One-Pot Three Component Synthesis, Biological Evaluation and Molecular Docking Studies of Novel Indolyl-3,4-dihydroacridinones

Shaik Rahber[1] and Jaiprakash Sharanappa Biradar[1*]
[1] Central Research Lab, Department of Chemistry, Gulbarga University, Kalaburagi-585 106 Karnataka State, INDIA

Abstract: *Simple and efficient one-pot method has been developed for the synthesis of indolyl-3,4-dihydroacridinone analogues* ***(4a-4p)*** *in good yields using 2,5-Disubstituted indole-3-carboxaldehyde, 5,5-dimethylcyclohexane-1,3-dione and 4-substitued anilines. The structures of all newly synthesized compounds have been characterized based on IR, ^{1}H, ^{13}C NMR, Mass spectral data and elemental analysis. All the compounds have shown very good in vitro antimicrobial and antioxidant activities. Further, in silico molecular docking study indicates that the synthesized compounds are novel inhibitors of the enzyme Glucosamine-6-phosphate synthase.*

Keywords: *Indole-3-carboxaldehyde, Dihydroacridinone, Antimicrobial Activity, Antioxidant Activity, Molecular Docking.*

INTRODUCTION

Over a period of past few decades microbial infections have been evolved on terrifying levels across the world as a result of antimicrobial resistance[1-2]. However, literature survey reveals that the slow rate of novel antimicrobials discovery coupled with rapid multidrug resistance signifies an urgent need to develop new and efficient antimicrobial agents with less side effects[3]. This framework involves the design of novel motifs with different mode of action than that of contemporary medicines. In search of a new potential target for antimicrobial therapy, the enzyme Glucosamine-6-phosphate synthase has been found to involve in constituting cell wall[4-5]. The product of this enzyme D-glucosamine-6-phosphate (GlcN-6-P), which is a precursor for the synthesis of essential macromolecules containing chitin in fungi and peptidoglycan in

bacteria, as well as glycoproteins in mammals [6]. Inhibition of GlcN-6-P synthase leads to disruption of microbial cell wall and subsequently, cell lysis without damaging mammalian cell.

Indole and its derivatives have emerged as important skeletons of various biologically active compounds such as antimicrobials[7], antioxidants[8], anti-carcinogens[9] and antivirals[10]. Moreover, indole nucleus has fascinated increased attention due to its various potential bioactivities, like anti-rheumatoidal[11], anti-inflammatory[12], and anti-HIV[13] activities and also play a vital role in tubulin polymerization inhibitory action[14]. On the other hand, acridine and acridinone derived scaffolds have acquired therapeutic success due to various interesting biological properties such as antioxidant[15], antimalarial[16], antihelmintic[17], and antitumor[18]. In addition many acridines have reported as DNA intercalators, topoisomerase inhibitors[19] and drugs in the treatment of Alzheimer's disease[20].

Incited by these studies and our research interest to launch the programs of design and synthesis of bioactive privileged structures containing indole ring[21-24], we have devised an efficient and economically viable protocol for the synthesis of indole integrated acridinones with a view to generate promising biologically active analogues. All compounds were evaluated for their *in vitro* antimicrobial and antioxidant activities. Molecular docking studies were performed on aforesaid GlcN-6-P synthase in order to investigate the better understanding of protein-drug binding affinity.

MATERIALS AND METHODS

All chemicals and reagents were bought commercially from Himedia, Spectrochem and SD Fine chemical companies and used without further purification. Open capillaries were used to determine the melting points of synthesized compounds and are uncorrected. The completion of reaction was examined by thin-layer chromatography (TLC) using pre-coated aluminium sheets with silica gel 60 F_{254} (MERCK). Mixture of ethyl acetate and hexane (1:1) was used as mobile phase and visualised under UV light and iodine. Infrared spectra (in KBr-pellet) were recorded using Perkin Elmer - Spectrum RX-IFTIR (ν_{max} in cm^{-1}). ^{1}H and ^{13}C NMR spectra recorded on BRUKER Avance II NMR Spectrometer. The LC-MSD-Trap-SL instrument was used to record Mass spectra. The elemental analysis was performed using FLASH EA 1112 SERIES instrument. All the compounds furnished C, H and N values within ±0.5% of the theoretical values.

General synthesis of 2,5-substituted lindole-3 carboxaldehydes (1a-1d)

The 2,5-disubstituted indoles were fomylated under Vilsmeier-Haack conditions to afford precursors 2,5-disubstituted indole-3-carboxaldehydes **(1a-1d)** which are used to obtain the target compounds[25].

General synthesis of 3,3-dimethyl-9-(2,5-disubstituted-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4a-4p)

A mixture of 2,5-disubstituted-indole-3-carbaxaldehyde **(1a-1d)** (0.01mol) 5,5-dimethylcyclohexane-1,3-dione **(2)** (0.01mol) and 4-substitued anilines **(3a-3d)** (0.01mol) in 12ml of ethanol was refluxed for 5-7hrs with catalytic amount of acetic acid. The progress of the reaction is monitored by TLC. After completion of the reaction, the excess of solvent was removed under reduced pressure. Crude product obtained after cooling to room temperature was filtered and then recrystallized from suitable solvent to get the target compounds **(4a-4p)**.

Compound	4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	4k	4l	4m	4n	4o	4p
R_1	Ph	Ph	Ph	H	Ph	Ph	Ph	H	Ph	Ph	Ph	H	Ph	Ph	Ph	H
R_2	Cl	CH_3	H	H	Cl	CH_3	H	H	Cl	CH_3	H	H	Cl	CH_3	H	H
R_3	Cl	Cl	Cl	Cl	Br	Br	Br	Br	CH_3	CH_3	CH_3	CH_3	OCH_3	OCH_3	OCH_3	OCH_3

[Reaction Scheme]

Antimicrobial Activities

Antimicrobial activities of the synthesized compounds were evaluated according to broth dilution assay method[26]. The medium containing various concentrations of synthesized compounds *viz.*, 1000 µg – 62.5 µg per ml in DMSO prepared by serial dilution. After inoculation of culture, the tubes were incubated for 72 hours at 28ºC. The minimum inhibitory concentration (MIC) of each sample was determined by measuring the optical density in the spectrophotometer (Electronics India) at 520 nm and compared the result with those of the non-inoculated broth used as blank. Control was prepared using media and inoculum without synthetic compound.

DPPH Radical Scavenging activity

The synthesized compounds were screened for free radical scavenging activity by DPPH method[27]. 1 mL of different concentrations (25 µg/mL, 50 µg/mL 75 µg/mL and 100 µg/mL) of each compound as well as reference

standards Ascorbic Acid (AA) and Butylated Hydroxy Anisole (BHA) in methanol were taken in different test tubes. 1 mL methanolic solution of DPPH (0.1 mM) was added to these test tubes. The volume was adjusted to 5 mL by adding 3 mL of methanol and shaken vigorously. After incubation for 30 minutes at 27 ºC, the absorbance was measured at 517 nm. The control was prepared as above without any compound. The percentage of radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = [(\text{Control OD} - \text{Sample OD}) / (\text{Control OD})] \times 100$$

Ferric reducing antioxidant power activity

Compounds were evaluated for ferric reducing antioxidant power activity by using Ascorbic Acid (AA) and Butylated Hydroxy Anisole (BHA) as standards. Different concentrations of compounds along with standards (25 µg/mL, 50 µg/mL 75 µg/mL and 100 µg/mL) were mixed with 2.5 mL of 1% potassium ferricyanide in 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6). The mixture was incubated at 50 ºC for 20 minutes and then 2.5 mL of trichloroacetic acid (10%, w/v) was added. Next, the contents were centrifuged at 3000 rpm for 10 minutes and supernatant layer (5 mL) mixed with 5 mL of distilled water followed by 1 mL of 0.1% ferric chloride solution. Absorbance was measured at 700 nm against the blank.

Molecular Docking Study

The ligands 2D structures were drawn using ChemDraw Ultra 12.0 and converted to energy minimized 3D structures with proper orientations using Marvin view (Chem Axon) program. The crystal structure of receptor enzyme D-glucosamine-6-phosphate synthase (PDB ID: 2VF5) was obtained from the RCSB Protein Data Bank, http://www.rcsb.org/pdb in pdb file format. To make the receptor enzyme free from any ligand, all heteroatoms and water molecules were removed while the Kollman charges, polar hydrogen atoms and solvation parameters added. All ligand's single bonds were assigned as free to revolve. AutoDock 4.2 and AutoDock Tools 1.5.6 docking programs were used to perform the docking simulation[28]. The docking grid box was set at 70, 64, and 56 Å for x, y and z respectively with a spacing of 0.375 Å which cantered at active site and surrounded by the residues Ala 602, Val 399, Ala 400, Gly 301, Thr 302, Ser 303, Cys 300, Gln 348, Ser 349, Thr 352, Ser 347 and Lys 603. AutoGrid 4 program was used in order to set the grid maps and other parameters were fixed to default standard values. The AutoDock 4 and Lamarckian genetic algorithm (LGA) was used for energy calculation with a maximum of ten runs for each tested

ligand. The outputs were visualized by means of the Accelrys Discovery Studio client package and inspected for the binding modes with possible hydrogen bonding, polar and hydrophobic interactions of ligands with receptor active site. All these AutoDock simulation were performed in Intel (R) Core(TM) *i*3 CPU M380 @ 2.53 GHz of Dell system origin, with 2 GB DDR3 RAM. AutoDock 4.2 was compiled and run under Windows 7 Ultimate operating system.

RESULTS AND DISCUSSION

Synthesis of novel dihydroacridinone analogues of 2,5-disubstituted indoles **(4a-4p)** via one-pot three component methodology as outlined in Reaction Scheme. The progress of the reaction was monitored by TLC. The excess of solvent was removed under reduced pressure and reaction mixture was cooled to room temperature. The resulting crude product obtained was filtered, washed and recrystallized from suitable solvent. The structures of the synthesized compounds were confirmed by their melting points, IR, ^{1}H, ^{13}C NMR, Mass and analytical data. This environmentally benign protocol presents mild reaction condition, simple procedure, with good yields and purity.

The IR spectrum of 7-chloro-9-(5-chloro-2-phenyl-1H-indol-3-yl)-3,3-dimethyl-3,4-dihydroacridin-1(2H)-one **(4a)** showed a strong absorption at 3443 cm^{-1} corresponding to indole NH, absorptions at 1663 cm^{-1} and 1593 cm^{-1} correspond to carbonyl and aromatic C=N stretching respectively. The ^{1}H NMR spectrum of **(4a)** has exhibited a singlet at δ 12.39 due to indole NH which is also D_2O exchangeable. A singlet at δ 8.21, doublet at δ 8.10, and multiplet between 7.96-7.11 for eleven aromatic protons in the molecule. Signals between 2.43-2.36 and at δ 1.01 were assigned for two methylene and methyl protons respectively. The ^{13}C NMR of the compound **(4a)** has shown a signal at 193 is due to carbonyl carbon and at δ 164 corresponds to C=N. The two methylene carbons appeared at δ 55 and 51 whereas two equivalent methyl carbon displayed a peak at δ 31. The Mass spectrum of **(4a)** has shown molecular ion peak at *m/z* = 484 corresponds to its molecular weight, with isotopic peaks at *m/z* = 486 and 488. These spectral data support the structure of compound **(4a)**.

7-Chloro-9-(5-chloro-2-phenyl-1H-indol-3-yl)-3,3-dimethyl-3,4-dihydroacridin-1(2H)-one (4a)

Yield 69%, mp 194-195 ºC. IR spectrum, *ν*, cm^{-1}: 3443(NH), 1663(C=O), 1593(C=N). ^{1}H NMR spectrum, *δ*, ppm: 12.39 (s, 1H, indole NH), 8.21 (s, 1H, Ar-H), 8.10 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.96 (dd, *J* = 9.0 and 2.0 Hz, 1H, Ar-H), 7.75-7.72 (m, 2H, Ar-H), 7.54-7.47 (m, 3H, Ar-H), 7.39 (s, 1H, Ar-H), 7.34 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.11 (dd, *J* = 8.4 and 1.6 Hz, 1H, Ar-H), 2.43 (s, 2H, CH_2), 2.36 (s, 2H, CH_2), 1.01 (s, 6H, $2CH_3$). ^{13}C NMR spectrum, *δ*, ppm:

193, 164, 146, 144, 138, 136, 137, 134, 132, 130, 125, 121, 55, 51, 33, 31. Found, %: C 71.69; H 4.52; N 5.80. Calculated, %: C 71.76; H 4.57; N 5.77. MS, *m/z*: 484 $[M]^+$, 486 $[M+2]^+$, 488 $[M+4]^+$ (10:6.3:1).

7-Chloro-3,3-dimethyl-9-(5-methyl-2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4b)

Yield 60%, mp 196-198 ºC. IR spectrum, *ν*, cm^{-1}: 3432(NH), 1652(C=O), 1601(C=N). ^1H NMR spectrum, *δ*, ppm: 12.42 (s, 1H, indole NH), 8.13 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.99 (s, 1H, Ar-H), 7.92 (dd, *J* = 8.7 and 2.0 Hz, 1H, Ar-H), 7.71-7.68 (m, 2H, Ar-H), 7.58-7.49 (m, 3H, Ar-H), 7.41 (s, 1H, Ar-H), 7.31 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.10 (dd, *J* = 8.3 and 1.6 Hz, 1H, Ar-H), 2.40 (s, 2H, CH_2), 2.33 (s, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.00 (s, 6H, $2CH_3$). Found, %: C 77.51, H 5.38, N 6.05. Calculated, %: C 77.49, H 5.42, N 6.02. MS, *m/z*: 465 $[M+1]^+$, 467 $[M+3]^+$ (3:1).

7-Chloro-3,3-dimethyl-9-(2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4c)

Yield 64%, mp 190-192 ºC. IR spectrum, *ν*, cm^{-1}: 3448(NH), 1658(C=O), 1599(C=N). ^1H NMR spectrum, *δ*, ppm: 12.39 (s, 1H, indole NH), 8.10 (d, *J* = 9.0 Hz, 1H, Ar-H), 7.98 (dd, *J* = 9.0 and 2.2 Hz, 1H, Ar-H), 7.85 (d, *J* = 8.6 Hz, 1H, Ar-H) 7.78-7.62 (m, 5H, Ar-H), 7. 51 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.42 (s, 1H, Ar-H) 7.28-7.19 (m, 2H, Ar-H), 2.43 (s, 2H, CH_2), 2.31 (s, 2H, CH_2). 0.98 (s, 6H, $2CH_3$). Found, %: C 77.21, H 5.10, N 6.18. Calculated, %: C 77.24, H 5.14, N 6.21. MS, *m/z*: 451 $[M+1]^+$, 453 $[M+3]^+$ (3:1).

7-Chloro-9-(1H-indol-3-yl)-3,3-dimethyl-3,4-dihydroacridin-1(2H)-one (4d)

Yield 59%, mp 210-213 ºC. IR spectrum, *ν*, cm^{-1}: 3258(NH), 1654(C=O), 1592(C=N). ^1H NMR spectrum, *δ*, ppm: 12.20 (s, 1H, indole NH), 8.29 (s, 1H), 8.06 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.94 (dd, *J* = 8.7 and 1.8 Hz, 1H, Ar-H), 7.87-7.84 (m, 1H, Ar-H), 7.59-7.56 (m, 1H, Ar-H), 7.46 (s, 1H), 7.30-7.27 (m, 2H, Ar-H), 2.43 (s, 2H, CH_2), 2.36 (s, 2H, CH_2), 1.01 (s, 6H, $2CH_3$). Found, %: C 73.65, H 5.16, N 7.50. Calculated, %: C 73.69, H 5.11, N 7.47. MS, *m/z*: 374 $[M]^+$ 376 $[M+2]^+$ (3:1).

7-Bromo-9-(5-chloro-2-phenyl-1H-indol-3-yl)-3,3-dimethyl-3,4-dihydroacridin-1(2H)-one (4e)

Yield 70%, mp 198-199 ºC. IR spectrum, *ν*, cm^{-1}: 3413(NH), 1649(C=O), 1610(C=N). ^1H NMR spectrum, *δ*, ppm: 12.41 (s, 1H, indole NH), 8.21 (s, 1H, Ar-H), 8.07 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.92 (dd, *J* = 9.2 and 2.0 Hz, 1H, Ar-H), 7.78-7.74 (m, 2H, Ar-H), 7.60-7.55 (m, 3H, Ar-H), 7.42 (s, 1H, Ar-H), 7.36 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.02 (dd, *J* = 8.4 and 1.6 Hz, 1H, Ar-H), 2.42 (s, 2H, CH_2), 2.35 (s, 2H, CH_2), 1.00 (s, 6H, $2CH_3$). ^{13}C NMR spectrum, *δ*, ppm:

196, 167, 143, 141, 140, 139, 138, 136, 131, 130, 126, 125, 54, 51, 32, 30. Found, %: C 65.75, H 4.17, N 5.30. Calculated, %: C 65.74, H 4.19, N 5.29. MS, *m/z*: 528 $[M]^+$, 530 $[M+2]^+$, 532 [M+4] (10:13:3).

7-Bromo-3,3-dimethyl-9-(5-methyl-2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4f)

Yield 65%, mp 202-203 ºC, IR spectrum, *ν*, cm^{-1}: 3376(NH), 1652,(C=O), 1586(C=N). ^{1}H NMR spectrum, *δ*, ppm: 12.38 (s, 1H, indole NH), 8.15 (d, *J* = 8.8 Hz, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 7.91 (dd, *J* = 8.7 and 2.0 Hz, 1H, Ar-H), 7.69-7.65 (m, 2H, Ar-H), 7.50-7.41 (m, 4H, Ar-H), 7.28 (d, *J* = 8.3 Hz, 1H, Ar-H), 7.09 (dd, *J* = 8.3 and 1.6 Hz, 1H, Ar-H), 2.41 (s, 2H, CH_2), 2.34 (s, 2H, CH_2), 2.30 (s, 3H, CH_3), 1.00 (s, 6H, $2CH_3$). Found, %: C 70.70, H 4.97, N 5.55. Calculated, %: C 70.73, H 4.95, N 5.50. MS, *m/z*: 508 $[M]^+$, 510 $[M+2]^+$ (1:1).

7-Bromo-3,3-dimethyl-9-(2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4g)

Yield 67%, mp 204-206 ºC, IR spectrum, *ν*, cm^{-1}: 3365(NH), 1670(C=O), 1594(C=N). ^{1}H NMR Spectrum, *δ*, ppm: 12.37 (s, 1H, indole NH), 8.02 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.93 (dd, *J* = 8.8 and 2.0 Hz, 1H, Ar-H), 7.86 (d, *J* = 8.2 Hz, 1H, Ar-H) 7.76-7.61 (m, 5H, Ar-H), 7. 49 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.40 (s, 1H, Ar-H) 7.29-7.23 (m, 2H, Ar-H), 2.43 (s, 2H, CH_2), 2.31 (s, 2H, CH_2). 0.98 (s, 6H, $2CH_3$). Found, %: C 70.36, H 4.65, N 5.68. Calculated, %: C 70.31, H 4.68, N 5.65. MS, *m/z*: 494 $[M]^+$, 496 $[M+2]^+$ (1:1).

7-Bromo-9-(1H-indol-3-yl)-3,3-dimethyl-3,4-dihydroacridin-1(2H)-one (4h)

Yield 71%, mp 218-220 ºC. IR spectrum, *ν*, cm^{-1}: 3298(NH), 1638(C=O), 1616(C=N). ^{1}H NMR spectrum,*δ*, ppm: 12.20 (s, 1H, indole NH), 8.25 (s, 1H, Ar-H), 8.01 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.91 (dd, *J* = 8.8 and 1.8 Hz, 1H, Ar-H), 7.85-7.82 (m, 1H, Ar-H), 7.60-7.57 (m, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.33-7.30 (m, 2H, Ar-H), 2.43 (s, 2H, CH_2), 2.34 (s, 2H, CH_2), 0.99 (s, 6H, $2CH_3$). Found, %: C 65.90, H 4.55, N 6.71. Calculated, %: C 65.88, H 4.57, N 6.68. MS, *m/z*: 418 $[M]^+$, 420 $[M+2]^+$ (1:1).

9-(5-Chloro-2-phenyl-1H-indol-3-yl)-3,3,7-trimethyl-3,4-dihydroacridin-1(2H)-one (4i)

Yield 63%, mp 280-282 ºC. IR spectrum, *ν*, cm^{-1}: 3350(NH), 1655(C=O), 1603(C=N). ^{1}H NMR spectrum, *δ*, ppm: 12.42 (s, 1H, indole NH), 8.17 (s, 1H, Ar-H), 8.00 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.86 (dd, *J* = 8.6 and 1.8 Hz, 1H, Ar-H), 7.69-7.63 (m, 2H, Ar-H), 7.55-7.49 (m, 3H, Ar-H), 7.34 (s, 1H, Ar-H), 7.21 (d, *J* = 8.0 Hz, 1H, Ar-H), 7.01 (dd, *J* = 8.0 and 1.6 Hz, 1H, Ar-H), 2.47 (s, 2H, CH_2), 2.35 (s, 2H, CH_2), 2.31 (s, 3H, CH_3), 1.02 (s, 6H, $2CH_3$). ^{13}C NMR

spectrum, *δ*, ppm: 195, 167, 143, 141, 140, 139, 138, 136, 131, 130, 126, 125, 55, 50, 32, 30, 22. Found, %: C 77.45, H 5.46, N 5.95. Calculated, %: C 77.49, H 5.42, N 6.02. MS, *m/z*: 463 $[M-1]^+$, 465 $[M+1]^+$ (3:1).

3,3,7-Trimethyl-9-(5-methyl-2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4j)

Yield 58%, mp 202-204 ºC. IR spectrum, *ν*, cm^{-1}: 3387(NH), 1673(C=O), 1599(C=N). ^1H NMR spectrum, *δ*, ppm: 12.34 (s, 1H, indole NH), 8.08 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.95 (s, 1H, Ar-H), 7.86 (dd, *J* = 8.7 and 2.0 Hz, 1H, Ar-H), 7.72-7.69 (m, 2H, Ar-H), 7.60-7.52 (m, 3H, Ar-H), 7.32 (s, 1H, Ar-H), 7.23 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.03 (dd, *J* = 8.2 and 1.4 Hz, 1H, Ar-H), 2.41 (s, 2H, CH_2), 2.34 (s, 2H, CH_2), 2.30 (s, 3H, CH_3), 2.29 (s, 3H, CH_3) 1.00 (s, 6H, $2CH_3$). Found, %: C 83.70, H 6.38, N 6.34. Calculated, %: C 83.75, H 6.35, N 6.30. MS, *m/z*: 444 $[M]^+$.

3,3,7-Trimethyl-9-(2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4k)

Yield 68%, mp 148-150 ºC. IR spectrum, *ν*, cm^{-1}: 3340(NH), 1658(C=O), 1591(C=N). ^1H NMR spectrum, *δ*, ppm: 12.36 (s, 1H, indole NH), 8.00 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.90 (dd, *J* = 8.6 and 2.0 Hz, 1H, Ar-H), 7.86 (d, *J* = 8.2 Hz, 1H, Ar-H) 7.76-7.60 (m, 5H, Ar-H), 7. 52 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.40 (s, 1H, Ar-H) 7.26-7.15 (m, 2H, Ar-H), 2.42(s, 2H, CH_2), 2.33(s, 2H, CH_2) 2.30 (s, 3H, CH_3), 1.00 (s, 6H, $2CH_3$). Found, %: C 83.63, H 6.18, N 6.46. Calculated. %: C 83.69, H 6.09, N 6.51. MS, *m/z*: 429 $[M-1]^+$.

9-(1H-Indol-3-yl)-3,3,7-trimethyl-3,4-dihydroacridin-1(2H)-one (4l)

Yield 62%, mp 212-214 ºC. IR spectrum, *ν*, cm^{-1}: 3359(NH), 1664(C=O), 1597(C=N). ^1H NMR spectrum, *δ*, ppm: 12.29 (s, 1H, indole NH), 8.24 (s, 1H, Ar-H), 8.00 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.94 (dd, *J* = 8.6 and 1.8 Hz, 1H, Ar-H), 7.87-7.84 (m, 1H, Ar-H), 7.60-7.57 (m, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.29-7.26 (m, 2H, Ar-H), 2.40 (s, 2H, CH_2), 2.32 (s, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.01 (s, 6H, $2CH_3$). Found, %: C 81.30, H 6.30, N 7.83. Calculated, %: C 81.33, H 6.26, N 7.90. MS, *m/z*: 354 $[M]^+$.

9-(5-Chloro-2-phenyl-1H-indol-3-yl)-7-methoxy-3,3-dimethyl-3,4-dihydroacridin-1(2H)-one (4m)

Yield 63%, mp 272-274 ºC. IR spectrum, *ν*, cm^{-1}: 3424(NH), 1643(C=O), 1589(C=N). ^1H NMR spectrum, *δ*, ppm: 12.26 (s, 1H, indole NH), 8.15 (s, 1H, Ar-H), 8.00 (d, *J* = 9.2 Hz, 1H, Ar-H), 7.93 (dd, *J* = 9.0 and 2.0 Hz, 1H, Ar-H), 7.80-7.76 (m, 2H, Ar-H), 7.58-7.54 (m, 3H, Ar-H), 7.40 (s, 1H, Ar-H), 7.35 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.00 (dd, *J* = 8.4 and 1.6 Hz, 1H, Ar-H), 3.49 (s, 3H, OCH_3), 2.43 (s, 2H, CH_2), 2.36 (s, 2H, CH_2), 1.01 (s, 6H, $2CH_3$). ^{13}C NMR

spectrum, *δ*, ppm: 195, 164, 145, 143, 142, 141, 139, 138, 133, 132, 126, 125, 56, 49, 34, 32, 29. Found, %: C 74.93, H 5.21, N 5.80. Calculated, %: C 74.91, H 5.24, N 5.82. MS, *m/z*: 480 $[M]^+$, 482 $[M+2]^+$ (3:1).

7-Methoxy-3,3-dimethyl-9-(5-methyl-2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4n)

Yield 65%, mp 206-208 ºC. IR spectrum, *ν*, cm^{-1}: 3369(NH), 1664(C=O), 1613(C=N). ^1H NMR spectrum, *δ*, ppm: 12.36 (s, 1H, indole NH), 8.10 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 7.90 (dd, *J* = 8.6 and 1.8 Hz, 1H, Ar-H), 7.71-7.66 (m, 2H, Ar-H), 7.49-7.40 (m, 4H, Ar-H), 7.26 (d, *J* = 8.2 Hz, 1H, Ar-H), 7.02 (dd, *J* = 8.2 and 1.4 Hz, 1H, Ar-H), 3.48, (s, 3H, OCH_3), 2.40 (s, 2H, CH_2), 2.33 (s, 2H, CH_2), 2.29 (s, 3H, CH_3), 1.00 (s, 6H, $2CH_3$). Found, %: C 80.85, H 6.10, N 6.06. Calculated, %: C 80.84, H 6.13, N 6.08. MS, *m/z*: 460 $[M]^+$.

7-Methoxy-3,3-dimethyl-9-(2-phenyl-1H-indol-3-yl)-3,4-dihydroacridin-1(2H)-one (4o)

Yield 59%, mp 194-195 ºC. IR spectrum, *ν*, cm^{-1}: 3290(NH), 1656(C=O), 1602(C=N). ^1H NMR spectrum, *δ*, ppm: 12.36 (s, 1H, indole NH), 8.02 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.93 (dd, *J* = 8.8 and 2.0 Hz, 1H, Ar-H), 7.86 (d, *J* = 8.4 Hz, 1H, Ar-H) 7.77-7.62 (m, 5H, Ar-H), 7. 48 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.40 (s, 1H, Ar-H) 7.28-7.20 (m, 2H, Ar-H), 3.51 (s, 3H, OCH_3), 2.42(s, 2H, CH_2), 2.33(s, 2H, CH_2), 1.00 (s, 6H, $2CH_3$). Found, %: C 80.71, H 5.90, N 6.29. Calculated, %: C 80.69, H 5.87, N 6.26. MS, *m/z*: 446 $[M]^+$.

9-(1H-Indol-3-yl)-7-methoxy-3,3-dimethyl-3,4-dihydroacridin-1(2H)-one (4p)

Yield 66%, mp 185-186 ºC. IR spectrum, *ν*, cm^{-1}): 3427(NH), 1669(C=O), 1600(C=N). ^1H NMR spectrum, *δ*, ppm: 12.39 (s, 1H, indole NH), 8.26 (s, 1H, Ar-H), 8.03 (d, *J* = 8.8 Hz, 1H, Ar-H), 7.94 (dd, *J* = 8.8 and 1.8 Hz, 1H, Ar-H), 7.82-7.79 (m, 1H, Ar-H), 7.59-7.55 (m, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.31-7.28 (m, 2H, Ar-H), 3.49 (s, 3H, OCH_3), 2.40 (s, 2H, CH_2), 2.32 (s, 2H, CH_2), 1.01 (s, 6H, $2CH_3$). Found, %: C 77.79, H 6.02, N 7.53. Calculated, %: C 77.81, H 5.99, N 7.56. MS, *m/z*: 370 $[M]^+$.

Antimicrobial activity

Newly synthesized compounds **(4a-4p)** were assessed for *in vitro* antimicrobial activity against a group of two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria using Streptomycin sulphate as standard. Antifungal activity was performed against three fungal species (*Aspergillus niger*, *Aspergillus flavus* and *Fusarium oxysporum*) by using Ketoconazole as standard. Minimum Inhibitory Concentration (MICs) were determined by broth dilution assay method[26].

The results of antimicrobial activity are presented in **Table 1**. Among the screened compounds **4a, 4e, 4f, 4g** and **4m** (MIC = 125 µg/mL) were found to be active against the various bacterial strains under study compared to Streptomycin sulphate (MIC = 62.5 µg/mL). However, the compounds **4a, 4b, 4f, 4g, 4i, 4j, 4m** and **4n** showed inhibition effect at MIC = 250 µg/mL against fungal strains *A. niger* and *A. flavus* while the compound **4e** exhibited same potency as the standard Ketoconazole at MIC = 125 µg/mL against *A. niger*. Analysis of these results shows that indole ring with chloro substitution at five position accounts for increased antibacterial activity. Chloro and methyl groups are responsible for enhanced antifungal activity.

DPPH radical scavenging activity

The interaction of synthesized compounds **(4a-4p)** with the stable DPPH free radical was investigated *in vitro*[27]. These interactions will generate α,α-diphenyl-β-picryl hydrazine which leads to discoloration. The degree of discoloration is considered as an indicator of free-radical-scavenging potential of the antioxidant. Butylated hydroxy anisole (BHA) and Ascorbic Acid (AA) were taken as standard antioxidants. Among the compounds **4a** and **4i** with chloro group at five position of indole ring have shown excellent radical scavenging potential, whereas the compounds **4b, 4c, 4i** and **4n** with methyl and hydrogen at five position of indole ring exhibited relatively low scavenging ability. In contrast, rest of the compounds have shown least scavenging property. These findings are illustrated in **Figure 1**.

Ferric reducing antioxidant power activity

All the synthesized compounds were screened *in vitro* to explore their ability to reduce preformed ferric to ferrous ion by ferric reducing antioxidant power assay method[29]. Butylated hydroxy anisole (BHA) and Ascorbic Acid (AA) were used as reference standard. The compounds showed reducing capabilities in concentration dependent mode. Similar to DPPH assay results, compounds **4a, 4e** and **4i** with chloro substituted indole ring revealed excellent activity and moderate activity is observed for methyl indolylacridinone analogues **4b, 4f** and **4j**. Alongside, other indolylacridinone analogues showed least reducing power. The results are compared with standard in **Figure 2**.

Molecular Docking Study

To recognize the structural insight into the potency of synthesized novel indolylacridinone analogues **(4a-4p)**, their binding interactions with the GlcN-6-P synthase crystal structure (PDB ID: 2VF5) were investigated. In the present study, the AutoDock 4.2 software instilled with Lamarckian genetic algorithm was used to envisage best fit ligand's conformation inside the active packet of target enzyme. The identified active packet of GlcN-6-P synthase comprises

12 amino acid residues Ala 602, Val 399, Ala 400, Gly 301, Thr 302, Ser 303, Cys 300, Gln 348, Ser 349, Thr 352, Ser 347 and Lys 603[30].

Table 2 summarizes database generated from a total set of 16 compounds docked with the known GlcN-6-P synthase receptor active site. The docking results obtained from ten different ligand-receptor complex conformations of each tested compound were scrutinized and conformation with lowest binding energy was further analyzed for the binding interactions, including hydrogen bonding, hydrophobic and electrostatic interactions. It has been observed that all ligands as well as the reference drug *i.e.* Streptomycin interact inside the receptor active site with binding energies ranging from -8.89 to -4.29 kcal/mol. Best docked conformations of compounds (**4a-4p)** with GlcN-6-P synthase displayed multiple hydrophobic interactions and hydrogen bonding with active site residues such as Ala 602, Thr352 and Lys 603 by means of one hydrogen bond whereas Streptomycin interacts with residues Ala 602, Cys 300, Thr 352 and Val 399 by four hydrogen bonds (**Table 3**). The nitrogen of acridinone and indole rings of compounds **4a** and **4e** were able to form hydrogen bond with residues Thr 352 and Ala 602 respectively. However, the oxygen of methoxy group present at acridinone ring of compound **4p** forming hydrogen bond with Ala 602. **Figure 3** illustrates the binding interactions of compounds **4a, 4e, 4p** and **Streptomycin**.

Table 1. The antimicrobial activity results (MIC, µg/mL) of compounds **(4a-4p)**

	Gram positive bacteria		**Gram negative bacteria**		**Fungi**		
Compound	***B. subtilis***	***S. aureus***	***E. coli***	***P. aeruginosa***	***A. niger***	***A. flavus***	***F. oxysporum***
4a	**125**	**250**	**125**	**125**	**250**	500	500
4b	**250**	**250**	**250**	**250**	**250**	**250**	500
4c	500	500	**250**	500	500	500	>1000
4d	500	**250**	500	500	1000	500	>1000
4e	**125**	**125**	**250**	**250**	**125**	500	500
4f	**250**	**250**	**125**	**125**	**250**	**250**	500
4g	**250**	500	**250**	**125**	500	**250**	>1000
4h	1000	500	500	500	500	500	>1000
4i	500	500	1000	500	**250**	**250**	500
4j	1000	1000	500	500	>1000	**250**	500
4k	500	500	500	1000	500	500	500
4l	500	500	**250**	500	500	500	>1000
4m	**125**	**250**	**125**	**250**	**250**	**250**	500
4n	500	500	**250**	**250**	**250**	>1000	500
4o	**250**	500	1000	500	500	500	500
4p	1000	500	1000	1000	500	1000	>1000
[A]	n.t.	n.t.	n.t.	n.t.	**125**	**125**	**250**
[B]	**62.5**	**62.5**	**62.5**	**62.5**	n.t.	n.t.	n.t.

B. subtilis: *Bacillus subtilis*, ***S. aureus***: *Staphylococcus aureus*, ***E. coli***: *Escherichia coli*, ***P. aeruginosa***: *Pseudomonas aeruginosa*, ***A. niger***: *Aspergillus niger*, ***A. flavus***: *Aspergillus flavus*, ***F. oxysporum***: *Fusarium oxysporum.*

[A]: Streptomycin sulphate, [B]: Ketoconazole. n.t.: not tested

Figure 1. DPPH Radical Scavenging Activity of the synthesized compounds **(4a-4p)**.

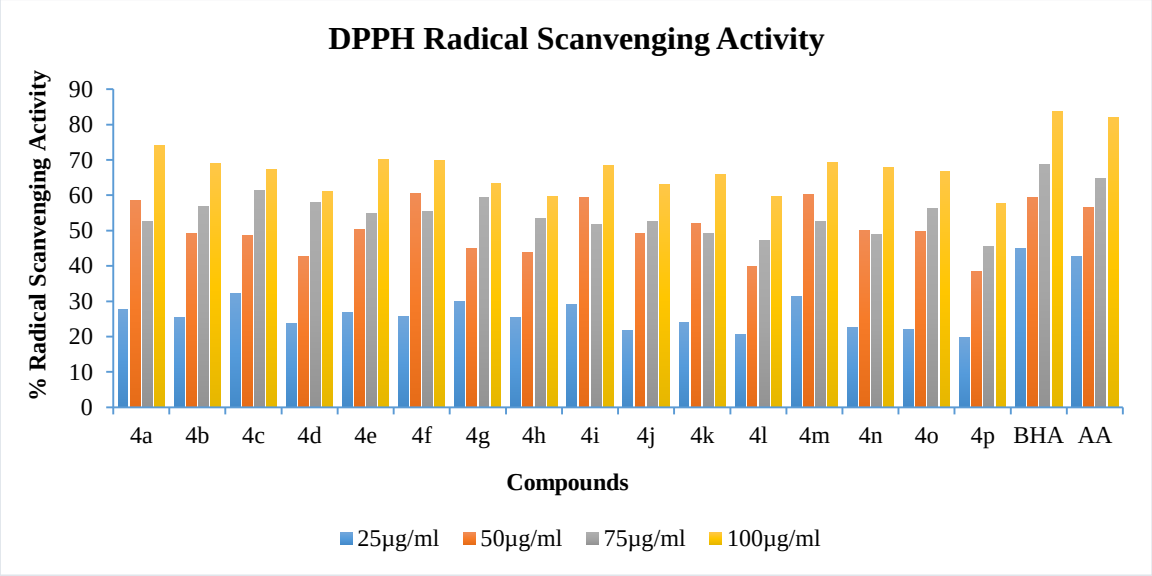

Figure 2. Ferric Reducing Antioxidant Power Activity of the synthesized compounds **(4a-4p)**.

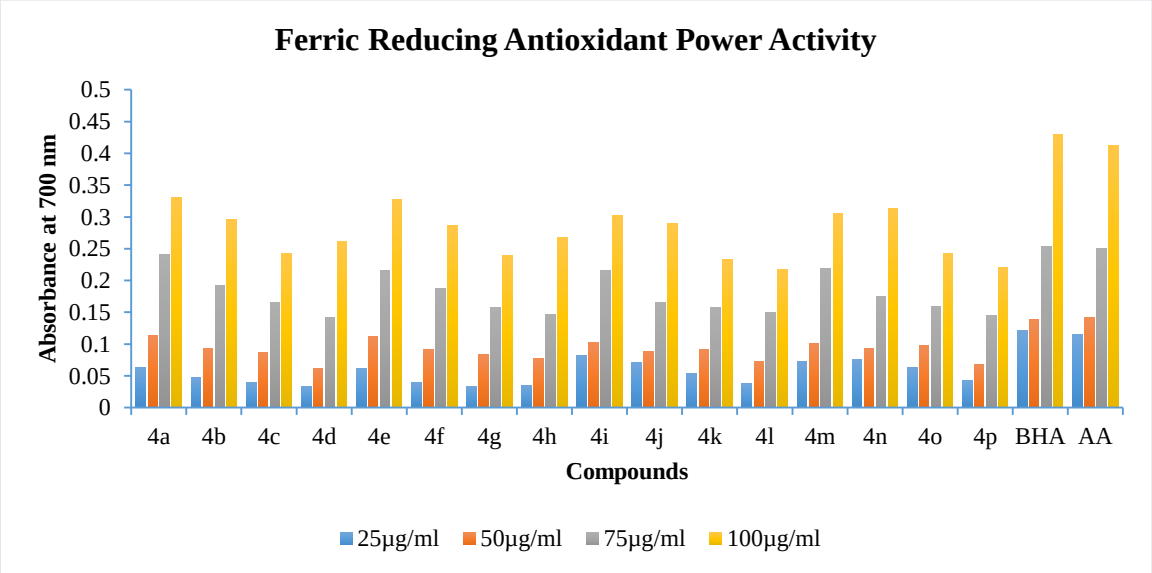

Table 2. Molecular Docking Interaction of synthesized novel compounds **(4a-4p)** against the GlcN-6-P synthase enzyme (2VF5) Active Site.

[a,b] Calculated by AutoDock. [c] Number of hydrogen bonds formed between the GlcN-6-P synthase active site and the novel leads.[d]

Compound	Binding Energy (Kcal/mol)[a]	Inhibition Constant (μM)[b]	No. of hydrogen bonds[c]	Bond distance[d]	Interacting residues of 2VF5[e]
4a	-8.00	1.36	1	1.86	Ala 602
4b	-8.64	0.46	1	1.96	Thr 352
4c	-8.47	0.623	1	1.95	Thr 352
4d	-7.71	2.23	1	2.06	Lys 603
4e	-8.89	0.306	1	2.05	Thr 352
4f	-8.81	0.346	1	2.00	Thr 352
4g	-8.75	0.387	1	2.08	Thr 352
4h	-7.79	1.93	1	2.07	Thr 352
4i	-8.56	0.530	1	1.97	Thr 352
4j	-8.56	0.527	1	1.96	Thr 352
4k	-7.74	2.13	1	1.87	Ala 602
4l	-7.74	2.12	1	2.01	Lys 603
4m	-8.36	0.745	1	1.94	Thr 352
4n	-8.54	0.547	1	1.96	Thr 352
4o	-8.12	1.12	1	2.14	Thr 352
4p	-8.87	0.315	1	1.74	Ala 602
Streptomycin Sulphate	-4.29	0.718	4	1.94 2.05 2.19 2.21	Ala 602 Cys 300 Thr 352 Val 399

Hydrogen bond distance [e] The interacting active site residues of GlcN-6-P synthase enzyme with novel leads in the ligand-receptor complex.

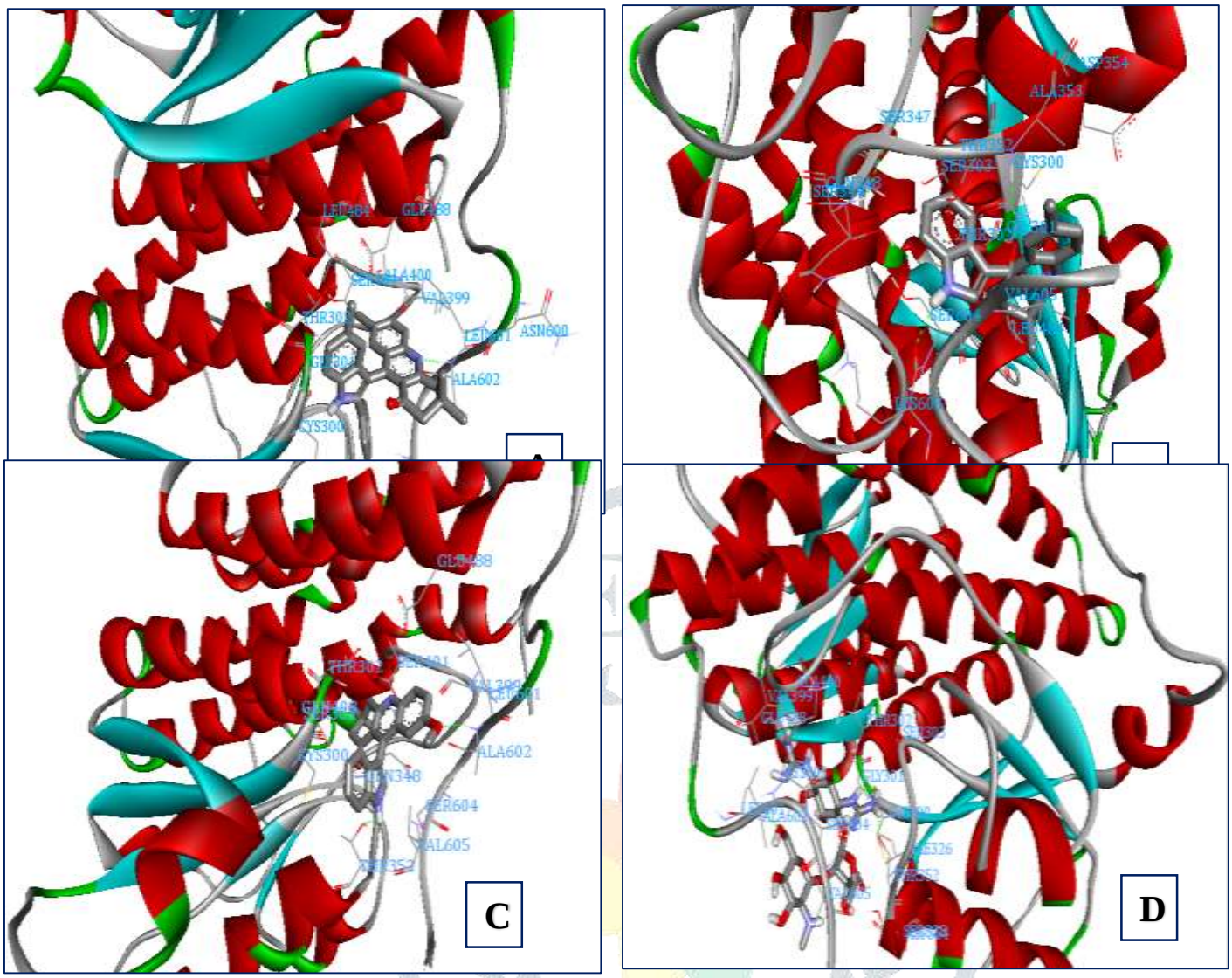

Figure 3. The stable and best conformers with least binding energy of the novel analogues 4a, 4e, 4p including the standard streptomycin. **(A)** 4a forming 1H-bond with Ala 602. **(B)** 4e forming 1H bond with Thr 352. **(C)** 4p forming 1H bond with Ala 602. **(D)** Streptomycin forming 4H bond with Ala 602, Cys 300, Thr 352 and Val399. The green dotted lines represent Hydrogen bonds.

CONCLUSION

In conclusion, we have developed a direct, one-pot three component and environmentally benign protocol for the synthesis of novel indole integrated acridinone scaffolds under mild reaction conditions, simple experimental procedure, with good yields and purity. The synthesized compounds were then evaluated for *in vitro* antimicrobial and antioxidant activities. The compounds **4a, 4e, 4f** and **4m** exhibited very good antimicrobial activity against the tested microbial strains whereas compounds **4a** and **4i** emerged as potent radical scavengers with excellent ferric ion reducing ability. Therefore, acridinone ring is responsible for the enhanced activity of indole system. The docking study revealed that all synthesized compounds exhibited strong affinity towards the enzyme GlcN-6-P synthase. Hence, these findings will attract chemists and pharmacologists for further studies in the indole field in search of strong antioxidant and anticancer agents.

ACKNOWLEDGEMENT

Authors are thankful to the Chairman, Department of Chemistry, Gulbarga University, Kalaburagi, for providing all the facilities. One of the authors Shaik Rahber is thankful to UGC, New Delhi for financial support through UGC-MANF (Moulana Azad National Fellowship, Ref. No. 2011-12-MANF-MUS-KAR-812). Authors are also thankful to the Director, IIT, Chennai and SAIF, Punjab University, Chandigarh for providing spectral data.

REFERENCES

1. Jomha M. Y., Yusef H. and Holail H. (2014) Antimicrobial and biocide resistance of bacteria in a Lebanese tertiary care hospital. *J. Glob. Antimicrob. Resist.* 2(4), 299-305.

2. Antibiotic / Antimicrobial Resistance. In *Healthcare-associated Infections (HAI).* May 7, 2015 ed.; US Centers for Disease Control and Prevention: Atlanta, GA, US state, http://www.cdc.gov/drugresistance/index.html.

3. Boucher H. W., Talbot G. H., Bradley J. S., Edwards J. E., Gilbert D., Rice L. B., Scheld M., Spellberg B. and Bartlett J. (2009) Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 48(1), 1-12.

4. Borowski E. (2000) Novel approaches in the rational design of antifungal agents of low toxicity. *Il Farmaco* 55(3), 206-208.

5. Chmara H., Andruszkiewicz R. and Borowski E. (1984) Inactivation of glucosamine-6-phosphate synthetase from Salmonella typhimurium LT 2 SL 1027 by Nβ-fumarylcarboxyamido-L-2,3-diaminopropionic acid. *Biochem. Biophys. Res. Commun.* 120(3), 865-872.

6. Milewski S. (2002) Glucosamine-6-phosphate synthase—the multi-facets enzyme. *Biochim. Biophys. Acta* 1597(2), 173-192.

7. Gurkok G., Altanlar N. and Suzen S. (2009) Investigation of Antimicrobial Activities of Indole-3-Aldehyde Hydrazide/Hydrazone Derivatives. *Chemotherapy* 55(1), 15-19.

8. Mor M., Silva C., Vacondio F., Plazzi P. V., Bertoni S., Spadoni G., Diamantini G., Bedini A., Tarzia G., Zusso M., Franceschini D. and Giusti P. (2004) Indole-based analogs of melatonin: in vitro antioxidant and cytoprotective activities. *J. Pineal Res.* 36(2), 95-102.

9. Zhang F., Zhao Y., Sun L., Ding L., Gu Y. and Gong P. (2011) Synthesis and anti-tumor activity of 2-amino-3-cyano-6-(1H-indol-3-yl)-4-phenylpyridine derivatives in vitro. *Eur. J. Med. Chem.* 46(7), 3149-3157.

10. Giampieri M., Balbi A., Mazzei M., La Colla P., Ibba C. and Loddo R. (2009) Antiviral activity of indole derivatives. *Antiviral Res.* 83(2), 179-185.

11. Buyukbingol E., Suzen S. and Klopman G. (1994) Studies on the synthesis and structure-activity relationships of 5-(3'-indolal)-2-thiohydantoin derivatives as aldose reductase enzyme inhibitors. *Il Farmaco* 49(6), 443-447.

12. Bhati S. K. and Kumar A. (2008) Synthesis of new substituted azetidinoyl and thiazolidinoyl-1,3,4-thiadiazino (6,5-b) indoles as promising anti-inflammatory agents. *Eur. J. Med. Chem.* 43(11), 2323-2330.

13. Suzen S. and Buyukbingol E. (1998) Evaluation of anti-HIV activity of 5- (2-phenyl-3′-indolal)-2-thiohydantoin. *Il Farmaco* 53(7), 525-527.

14. Zhang Q., Peng Y., Wang X. I., Keenan S. M., Arora S. and Welsh W. J. (2007) Highly Potent Triazole-Based Tubulin Polymerization Inhibitors. *J. Med. Chem.* 50(4), 749-754.

15. Wansi J. D., Wandji J., Mbaze Meva'a L., Kamdem Waffo A. F., Ranjit R., Khan S. N., Asma A., Iqbal C. M., Lallemand M.-C., Tillequin F. and Fomum Tanee Z. (2006) α-Glucosidase Inhibitory and Antioxidant Acridone Alkaloids from the Stem Bark of Oriciopsis glaberrima ENGL. (Rutaceae). *Chem. Pharm. Bull. (Tokyo)* 54(3), 292-296.

16. Fernández-Calienes A., Pellón R., Docampo M., Fascio M., D'Accorso N., Maes L., Mendiola J., Monzote L., Gille L. and Rojas L. (2011) Antimalarial activity of new acridinone derivatives. *Biomed. Pharmacother.* 65(3), 210-214.

17. Inman W. D., O'Neill-Johnson M. and Crews P. (1990) Novel marine sponge alkaloids. 1. Plakinidine A and B, anthelmintic active alkaloids from a Plakortis sponge. *J. Am. Chem. Soc.* 112(1), 1-4.

18. Martins E. T., Baruah H., Kramarczyk J., Saluta G., Day C. S., Kucera G. L. and Bierbach U. (2001) Design, Synthesis, and Biological Activity of a Novel Non-Cisplatin-type Platinum−Acridine Pharmacophore. *J. Med. Chem.* 44(25), 4492-4496.

19. Infante Lara L., Sledge A., Laradji A., Okoro C. O. and Osheroff N. (2017) Novel trifluoromethylated 9-amino-3,4-dihydroacridin-1(2H)-ones act as covalent poisons of human topoisomerase IIα. *Bioorg. Med. Chem. Lett.* 27(3), 586-589.

20. Rodríguez-Franco M. I., Fernández-Bachiller M. I., Pérez C., Hernández-Ledesma B. and Bartolomé B. (2006) Novel Tacrine−Melatonin Hybrids as Dual-Acting Drugs for Alzheimer Disease, with Improved Acetylcholinesterase Inhibitory and Antioxidant Properties. *J. Med. Chem.* 49(2), 459-462.

21. Biradar J. S. and Sharanbasappa B. (2009) MK-10 clay catalyzed, one pot, three component and efficient synthesis of novel 4-(2 ',5 '-disubstituted-1 ' H-indol-3 '-yl)-2,6-bis(2 ",5 "-disubstituted-1 " H-indol-3 "-yl) pyridine-3,5-dicarbonitrile under conventional and microwave irradiation. *Green Chem Lett Rev* 2(4), 237-241.

22. Biradar J. S., Sasidhar B. S. and Parveen R. (2010) Synthesis, antioxidant and DNA cleavage activities of novel indole derivatives. *Eur. J. Med. Chem.* 45(9), 4074-4078.

23. Biradar J., Rajesab P. and Sasidhar B. S. (2014) Design and synthesis of novel thiopheno-4-thiazolidinylindoles as potent antioxidant and antimicrobial agents. *Chem. Pap.* 68(3), 392-400.

24. Somappa S. B., Biradar J. S., Rajesab P., Rahber S. and Sundar M. (2015) A one-pot synthesis of indole-appended heterocycles as potent anti-inflammatory, analgesic, and CNS depressant agents. *Monatsh. Chem.* 146(12), 2067-2078.

25. Biradar J. S. (1982) Ph.D. Thesis, India (Gulbarga): Gulbarga University.

26. Reference method for broth dilution antifungal susceptibility testing of yeasts approved standard M27A. In *National Committee for Clinical Laboratory Standards, Approved Standard M27–A,* NCCLS: Wayne, PA, USA, 1997.

27. Singh R. P., Chidambara Murthy K. N. and Jayaprakasha G. K. (2002) Studies on the Antioxidant Activity of Pomegranate (Punica granatum) Peel and Seed Extracts Using in Vitro Models. *J. Agric. Food. Chem.* 50(1), 81-86.

28. Morris G. M., Goodsell D. S., Halliday R. S., Huey R., Hart W. E., Belew R. K. and Olson A. J. (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comput. Chem.* 19(14), 1639-1662.

29. Barreira J. C. M., Ferreira I. C. F. R., Oliveira M. B. P. P. and Pereira J. A. (2008) Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. *Food Chem. Toxicol.* 46(6), 2230-2235.

30. Teplyakov A., Obmolova G., Badet-Denisot M.-A., Badet B. and Polikarpov I. (1998) Involvement of the C terminus in intramolecular nitrogen channeling in glucosamine 6-phosphate synthase: evidence from a 1.6 å crystal structure of the isomerase domain. *Structure* 6(8), 1047-1055.