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Synthesis of Biologically Active Novel Indole Fused Heterocyclic Derivatives: Molecular Modeling Studies

ADAMSHAFI SHAIK[1,2], ALLAKA TEJESWARA RAO[3,*], S.V.M. MOHAN RAO[2] and PILLI V.V.N. KISHORE[2,*]

[1]Shilpa Medicare Ltd, API, R & D Unit, Modavalasa, Vizianagaram-531162, India
[2]Chemistry Division, Department of Sciences and Humanities, VFSTR (Deemed to be University), Vadlamudi, Guntur-522213, India
[3]Centre for Chemical Sciences and Technology, Institute of Science & Technology, Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad-500085, India

*Corresponding authors: E-mail: tejajntuh18@gmail.com; kishore1pvvn@gmail.com

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A novel series of fluoro/methoxy indole analogues **6** was synthesized and the final targets were confirmed by IR, ^{1}H & ^{13}C NMR and mass spectral analysis. Novel 3-substituted indole derivatives estimate for their antibacterial, antioxidant activities particularly the parent core combined with benzamides ring significantly. From antibacterial activities, compounds **6c**, **6e** and **6b** show the highest bacterial activity against *S. epidermidis, S. aureus, E. coli*, with zone of inhibition 34, 30, 28 mm, respectively. Novel fluoroindole derivatives **6c**, **6b**, **6i** shows an excellent antioxidant activity with % of inhibition 150.12, 139.04, 137.08 mmol/mL, respectively. The calculations for ligand-protein flexible of crystal structure of C(30) carotenoid dehydrosqualene synthase from *S. aureus* complexed with bisphosphonate BPH-700 (2ZCS). Among the designed compounds **6c** exhibited highest hydrogen bonding interactions 2.06 Å, 1.85 Å with amino acids Asp27, Lys273 and binding energy -6.38 kcal/mol, respectively. Fluoroindoles **6i**, **6e and 6f** shows highest ΔG = -7.90, -7.66, -7.47 kcal/mol with dissociation constants 10.32, 21.77, 22.68 μM and amino acid Lys273 interactions.

Keywords: Antibacterial activity, Antioxidant activity, Indole, Acetamide, Molecular docking.

INTRODUCTION

Indole is an important heterocyclic compound because it is built into proteins in the form of amino acid tryptophan on the basis of indomethacin and it provides indole alkaloids biological active compounds from plants including strychnine and lysergic acid diethylamide. The indole core is found in various biologically and pharmacologically active compounds [1]. Indole alkaloids are identified as one of the quickly growing groups of marine invertebrate metabolites for their broad spectrum of biological properties [2]. In particularly, 3-substituted indole analogues, which is important building blocks used for the synthesis of biologically dominant compounds. Some of the 3-substituted indole derivatives have showed significant cytotoxicity against several human cancer cell lines [3].

Various 3-substituted indoles have been used as starting materials for the synthesis of a number of agrochemicals, alkaloids, perfumes and pharmaceuticals. Also indoles have various types of broad spectrum of biological activities such as antitumor, antimicrobial, anti-inflammatory, hypoglycemic, antipyretic and analgesic activities [4]. In view of the predominant biological properties of the indoles, we planned to synthesize a novel series of 3-substituted indole analogues bearing side chains with different structures and possess interesting useful in antibacterials. Indole derivatives represent a main class of therapeutic agents in medicinal chemistry including analgesic [5], anti-inflammatory [6], anti-depressant [7], antimicrobial [8], antifungal [9], antitumor [10], antiviral [11], antihypertensive [12], anti-HIV [13], anti-proliferative [14], anti-psychotic [15], anti-leukamic [16], anticancer [17] activities, *etc.* While indole moiety is very small but it is mesmerize by scientists because of the diverse biological properties by not only indole but its varied substituted derivatives as well.

Consequently, 3-substituted indoles immobile represent a significant synthetic challenges of novel antibacterial agents are urgently needed. Here we report our studies on the efficient

synthesis, SAR studies and activity of novel class of indoles linked to acetamide analogs which show promising antibacterial as well as antioxidant properties. From molecular docking, it was found that target compounds have potential to inhibit the dehydrosqualene synthase from *S. aureus* complexed with bisphosphonate BPH-700 using autodock 4.2. The final compounds exhibited good affinity for this enzyme when compared with the kinetic energies of standard drug pefloxacin. The contrast of corresponding free energies bind to title compounds with target protein reveals from interact receptor. Almost all the molecules exhibited under free energy values, indicating thermodynamically more favoured interaction. Recently, many studies related to different heterocycles conjugated with fluoroindoles are known to have very good biological activities. In this study, we are intended to explore the antibacterial, antioxidant activity studies of benzamides conjugated fluoroindoles against Gram-positive, Gram-negative microorganisms, DPPH radicals along with molecular docking study calculations. Thus, suggesting these compounds in the present series can serve as an important gateway for the design and development of novel antibacterial, antioxidant targets make them valid leads for further optimization.

EXPERIMENTAL

The chemicals *viz*. 5-fluoro/methoxy indoles and triisopropylsilyl (TIPS) chloride, were commercially available and used without any further purification. ^{1}H NMR spectra were recorded on Bruker Avance 300-400 MHz spectrometer and ^{13}C NMR spectra were recorded on Bruker Avance 75-100 MHz spectrometer in DMSO-d_6 and $CDCl_3$ as a solvents. Flash column chromatography was performed on Merck silica gel 100-200 mesh and compounds were visualized with UV light. IR spectra were recorded on Perkin-Elmer spectrophotometer by using KBr pellets and melting points were determined in open capillary tubes. All the reactions were monitored by thin layer chromatographic (TLC) technique using silica gel plates.

Synthesis of 5-fluoro-1-(triisopropylsilyl)-1*H*-indole (2): 5-Fluoro-1*H*-indole (**1**) (3.0 g, 28 mmol) in dry THF (25 mL), LiHMDs (10 mL), triisopropyl silyl chloride (3.5 g, 28 mmol) and stirred at -30 ºC for 1 h. Reaction progress was monitored by TLC and then reaction mixture was quenched with saturated $NaHCO_3$ solution at 0 ºC. The reaction mixture was extracted with ethyl acetate (3 × 25 mL), solvent was dried over anhydrous Na_2SO_4 and evaporated the organics to yield crude product. Crude material was purified by column chromatography to get pure product **2** in 90% yield.

Synthesis of 2-(4-bromophenyl)-2-oxoacetyl chloride (3): Take a 100 mL round bottom flask sealed with a rubber septum, bromobenzene (8 mmol) was dissolved in dry diethyl ether (15 mL) with the help of syringe through septum at 0 ºC for 5-10 min. Addition of oxalyl chloride (8 mmol) was added with the help of a syringe and resulted in the formation of yellow coloured intermediate 2-(4-bromophenyl)-2-oxoacetyl chloride (84%) was formed and filtered immediately.

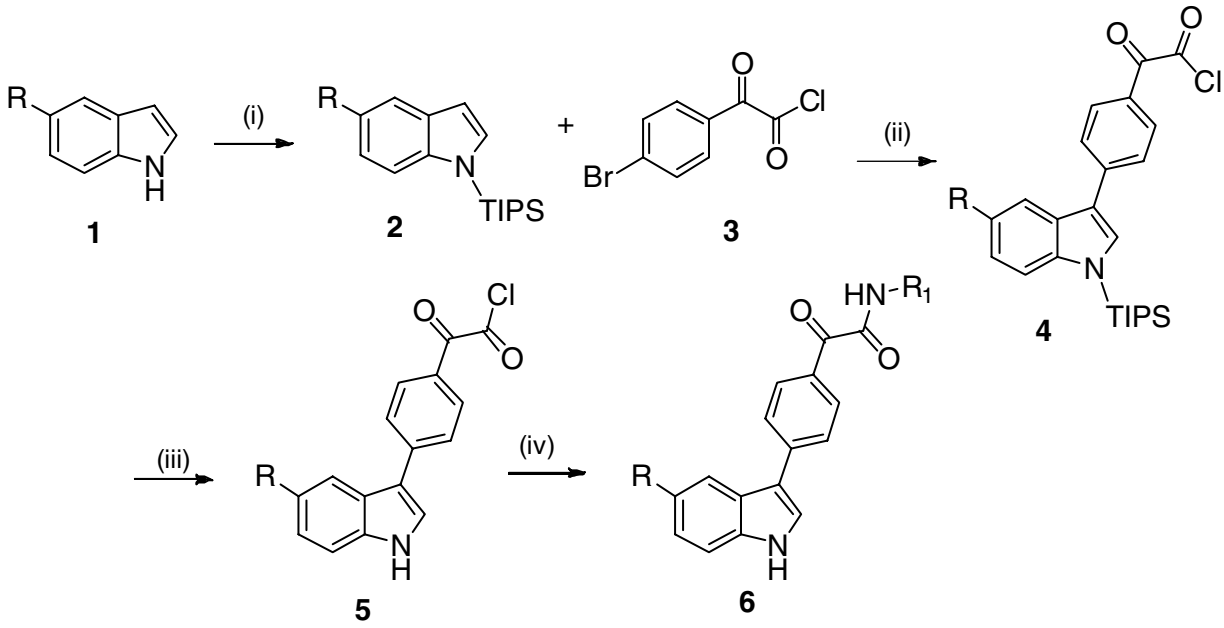
Synthesis of 2-(4-(5-fluoro-1-(triisopropylsilyl)-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (4): A mixture of 5-fluoro-1-(triisopropylsilyl)-1*H*-indole (**2**) (4.0 g, 19 mmol), in DME with methyl 4-bromobenzoate (**3**) (2 g, 23 mmol), Cs_2CO_3 (5 g, 28 mmol) at ambient temperature. Reaction mixture was de gassed by purging with argon gas for 10 min and added $PdCl_2$·DCM (0.8 g, 0.9 mmol). Reaction mixture was heated for 5 h, reaction mixture was cooled to room temperature and finally filtered through celite pad and evaporated under reduced pressure. The obtained residue was purified by column chromatography using petroleum ether: ethyl acetate (9:1) as eluent, to 2-(4-(5-fluoro-1-(triisopropylsilyl)-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (**4**) (75%) as a white solid.

Synthesis of 2-(4-(5-fluoro-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (5): To a solution of **4** (1 g, 8 mmol) in THF (12 mL) at 0 ºC was charged with TBAF (2 mL) and the reaction mixture was stirred at room temperature for 2 h. Reaction mixture was quenched with water and diluted with ethyl acetate (2 × 25 mL). The solvent was dried over anhydrous Na_2SO_4 and evaporated the combined organic layer to get 2-(4-(5-fluoro-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (**5**) (76%) as a white solid.

Synthesis of 6a-j: To a solution of 2-(4-(5-fluoro-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (**5**) (0.5 g, 0.78 mmol), HATU (0.3 g, 0.9 mmol), in the presence of DIPEA (0.3 g, 2 mmol) and the corresponding amines (0.9 mmol) in DMF (5 mL) stirred at room temperature for 2-5 h. Reaction progress was monitored by TLC and quenched with ice water and collected the obtained solid by filtration to yield compound **6** (**Scheme-I**).

2-(4-(5-Fluoro-1*H*-indol-3-yl)phenyl)-2-oxo-*N*-(2-(pyrrolidin-1-yl)ethyl)acetamide (6a): White colour solid, m.p.: 171-173 ºC, yield 80%, IR (KBr, ν_{max}, cm^{-1}): 3371.39, 3343.38 (NH *str.*), 3056.55 (=CH *str.*), 2966.78 (-CH *str.*), 1679.29 (CO *str.*), 1632.27 (CO *str.*), 1581.50, 1516.80 (C=C *str.*), 1403.24 (CN *str.*), 836.50 (C–F *str.*). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 11.56 (s, 1H, NH), 8.45-8.43 (t, 1H, *J* = 8 Hz, NH), 7.93-7.88 (m, 3H, ArH), 7.83 (m, 1H, ArH), 7.79-7.76 (m, 2H, ArH), 7.26-7.23 (m, 1H, ArH), 7.01-6.96 (m, 1H, ArH), 3.45-3.40 (2H, *J* = 9.5 Hz, CH_2), 2.67-2.64 (m, 2H, CH_2), 2.57 (m, 4H, CH_2), 1.70 (m, 4H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 181.22, 162.03, 157.66, 149.54, 139.40, 136.91, 136.18, 128.35, 127.67, 126.26, 123.24, 122.57, 121.72, 118.24, 112.86, 111.63, 52.07, 42.81, 34.82, 30.33, 23.60. ESI (*m/z*) calcd. for $C_{21}H_{22}FN_3O$ (M+1)$^+$: 352.17, found: 352.42.

2-(4-(5-Fluoro-1*H*-indol-3-yl)phenyl)-2-oxo-*N*-(4-(trifluoromethyl)benzyl)acetamide (6b): Light yellow solid, m.p.: 161-163 ºC, yield 68%, IR (KBr, ν_{max}, cm^{-1}): 3383.60, 3336.93 (NH *str.*), 3072.74 (=CH *str.*), 2932.22 (CH *str.*), 1703.42 (CO *str.*), 1636.68 (C=O *str.*), 1575.60, 1492.76 (C=C *str.*), 1412.12 (CN *str.*), 1334.73 (CN *str.*), 858.25 (CF *str.*), 782.10 (CF *str.*). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.57 (s, 1H, NH), 9.15-9.12 (t, 1H, *J* = 9 Hz, NH), 7.98-7.89 (m, 3H, ArH), 7.85-7.79 (m, 3H, ArH), 7.69-7.56 (m, 4H, ArH), 7.26-7.01 (m, 1H, ArH), 6.99-6.95 (m, 1H, ArH), 4.60-4.58 (2H, *J* = 6 Hz, $NHCH_2$), 3.10 (2H, *J* = 4 Hz, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 179.81, 152.66, 148.53, 138.27, 137.08, 135.11, 134.49, 130.90, 130.07, 128.82, 127.25, 124.44, 122.50, 120.56, 119.74, 115.42, 113.66, 110.24, 54.58. ESI (*m/z*) calcd. for $C_{23}H_{16}F_4N_2O_2$ (M+1)$^+$: 428.38, found: 428.11.



Scheme-I: Synthesis of 5-fluoro/methoxy indole linked to acetamide compounds: (i) LiHMDs, THF, -30 °C for 1 h, 90%; (ii) Cs_2CO_3, $PdCl_2$·DCM, 1,4-dioxane, reflux, 5 h, 75%; (iii) TBAF, THF, room temperature, 2 h, 76%; (iv) Corresponding amines, EDCI, DIPEA, CH_2Cl_2, DMF, 2-5 h, 62-82%

2-(4-(5-Fluoro-1*H*-indol-3-yl)phenyl)-*N*-(2-(furan-2-yl)ethyl)-2-oxoacetamide (6c): White colour solid, m.p.: 174-176 ºC, yield 66%, IR (KBr, ν_{max}, cm^{-1}): 3336.15 (NH *str.*), 3068.49 (=CH *str.*), 2964.14 (-CH *str.*), 1712.46 (CO *str.*), 1616.20 (CO *str.*), 1573.32, 1504.46 (C=C *str.*), 1425.89 (CN *str.*), 1331.75 (CN *str.*), 1168.46 (CSC *str.*), 880.41 (CF *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.54 (s, 1H, NH), 8.62-8.59 (1H, *J* = 12 Hz, NH), 7.94-7.89 (m, 3H, ArH), 7.83-7.82 (1H, *J* = 2.4 Hz, ArH), 7.78-7.76 (1H, *J* = 8 Hz, ArH), 7.35-7.33 m (1H, ArH), 7.25-7.22 (m, 1H, ArH), 6.98-6.65 (m, 2H, ArH), 6.93-6.92 (m, 1H, ArH), 3.53-3.51 (2H, *J* = 8 Hz, $NHCH_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 179.83, 156.02, 138.52, 135.60, 134.14, 131.42, 128.57, 127.34, 125.68, 122.96, 121.73, 119.32, 115.44, 112.05, 109.18, 54.72. ESI (*m/z*) calcd. for $C_{20}H_{15}FN_2OS$ $(M+1)^+$: 351.41, found: 351.09.

***N*-Benzyl-2-(4-(5-fluoro-1*H*-indol-3-yl)phenyl)-2-oxoacetamide (6d):** Colourless liquid, m.p.: 167-169 ºC, yield 72%, IR (KBr, ν_{max}, cm^{-1}): 3351.57, 3313.78 (NH *str.*), 3065.28 (=CH *str.*), 2951.26 (-CH *str.*), 1694.38 (CO *str.*), 1625.88 (CO *str.*), 1589.66, 1502.01 (C=C *str.*), 1498.82, 1409.52 (CN *str.*), 788.63 (CF *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.56 (s, 1H, NH), 9.08 (1H, *J* = 5.8 Hz, NH), 7.98-7.89 (m, 4H, ArH), 7.84-7.78 (m, 2H, ArH), 7.34-7.33 (2H, *J* = 4.0 Hz, ArH), 7.25-7.23 (m, 4H, ArH), 6.98-6.96 (m, 1H, ArH), 4.51-4.50 (2H, *J* = 5.6 Hz, $NHCH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 180.80, 160.23, 139.12, 136.45, 133.74, 131.67, 129.65, 127.94, 126.83, 126.04, 124.25, 123.80, 123.13, 122.28, 121.36, 118.83, 113.74, 111.71, 58.37. ESI (*m/z*) calcd. for $C_{22}H_{17}FN_2O$ $(M+1)^+$: 345.13, found: 344.38.

***N*-(3-Chlorobenzyl)-2-(4-(5-fluoro-1*H*-indol-3-yl)-phenyl)-2-oxoacetamide (6e):** White crystalline solid, m.p.: 180-181 ºC, yield 82%, IR (KBr, ν_{max}, cm^{-1}): 3443.10, 3332.40 (NH *str.*), 3051.23 (=CH *str.*), 2926.46 (-CH *str.*), 1731.46 (CO *str.*), 1622.34 (CO *str.*), 1582.79, 1495.48 (C=C *str.*), 1413.53 (CN *str.*), 917.38 (CF *str.*), 834.49 (CCl *str.*). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.56 (s, 1H, NH), 9.08-9.05 (t, 1H, *J* = 9 Hz, NH), 7.98-7.96 (m, 2H, ArH), 7.92-7.78 (m, 4H, ArH), 7.41-7.35 (m, 2H, ArH), 7.26-7.19 (m, 2H, ArH), 7.17-7.09 (m, 1H, ArH), 7.05-6.96 (m, 1H, ArH), 4.52 (2H, *J* = 4.5 Hz, $NHCH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 179.52, 163.23, 148.40, 141.12, 139.60, 136.73, 135.34, 132.27, 130.04, 128.36, 126.85, 126.14, 124.27, 123.34, 121.84, 119.81, 113.87, 112.23, 57.20. ESI (*m/z*) calcd. for $C_{22}H_{16}ClFN_2O$ $(M+2)^+$: 380.09, found: 378.83.

2-(4-(5-Methoxy-1*H*-indol-3-yl)phenyl)-2-oxo-*N*-(4-(trifluoromethyl)benzyl)acetamide (6f): Colourless liquid, m.p.: 170-172 ºC, yield 67%, IR (KBr, ν_{max}, cm^{-1}): 3402.26, 3341.14 (NH *str.*), 3048.49 (=CH *str.*), 2920.49 (-CH *str.*), 1750.46 (CO *str.*), 1591.47, 1490.23 (C=C *str.*), 1410.76 (CN *str.*), 910.02 (CF *str.*), 830.48 (CF *str.*). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.58 (s, 1H, NH), 9.16-9.13 (1H, *J* = 9 Hz, NH), 8.00-7.85 (m, 6H, ArH), 7.82-7.79 (m, 2H, ArH), 7.72-7.70 (m, 1H, ArH), 7.57-7.54 (m, 1H, ArH), 7.26-7.02 (m, 1H, ArH), 6.99-6.96 (m, 1H, ArH), 4.60-4.58 (2H, *J* = 6 Hz, $NHCH_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 170.53, 163.27, 148.06, 138.73, 136.31, 132.76, 130.54, 129.21, 127.13, 126.50, 125.14, 124.65, 122.21, 120.57, 117.81, 57.26. ESI (*m/z*) calcd. for $C_{23}H_{16}F_4N_2O$ $(M+1)^+$: 413.12, found: 413.38.

2-(4-(5-Methoxy-1*H*-indol-3-yl)phenyl)-2-oxo-*N*-(piperidin-4-ylmethyl)acetamide (6g): Brown colour solid, m.p.: 163-165 ºC, yield 71%, IR (KBr, ν_{max}, cm^{-1}): 3384.16, 3331.40 (NH *str.*), 3040.25 (=CH *str.*), 2953.30 (-CH *str.*), 1681.12 (CO *str.*), 1621.40 (CO *str.*), 1583.25, 1510.31 (C=C *str.*), 1406.18 (CN *str.*), 872.42 (CF *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.55 (s, 1H, NH), 8.37 (1H, *J* = 10.5 Hz, NH), 7.97-7.88 (m, 3H, ArH), 7.82-73 (m, 3H, ArH), 7.49-7.48 (m,

1H, ArH), 7.34 (m, 1H, ArH), 7.25-7.20 (m, 1H, ArH), 7.00-6.94 (m, 1H, ArH), 4.75 (s, 1H, NH), 3.42-3.16 (m, 4H, CH_2), 1.98-1.77 (m, 2H, CH_2), 1.33 (m, 1H, CH), 1.23-1.16 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 170.12, 154.54, 138.36, 137.50, 135.57, 131.26, 129.28, 126.30, 125.11, 121.04, 120.52, 119.27, 114.04, 58.56, 48.28, 42.16, 29.94. ESI (*m/z*) calcd. for $C_{21}H_{22}FN_3O$ $(M+1)^+$: 351.17, found: 351.42.

***N*-Isobutyl-2-(4-(5-methoxy-1*H*-indol-3-yl)phenyl)-2-oxoacetamide (6h):** Brown colour solid, m.p.: 160-161 ºC, yield 69%, IR (KBr, ν_{max}, cm^{-1}): 3403.20 (NH *str.*), 3066.78 (=CH *str.*), 2912.52, 2873.20 (CH *str.*), 1721.14 (CO *str.*), 1593.46, 1546.74 (C=C *str.*), 1389.49 (CN *str.*), 889.40 (CF *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 11.55 (s, 1H, NH), 8.44 (t, 1H, J = 4 Hz, NH), 7.92-7.91 (s, 1H, ArH), 7.90-7.82 (m, 1H, ArH), 7.77-7.75 (d, 2H, ArH), 7.26-7.22 (m, 1H, ArH), 7.01-6.95 (m, 1H, ArH), 3.12-3.09 (t, 2H, J = 12 Hz, CH_2CH), 1.90-1.83 (1H, J = 8.0 Hz, $CH(CH_3)_2$), 0.91-0.89 (m, 6H, $CH(CH_3)_2$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 178.23, 160.17, 137.56, 135.60, 132.34, 131.52, 128.63, 125.31, 123.30, 122.04, 119.53, 115.08, 55.61, 48.23, 29.41. ESI (*m/z*) calcd. for $C_{19}H_{19}FN_2O$ $(M+1)^+$: 311.15, found: 311.37.

2-(4-(5-Methoxy-1*H*-indol-3-yl)phenyl)-*N*-(4-nitrobenzyl)-2-oxoacetamide (6i): Yellow colour solid, yield 66%, m.p.: 168-170 ºC. IR (KBr, ν_{max}, cm^{-1}): 3436.20 (NH *str.*), 3086.34 (=CH *str.*), 2920.45, 2879.75 (-CH *str.*), 1698.46 (CO *str.*), 1621.12 (CO *str.*), 1581.63, 1474.10 (C=C *str.*), 1402.30 (CN *str.*), 1330.48 (CN *str.*), 850.25 (CF *str.*). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 11.56 (s, 1H, NH), 9.04 (t, 1H, J = 5.3 Hz, NH), 7.97-7.90 (m, 3H, ArH), 7.88-7.77 (m, 3H, ArH), 7.39-7.35 (m, 2H, ArH), 7.25-7.01 (m, 3H, ArH), 6.98-6.95 (1H, J = 9 Hz, ArH), 4.49-4.47 (2H, J = 6 Hz, $NHCH_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 171.28, 148.55, 136.46, 135.21, 134.62, 130.53, 129.10, 127.07, 125.85, 122.97, 121.64, 120.15, 116.36, 115.31, 110.63, 60.12. ESI (*m/z*) calcd. for $C_{22}H_{16}FN_3O_3$ $(M+1)^+$: 389.12, found: 389.38

2-(4-(5-Methoxy-1*H*-indol-3-yl)phenyl)-*N*-(3-methylbenzyl)-2-oxoacetamide (6j): White solid, m.p.: 160-162 ºC, yield 76%, IR (KBr, ν_{max}, cm^{-1}): 3413.12, 3326.40 (NH *str.*), 3066.94 (=CH *str.*), 2922.48 (CH *str.*), 1725.45 (CO *str.*), 1634.79 (CO *str.*), 1581.49, 1502.45 (C=C *str.*), 1423.76s (CN *str.*), 1308.72 (CN *str.*), 889.45s (CF *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 1.40-1.39 m (2H, CH_2), 1.52-1.50 m (4H, CH_2), 2.44 m (4H, CH_2), 3.42-3.32 m (4H, CH_2), 7.00–6.95 m (1H, ArH), 7.25-7.22 m (1H, ArH), 7.78-7.76 m (2H, ArH), 7.83-7.82 m (1H, ArH), 8.37 t (1H, J = 6 Hz, NH), 11.55 s (1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.06, 155.12, 137.87, 136.14, 133.48, 130.85, 128.42, 126.27, 124.55, 122.06, 121.81, 118.10, 116.87, 111.65, 53.51. ESI (*m/z*) calcd. for $C_{23}H_{19}FN_2O$ $(M+1)^+$: 358.41, found: 358.67.

Biological activity profiles

Antibacterial activity: In the present study, six organisms were chosen including three Gram-positive and three Gram-negative organisms. *Klebsiella aerogenes, Escherichia coli* and *Klebsiella pneumonia* were obtained from Microbial Type Culture Collection, IMTECH and Chandigarh, India. Clinical isolates such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Staphylococcus epidermidis* were obtained from Microbiology laboratory of Global Hospital, Hyderabad, India. All the strains were tested for purity by standard microbiological methods and employed for evaluation of antibacterial activity by Agar-well diffusion method [18]. The bacterial stock cultures were maintained by Mueller-Hinton Agar (MHA) slants and stored at 4 ºC.

Antibacterial activity: These bacterial strains were reactivated from stock cultures by transferring into Mueller Hinton Broth and incubated at 37 ºC for 18 h. A final inoculum contains 10^6 colony forming units (1×10^6 CFU/mL) was added aseptically to MHA and poured into sterile Petri dishes. Different test extracts at a concentration of 0.4 mg/50 µL were added to wells (8 mm in diameter) punched on agar surface. Plates were incubated overnight at 37 ºC and diameter of inhibition zone around each well was measured. Experiments were performed in triplicates. Antibiotics such as ciprofloxacin at a concentration of 0.4 mg/50 µL were used as positive reference to determine sensitivity of microorganisms tested. DMSO was used as negative control.

Antioxidant activity

2,2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay: All the test samples were estimated according to the reported method [19]. The samples (10, 25, 50, 100 and 200 µg/mL for sample; 0-5 µg/mL for BHT) in 100 µL aliquot mixed with 100 mM tris-HCl buffer (800 µL, pH 7.4) and added 1 mL of 200 µM DPPH in methanol (final concentration of 150 µM). The mixture was vigorously shaken and incubated in the dark at room temperature for 20 min. A DPPH blank solution (control) was prepared above without the sample and methanol was used for the baseline correction. The absorbance of the test solutions were measured spectrophotometrically at 517 nm and results were expressed as mean of three determinations. The DPPH radical scavenging activities were calculated using the equation:

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_c - A_s}{A_c} \times 100$$

where, A_s is the absorbance of the test samples and A_c is the absorbance of the control. The inhibition concentration of the samples for 50% (IC_{50}) DPPH radical scavenging was also calculated.

Docking studies: Molecular docking was performed to explain the binding mode of proteins and synthesized compounds and all the molecules were docked individually by Autodock 4.2 [20,21]. The modelled 3D structure of nitrate reductase protein was imported to Autodock 4.2 and structurally optimized by adding hydrogens to protein allocated with Kollaman charges. After adding the hydrogens the model was saved in PDBQT format, later ligands were prepared by optimizing the torsion angles and saved them in PDBQT format. Potential binding site of proteins were selected by using PDBSUM [22] and a grid was generated around to identify XYZ-coordinates, around the binding site of proteins individually. Lamarckian genetic algorithm (LGA) was selected for docking, freezing and default parameters used by Autodock 4.2 and it is open

source of software for drug discovery, molecular docking. Docking has been use commonly performed for predicting binding modes to proteins and their binding energies of ligands. X,Y,Z-coordinates of PDB were selected by using SPDBV.

RESULTS AND DISCUSSION

Our initial synthetic efforts focused on the synthesis of target compounds containing an indole with *n*-alkyl acetamide linkers present in Fig. 1. In the structural design of target indole compounds **6a-j** was retained with acetamide characteristics. With 5-fluoro/methoxy-1*H*-indole as starting material, compounds **6a-j** were synthesized after three steps of triisopropylsilyl, acetylation and amide conjugation.

These compounds were synthesized from the following intermediates; 5-fluoro/methoxy-1*H*-indole (**1**) in dry THF, to a solution of LiHMDS at -25 ºC to gave 5-fluoro/methoxy-1-(triisopropylsilyl)-1*H*-indole (**2**) in good yields. A mixture of triisopropylsilyl-1*H*-indole in DME was charged with 2-(4-bromophenyl)-2-oxoacetyl chloride (**3**) and Cs_2CO_3 at room temperature to permit the compound 2-(4-(5-fluoro-1-(triisopropylsilyl)-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (**4**). Further the reaction intermediate 2-(4-(5-fluoro-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (**5**) was administer by using **4** in THF at 0 ºC was charged with TBAF and stirred at ambient temperature for 2.5 h to give in good yield. Finally, 5-fluoro/methoxy indole linked to *N*-alkyl acetamide derivatives **6a-j** from 2-(4-(5-fluoro-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (**5**), DMF in the presence of EDCI, DIPEA and the corresponding amines was added at room temperature as shown in **Scheme-I**.

The structures of the synthesized compounds were characterized by IR, ^{1}H & ^{13}C NMR and mass spectrometry. From IR spectrum, the stretching frequencies of the amine, acetyl carbonyl moiety of compound **6a** appeared at 3371, 1679, 1632 cm^{-1}, respectively. Arrival a triplet, quartet at δ 8.45, 3.45 ppm for amine, CH_2 groups, respectively. Singlet at δ 11.56 ppm was due to presence of one NH group, respectively. Three -CH_3 were substantiated from multiple signals range at δ 2.67-1.70 ppm. In ^{13}C NMR spectrum of **3a**, 22 aromatic carbons, 7 aliphatic carbons and 1 carbonyl group for ester appeared. The acetamide carbonyl peak appeared at δ 181.22, 162.03 ppm; the signals for four methylene carbon atoms appeared at δ 52.07, 42.81, 34.82 and 30.33 ppm, respectively. Aromatic carbons, indole ring signals range were appeared at δ 157-111 ppm. Mass spectrum of **6a** showed protonated molecular ion peak at *m/z* 352.1. After conrming the structure **6a**, we have decided to apply this sequential reaction strategy to synthesize a library of molecules by varying indole with acetamide substituents.

Antibacterial activity: The new series of indoles were tested, ascertain to their *in vitro* antibacterial sensitivity by agar well diffusion method and their results are summarized in Table-1. From the assay, it was visible that all the designed compounds exhibit potent antibacterial activity probably due

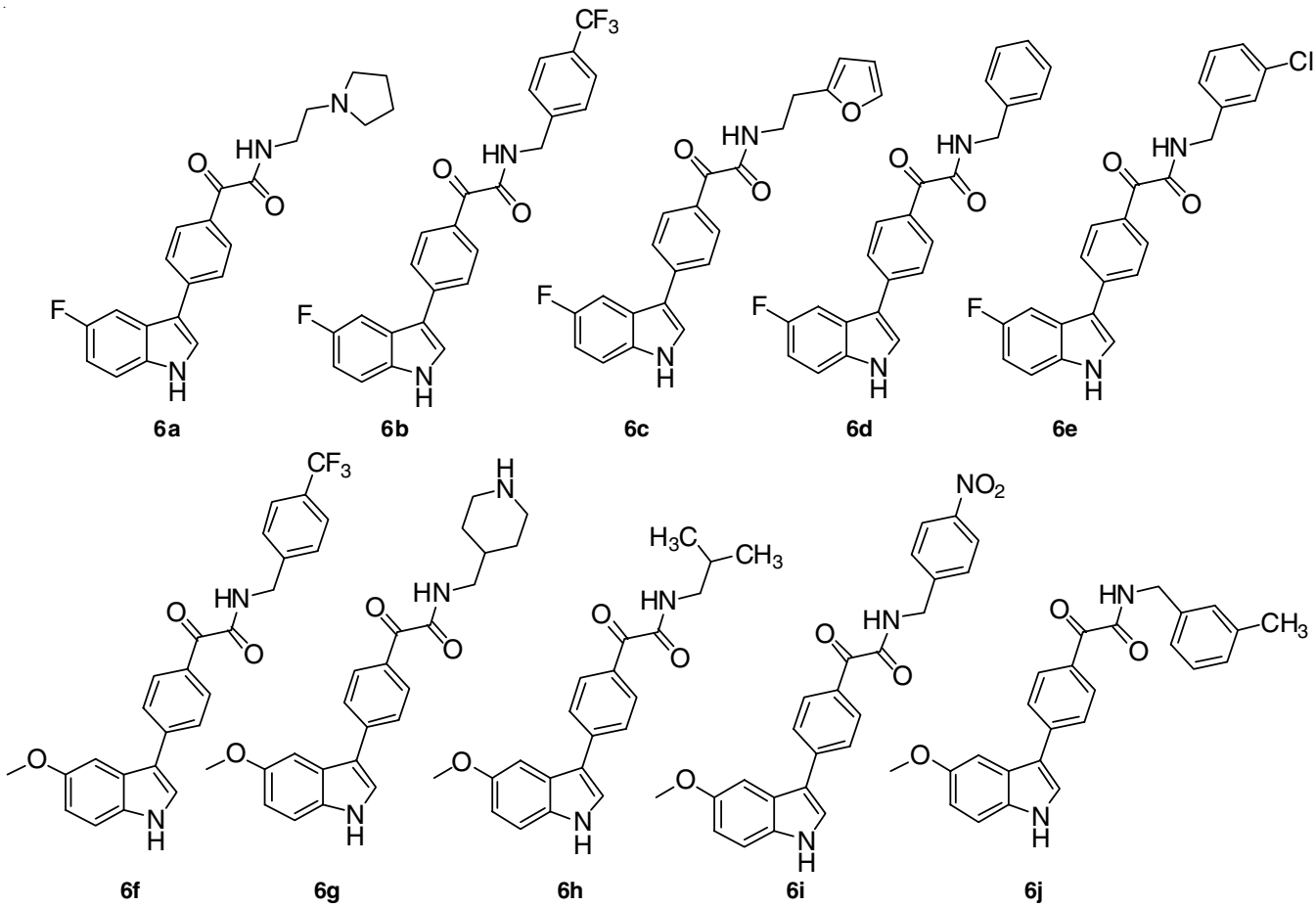


Fig. 1. List of synthesized compounds **6a-j**

TABLE-1
ANTIBACTERIAL SENSITIVITY OF FLUOROINDOLE DERIVATIVES

Entry	Zone of inhibition (mm)					
	S. aureus	*S. pneumoniae*	*S. epidermidis*	*K. aerogenes*	*E. coli*	*K. pneumonia*
6a	18 ± 0.2	15 ± 0.1	17 ± 0.2	20 ± 0.1	19 ± 0.2	20 ± 0.3
6b	16 ± 0.3	27 ± 0.1	25 ± 0.1	12 ± 0.3	22 ± 0.1	No inhibition
6c	15 ± 0.1	18 ± 0.1	16 ± 0.3	19 ± 0.2	16 ± 0.1	18 ± 0.3
6d	28 ± 0.2	22 ± 0.2	30 ± 0.2	No inhibition	22 ± 0.1	25 ± 0.1
6e	30 ± 0.2	16 ± 0.3	No inhibition	17 ± 0.1	15 ± 0.1	18 ± 0.1
6f	17 ± 0.2	18 ± 0.3	13 ± 0.1	14 ± 0.2	16 ± 0.1	17 ± 0.3
6g	19 ± 0.3	No inhibition	28 ± 0.3	14 ± 0.1	28 ± 0.3	28 ± 0.1
6h	No inhibition	18 ± 0.1	34 ± 0.2	21 ± 0.1	18 ± 0.3	No inhibition
6i	22 ± 0.3	15 ± 0.2	24 ± 0.1	22 ± 0.3	No inhibition	21 ± 0.2
6j	16 ± 0.2	17 ± 0.3	No inhibition	13 ± 0.2	15 ± 0.1	16 ± 0.3
PEF	30 ± 0.3	32 ± 0.1	35 ± 0.2	31 ± 0.1	36 ± 0.2	38 ± 0.1

to their additional contribution of targets due to fluoroindole with benzamide substitutions. These analogues **6a-j** are against six organisms *K. aerogenes*, *E. coli and K. pneumonia*, *S. aureus*, *S. pneumoniae*, *S. epidermidis* were found to be more potent activity than pefloxacin. Compounds **6e**, **6d** and **6i** exhibited outstanding antibacterial activity against Gram-positive bacteria *S. aureus* 30, 28, 22 mm values, respectively. Whereas compounds **6h**, **6d**, **6g** has shown very good activity against *S. epidermidis* bacterial strain with zone of inhibition 34, 30, 28 mm. Further final analogues **6g** and **6d** having excellent activity against two Gram-negative antibacterial organisms *E. coli, K. pneumonia* 28, 22, 25 zone of inhibition**.** Compounds **6c** and **6h** have moderate active against the Gram-positive, Gram-negative bacterial organisms *S. pneumoniae*, *K. aerogenes* with 19, 18, 21 mm values, respectively. Some of the compounds did not show antibacterial activity against these bacterial organisms comparable to pefloxacin. Fluoroindole derivatives **6a**, **6f** and **6b** shows a moderate to good antibacterial activity for entire Gram-positive and Gram-negative bacterial organisms.

Antioxidant activity: Antioxidant activity of final fluoro/methoxy indole derivatives in mmol/mL are shown in Table-2. The highest antioxidant activity has been detected in compound **6h** with the value 150.12 ± 0.3 mmol/mL and the lowest in the compound **6j** with the value 15.84 ± 0.2 mmol/mL. Compounds **6i**, **6g** and **6d** show good antioxidant activity with % of inhibition 139.04 ± 0.1, 137.08 ± 0.2 and 135.56 ± 0.1 mmol/mL, respectively. Final targets **6e**, **6f** and **6a** reveal the moderate antioxidant activity % of inhibition range 92.31-38.61 mmol/mL.

TABLE-2
ANTIOXIDANT ACTIVITY, PHYSIOLOGICAL PROPERTIES OF **6a-j** BY DPPH METHOD

Compd. No.	Inhibition (%)	log p/Clog p
6a	85.46 ± 0.1	2.4/4.16
6b	68.84 ± 0.1	1.87/3.07
6c	38.61 ± 0.3	3.02/4.29
6d	135.56 ± 0.1	3.82/4.89
6e	92.31 ± 0.2	4.38/5.61
6f	77.64 ± 0.1	4.45/5.49
6g	137.08 ± 0.2	4.74/5.78
6h	150.12 ± 0.3	2.54/4.40
6i	139.04 ± 0.1	3.02/4.35
6j	15.84 ± 0.1	4.02/5.01
Ascorbic acid	140 ± 0.2	-3.36/-1.75

Docking results: Docking of all the synthesized compounds binding site into the protein was performed to estimate the binding affinity of the complex. We have to design the process of crystal structure of C(30) carotenoid dehydrosqualene synthase from *S. aureus* complexed with bisphosphonate BPH-700 (2ZCS). Docking is the most considerably used method for the calculation of protein-ligand interactions are summarized in Table-3. In this study, the native **10** ligands have been identify as a potent antibacterial inhibitors from binding energy assessment by AutoDock4.2 version and an efficient method to predict the potential ligand interactions. The docking score and H-bond interactions, π-π interactions, π-sigma interactions, wan der Waal forces were done for all the synthesized comp-

TABLE-3
MOLECULAR DOCKING INTERACTIONS OF SYNTHESIZED DERIVATIVES WITH THEIR ANTIBACTERIAL 2ZCS PROTEIN

Compd. No.	π- π, π-sigma Interacting amino acids	ΔG (Kcal/Mol)	KI (μM)	Hydrogen bonding interactions
6a	His18, Arg265	-6.47	18.37	Arg171, Ser21
6b	Lys20, Lys17	-6.35	16.77	Ser21, Asp49, Arg171
6c	Lys18, Lys20, Lys270	-6.38	4.21	Lys273, Asp27
6d	Ser273, Lys276, Lys20	-6.82	9.92	Lys273, Asp27
6e	Asp49, Arg265	-7.66	21.77	Lys273
6f	Lys270, Lys20	-7.47	22.68	Lys273, Asp27
6g	Asp49, Arg265	-6.25	25.40	Phe267
6h	Lys273, Asp27	-7.09	6.47	Lys273
6i	Arg265, Tyr24	-7.90	10.32	Lys16
6j	Arg45, Val266	-5.70	45.64	Ser21, Lys20
PEF	His76, Ser80	-6.21	28.02	Pro39

ounds used in this study. Binding energies have been measured like wan der Waal forces, hydrogen bondings, cation-π interactions, π-π interactions, *etc*. The hydrogen bondings of all molecules with protein are less than 2.1 Å. Docking score and hydrogen bonding interactions of final targets are against 2ZCS protein summarized in Table-3 and Fig. 2.

Compound **6c** shows highest hydrogen bonding interactions with amino acids Asp27 (2.06 Å), Lys273 (1.85 Å) and binding energy ΔG = -6.38 kcal/mol, respectively. Final targets **6a**, **6g**, **6h** having good hydrogen bonding interactions 1.91 Å, 1.53 Å, 1.34 Å with amino acids Ser21, Lys273, Lys20. Some compounds **6i**, **6e** and **6f** exhibited more binding energy

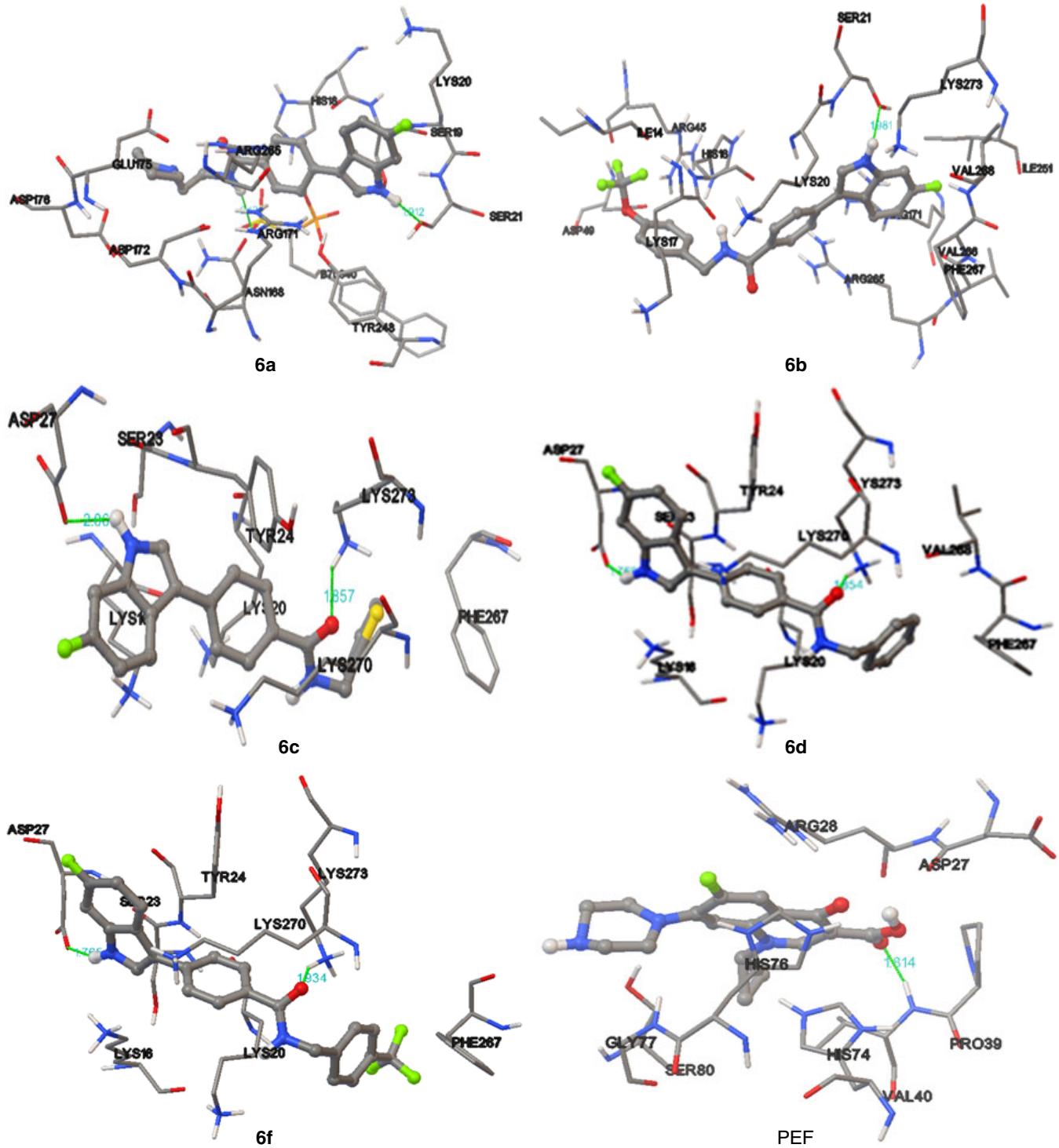


Fig. 2. The binding 3D-interactions-side chain flexibility of *S. aureus* complexed with active site of bisphosphonate BPH-700 (2ZCS); interactions types: light green colour: conventional hydrogen bonding; pink colour: π-π interactions; light pink colour: π-alkyl, alkyl-alkyl interactions; orange colour: π-anion interactions; purple colour: π-sigma interactions; cyan colour: van der waals; red colour-oxygen, light green colour-fluorine, blue colour-nitrogen, ash colour-carbon, white colour-hydrogen's indicated side chain residues of active site of 2ZCS protein

-7.90, -7.66, -7.47 with dissociation constants 10.32, 21.77, 22.68 μM and interacting amino acid Lys273. Final acetamide indole **6d** reveals excellent hydrogen bonding interactions with Lys273 (1.95 Å), Asp27 (1.74 Å) and π-π bindings with Ser273, Lys276, π-sigma bindings with Lys20 interactions. Final target indole **6f** shows Lys273, Asp27, Lys270, Lys20 amino acid interactions of hydrogen bondings (1.93 Å, 1.78 Å) and π-π bindings, respectively. Furthermore, compounds **6g**, **6h**, **6d**, **6c** and **6j** effective interactions with two amino acids Asp49, Arg265, Lys273, Asp27, Lys270, Ser21, Lys20, with binding energies -6.25, 7.09, -6.82, -6.38 and -5.70 Kcal/Mol values, respectively. The novel indole derivatives **6j**, **6f**, **6g** exhibit outstanding dissociation constant 45.64, 22.68, 25.40 μM when comparatively pefloxacin 51.74 μM.

Conclusion

In summary, a novel series of fluoro/methoxy indole acetamide (**6a-j**) was developed from key intermediate 2-(4-(5-fluoro-1*H*-indol-3-yl)phenyl)-2-oxoacetyl chloride (**5**). These compounds **6g, 6h, 6d, 6e, 6i** and **6c** showed very good activity against Gram-positive microorganisms with zone of inhibition 34, 28, 30, 22 mm, which is closer to standard drug pefloxacin. More interestingly, compounds **6a**, **6f**, **6b** exhibited a good activity for the entire Gram-positive and Gram-negative bacterial organisms. The products **6a-j** also showed an excellent antioxidant activity with% of inhibition range 15.84-150.12 mmol/mL when compared to reference compound ascorbic acid. Compounds **6i**, **6e** and **6c** showed highest binding energy of -7.90, -7.66 and -6.38 kcal/mol with interacting amino acid Lys273 and all the final compounds are expected to present good bioavailability profile. According to *in vitro* and docking studies, compounds **6g**, **6h** and **6c** stand out as potential orally bioavailable antimicrobial, antioxidant agents for further studies.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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