The obtained crude material was purified by using isopropyl alcohol as a solvent (Scheme-1).

6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine (2) was synthesized by reaction of aryl substituted pyrimidine with triethyl ortho formate in presence of Conc.HCl at room temperature for 8 hrs. They were purified by using methanol as a solvent (Scheme-1).

In the present article different purinyl derivatives of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine (3-13) were synthesized.

3-(2-amino-9-(3-chloro-4-fluorophenyl)-9H-purin-6-ylamino)butan-1-ol[3] was synthesized by the reaction of 6-chloro purine with 3-Amino butanol in presence of DIPEA. The IR spectra of **3** exhibited intense band at 3505 cm^{-1} confirming the presence of –OH. ^1H NMR showed one doublet at δ 1.18-1.20 (3H, -CH_3), another doublet at δ 3.46-3.48(2H, CH_2), one multiplet at δ 1.64-1.78 (2H, -CH_2), one singlet at δ 4.42 (1H, -OH) and one triplet at δ 4.47-4.50 (1H, CH) confirming the structure of the purinyl derivative.[3] Similarly ethanol amine derivative[4] was synthesized using ethanol amine and DIPEA as base.

N^6-butyl-9-(3-chloro-4-fluorophenyl)-9H-purine-2,6-diamine[5] was synthesized by the reaction of 6-chloro purine with n-butyl amine in presence of triethyl amine. The IR of 5 exhibited intense band at 3266 cm^{-1} confirming the presence of –NH. ^1H NMR showed one triplet at δ 0.88-0.93 (3H, -CH_3), one dubs of triplet at δ 1.27-1.40 (2H, -CH_2), one multiplet at δ 1.52-1.61 (2H, -CH_2), one triplet at δ 3.41-3.42(2H, CH_2), one broad signal at δ 7.37 (1H, -NH) confirming the structure of the purinyl derivative.[5] Similarly n-propyl amine derivative.[6] was synthesized using n-propyl amine and tri ethyl amine as base.

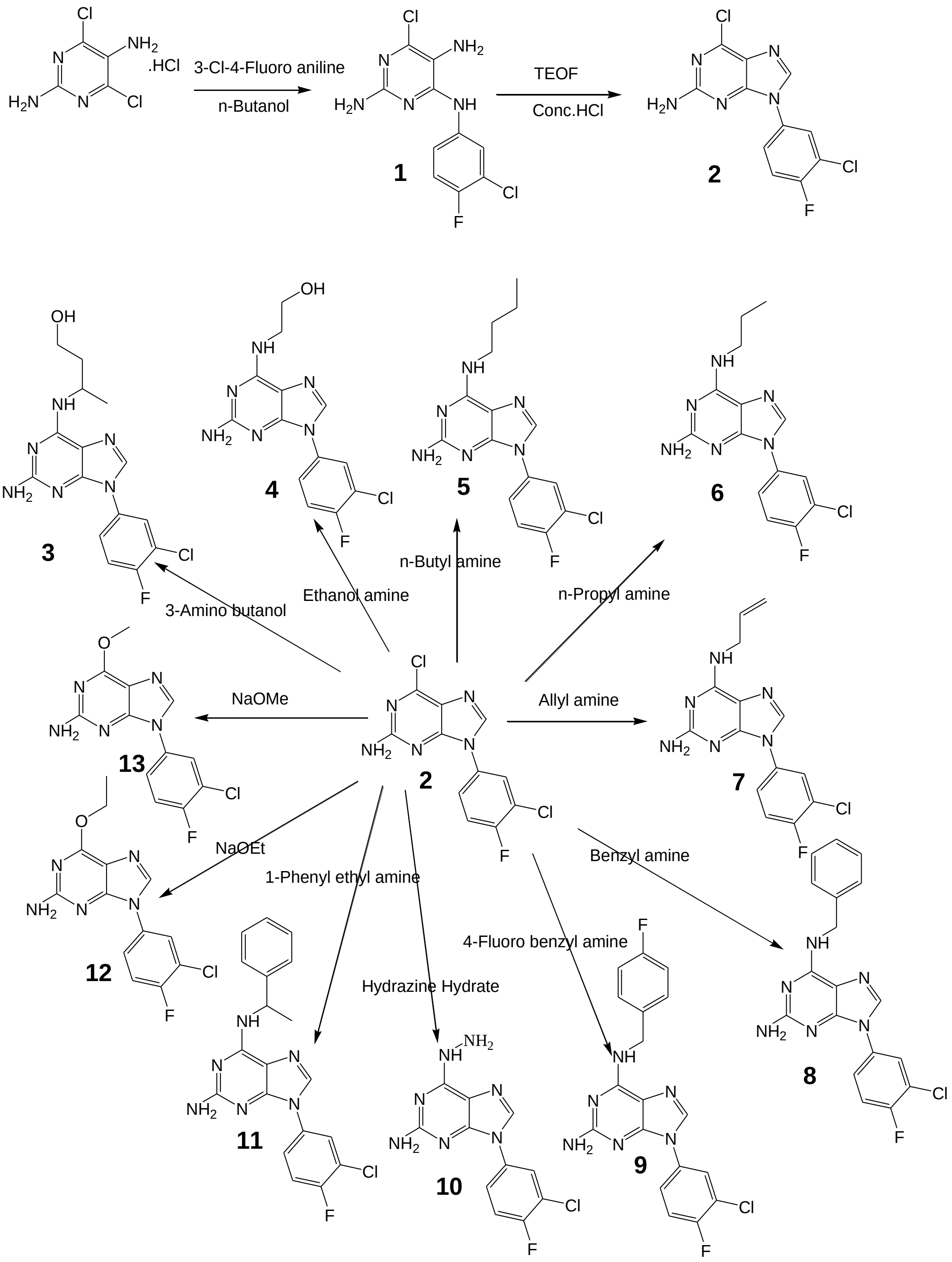
N^6-allyl-9-(3-chloro-4-fluorophenyl)-9H-purine-2,6-diamine[7] was prepared by the reaction of 6-chloro purine with allyl amine in presence of triethyl amine as base. The IR spectra of 7 exhibited intense bands at 3212cm^{-1} confirming the presence of NH. ^1H NMR showed one singlet at δ 4.09 (2H, -CH_2), one multiplet at δ 5.04-5.19 (2H, -CH_2), another multiplet at δ 5.89-6.02 (1H, CH), and broad signal δ 7.55 (1H, -NH) confirming the structure of the purinyl derivative.[7]

N^6-benzyl-9-(3-chloro-4-fluorophenyl)-9H-purine-2,6-diamine[8] was synthesized by the reaction of 6-chloro purine with benzyl amine in presence of tri ethyl amine. The IR spectra of **7** exhibited intense bands at 3210 cm^{-1} confirming the presence of NH. ^1H NMR showed one singlet at δ 4.67 (2H, -CH_2), one singlet at δ 8.20 (1H, -NH) confirming the structure of the purinyl derivative.[8] Similarly 4-fluoro benzyl amine derivative[9] was synthesized using 4-fluoro benzyl amine and tri ethyl amine as base.

9-(3-chloro-4-fluorophenyl)-N6-(1-phenylethyl)-9H-purine-2,6-diamine[10] was synthesized by reaction of 6-chloro purine with 1-phenyl ethyl amine. ^1H NMR of **10** showed one doublet at δ 1.52-1.54 (3H, CH_3) and one signal δ 5.52 (1H, -CH) confirming the structure of **10.** Hydrazine hydrate on reaction with 6-chloro purine **(2)** gave 9-(3-chloro-4-fluorophenyl)-6-hydrazinyl-9H-purin-2-amine.[11] The IR spectra of **11** exhibited intense bands at 3195cm^{-1} confirming the presence of NH. ^1H NMR showed one singlet at δ 8.63 (1H, -NH) and another singlet δ 4.46 (2H, -NH_2) confirming the structure of the hydrazinyl derivative.[11]

6-Alkoxy of purinyl derivatives[12-13] were synthesized by reaction 6-chloro purines with different alcohols in presence of sodium alcoxide. The IR spectra of 9-(4-bromophenyl)-6-ethoxy-9H-purin-2-amine[12]

Exhibited intense band at 1298 cm^{-1} confirming the presence of O-CH_2 group. ^1H NMR showed one triplet at δ 1.38 (3H, -CH_3) and one quartet δ 4.50 (2H, -CH_3) confirming the structure of the 6-ethoxy derivative. Similarly, 6-metoxy derivative[13] was synthesized from 6-chloro purine by reacting with sodium methoxide.

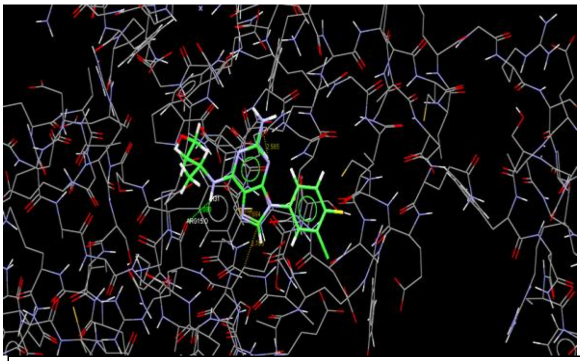


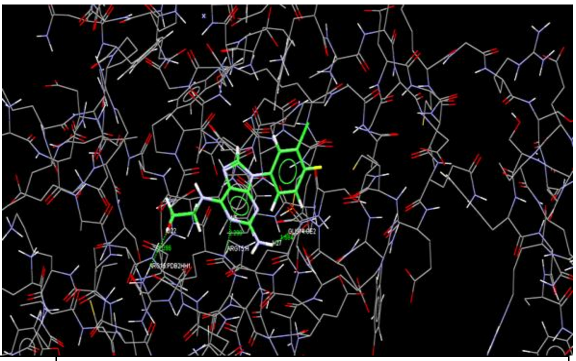
Scheme 1: Reaction path way for the preparation of 6-Chloro purine derivatives.

ANTI-FUNGAL ACTIVITY

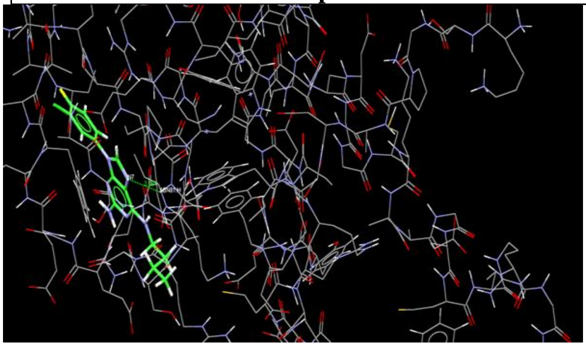
Docking study

Docking of the inhibitors (synthesized compounds) with Phytase domain was performed using GOLD 3.0.1, which is based on genetic algorithm (GA). This program generates an ensemble of different poses (rigid body orientations) for each compound conformer within the binding pocket. Then this program passes each molecule against a negative image of the binding site. The orientations surviving the bump test are then scored and ranked with a Gaussian shape function. Docking of best inhibitor (synthesized compound) with the active site of protein showed the activity of the molecule on protein function. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The bond distances (Å) and bond angles (Å) of hydrogen bonds present in the Inhibitors - Phytase complexes are listed in Tab.1. The binding 3D conformations of all Inhibitors (synthesized compounds) with Phytase molecule having good binding affinity were saved and given below (Fig 2).

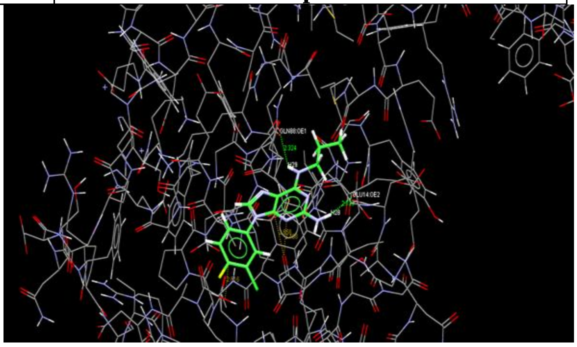




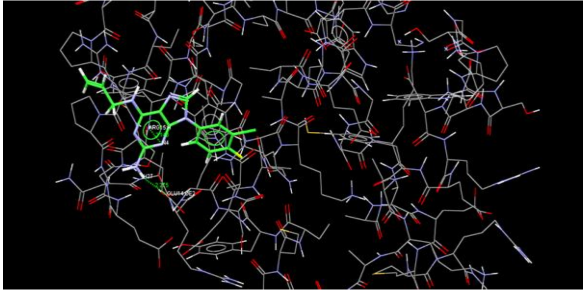
Comp.no.3 **Comp.no.4**



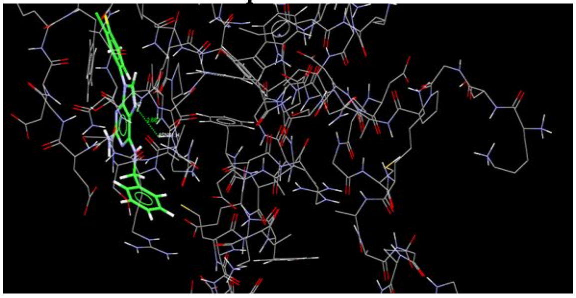
Comp.no.5



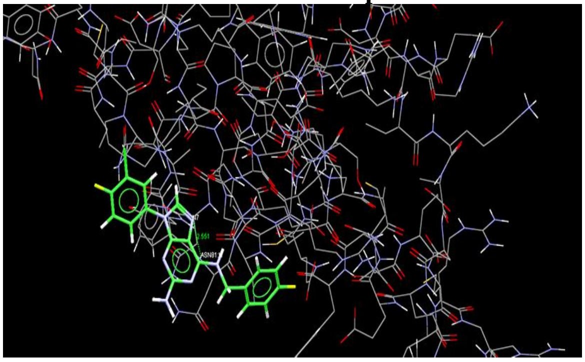
Comp.no.6



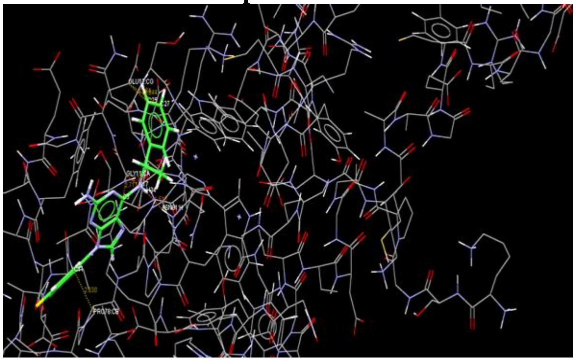
Comp.no.7



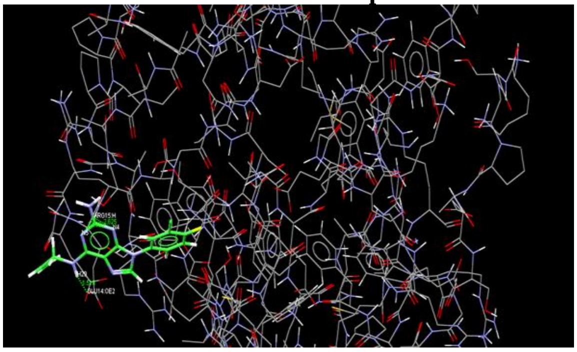
Comp.no.8



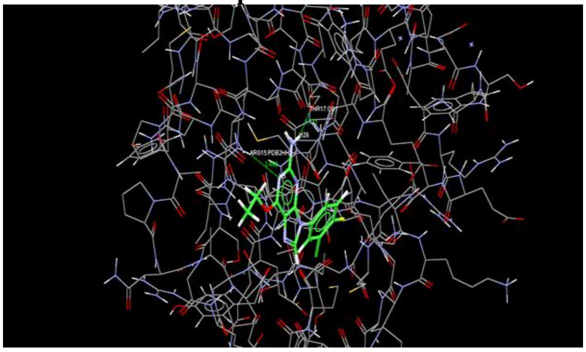
Comp.no.9



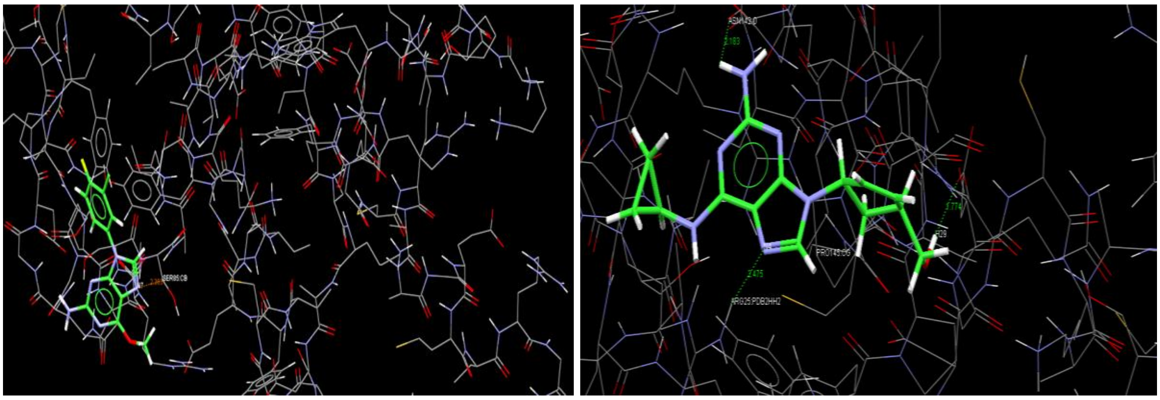
Comp.no.10



Comp.no.11



Comp.no.12



Comp.no.13 **Abacavir (std)**

Fig 2: Shows binding of Gastrotropin molecule to synthesized compounds having good affinity.

Table 1: The fitness of the top-ranked individual for each ligand docked in GOLD.

Comp. no.	Fitness	S(hb_ext)	S(vdw_ext)	S(hb_int)	S(int)
3	32.27	0.28	26.35	0.00	-4.25
4	33.18	6.75	22.91	0.00	-5.08
5	29.24	0.00	25.29	0.00	-5.53
6	29.89	1.05	24.26	0.00	-4.51
7	29.36	3.75	21.65	0.00	-4.16
8	34.24	0.00	27.57	0.00	-3.66
9	32.74	0.00	26.63	0.00	-3.88
10	31.59	0.00	27.72	0.00	-6.52
11	28.60	6.06	18.48	0.00	-2.86
12	29.46	2.11	21.23	0.00	-1.85
13	27.98	0.00	22.67	0.00	-3.19
Abacavir (Std.)	44.11	2.00	34.42	0.00	-5.21

The results of docking study of newly synthesized derivatives of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine reveals that all the compounds are having good interaction in favourable pose with Phytase. Among eleven derivatives of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine, two derivatives (4&8) showed better activity, three derivatives(3,9 and 10) showed slightly less activity when compared to standard whereas rest of six derivatives (5, 6, 7, 11, 12 and 13) showed less activity when compared to standard.

CONCLUSION

A new series of derivatives of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine were synthesized. The structure of these compounds was confirmed by their spectral data. The molecular docking study of newly synthesized derivatives of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine reveals that all the compounds are having good interaction in favourable pose with Phytase. Among eleven derivatives of 6-chloro-9-(3-chloro-4-fluorophenyl)-9H-purin-2-amine two derivatives (4&8) showed better activity which indicates these can be used as lead compounds for further development.

ACKNOWLEDGEMENTS

The authors are thankful to Laurus labs Pvt Ltd (Hyd) for providing facilities to do this work. And also thankful to JNT University authorities for providing facilities, Anantapur. The authors are thankful to Mr. Jayasimha Rayalu for supporting to do docking studies in time.

REFERENCES

1. Alicja Kowalska, Małgorzata Latocha, Krystian Pluta. Synthesis and anticancer activity of thiosubstituted purines, Med Chem Res (2015) 24:3107–3116.
2. Tohru Ueda, Kazunobu Miura, and Tsuguo Kasai. Synthesis of 6-Thioguanine and 2,6-Diaminopurine Nucleosides and Nucleotides from Adenine Counterparts via a Facile Rearrangement in the Base Portion, Chem. Pharm. Bull., 1978; 26(7): 2122-2127.
3. Kay NE, Geyer SM, Call TG, et al, Combination chemo immunotherapy with pentostatin, cyclophosphamide, and rituximab shows significant clinical activity with low accompanying toxicity in previously untreated B chronic lymphocytic leukemia, *Blood*, 2007; 109(2): 405–411.
4. Sauter C, Lamanna N, Weiss MA, Pentostatin in chronic lymphocytic leukemia Expert Opin Drug Metab Toxicol, 2008; 4(9): 1217–1222.
5. Cannon T, Mobarek D, Wegge J, Tabbara IA, Hairy cell leukemia: current concepts, Cancer Investigation, 2008; 26(8): 860–865.
6. Liliemark J, The clinical pharmacokinetics of cladribine, *ClinicalPharmacokinetics*, Jan, 1997; 32(2): 120–131.

7. Leist, TP; Weissert, R, Cladribine: mode of action and implications for treatment of multiple sclerosis, Clinical Neuropharmacology, 2010; 34(1): 28–35.
8. Shaohong Xu Peng Yao Gairong Chen &Hui Wang, A New Synthesis of 2-chloro-2′-deoxyadenosine (Cladribine), CdA), Nucleosides, Nucleotides and Nucleic acids, 2011; 30(5): 353-359.