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Synthesis of Benzoxazole Associated Benzothiazine-4-ones and their *in vitro* and *in silico* Antimicrobial, Antioxidant Activities

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In present study, a series of 3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2-phenyl-2,3-dihydro-4*H*-1,3-benzothiazin-4-ones (**6a-n**) were synthesized and elucidated by elemental, FT-IR, ^{1}H & ^{13}C NMR and LC-MS studies. The *in vitro* antibacterial screening against Gram-positive bacterial strains such as *B. subtilis*, *S. aureus*, *S. epidermidis* and Gram-negative bacteria such as *E. coli*, *P. aeruginosa* was carried in comparison with tetracycline as reference standard. Antifungal activities against different fungal strains namely *R. oryzae*, *A. niger*, *A. favus*, *C. albicans* and *S. cerevisiae* have been evaluated by comparing with fluconazole as reference standard. Compounds **6b**, **6c**, **6e**, **6j**, **6m** and **6n** emerged as highly potent antimicrobial agents. The DPPH radical scavenging assay of the synthesized moieties showed good antioxidant potency comparable to standard ascorbic acid. The molecular docking simulation studies of all the title compounds in their active conformation analogues with target proteins (PDB ID 2XCT-antibacterial, PDB ID 1IYL-antifungal, PDB ID 2HCK-antioxidant) exhibited good binding interactions in top scoring poses. The pharmacokinetic properties prediction by ADMET descriptors and Lipinski′s rule of five endorse the properties of newly synthesized compounds to a drug molecule. The results of the docking protocols were compatible with the *in vitro* studies which validates the potency of the molecules.

Keywords: Benzoxazole, Benzothiazine, Antimicrobial activity, Antioxidant activity, Molecular docking.

INTRODUCTION

The dramatic ascendance of the infections caused by microbes resistant to various drugs in the former few decades has turned into a deliberate health complication. Particularly, the advent of multiple drug resistant strains of Gram-positive bacterial pathogens like methicillin resistant *Staphylococcus aureus*, *Staphylococcus epidermis* and also certain vancomycin resistant *Enterococcus* strains have been an everlasting challenge in the clinic [1-5]. To avert this critical problem, the elaboration of new varieties of the formerly known drug molecules is a substantive task. The organic moieties possessing benzoxazole scaffold as a core unit have significantly competent use in medicinal, bioorganic and optical fields, *etc*. Hence, these captivated remarkable attention of researchers for their profound bioorganic medicinal and pharmaceutical applications including the important biological activities such as antibiotic [6], antimicrobial [7-9], antiviral [10], topoisomerase I and II inhibitor [11], multiple drug resistant cancer cell activities [12] and antioxidant activities [13]. Benzothiazines also establish a significant class of therapeutically interesting heterocyclic moiety and known for their applications as antifungal [14,15], antibacterial [16], antimicrobial [17], anticancer [18-20], cardiovascular [21] drugs, *etc*. Combating against microbial infections has brought about the advancement of a wide range of antibiotics, still there is an urgency to develop new anti-mycobacterial agents. Therefore, those antimicrobial agents have been under investigation till today.

The benzoxazole core structure was found to be active particularly against some Gram-positive bacteria by acting as a receptive ionophore [22-25] as well as the benzothiazines were also found to be good antimicrobial agents from the literature [14-17]. Herein, we planned to ally both the moieties together to accomplish new antimicrobial, antioxidant agents. In the present work, we designed and synthesized the new target compounds 3-(5-methylbenzo[*d*]oxazol-2-ylamino)-2,3-

dihydro-2-phenylbenzo[*e*][1,3]thiazin-4-ones (**6a-n**), evaluated for their *in vitro* antimicrobial potency and antioxidant efficiency. Docking studies of all the synthesized analogues through Discovery studio docking suite has been performed on target proteins (PDB ID 2XCT-antibacterial, PDB ID 1IYL-antifungal, PDB ID 2HCK-antioxidant) with the purpose to bring the active conformations of the analogues in their top scoring poses. The data obtained from *in vitro* and *in silico* studies are compatible with one another. The potency of the molecules has also been upheld by prediction of ADMET properties.

EXPERIMENTAL

The AR grade chemicals and reagents were bought commercially from Sigma-Aldrich, Merck and Himedia, and used without additional purification. All the synthetic and purification purposes were accomplished by using freshly distilled solvents.

Electro thermal melting point apparatus was used to detect the melting points, and are uncorrected. Silica gel GF_{254} thin plates from Merck were employed for TLC and the eluted spots were visualized in UV. The elemental analysis was carried using VarioMICROV1.7.0 (Elemental Analyser systeme GmbH) instrument. The ^1H & ^{13}C NMR spectra were recorded on JNM-ECS 400 MHz and Bruker Avance III, 400MHz, respectively. Shimadzu Fourier Transform Infrared (FTIR) spectrometer was used to record the FT-IR spectra using KBr pellet (100 mg). Varian Inc, 410 Prostar Binary LC with 500 MS IT PDA detectors has been employed for the LC-MS spectral analysis.

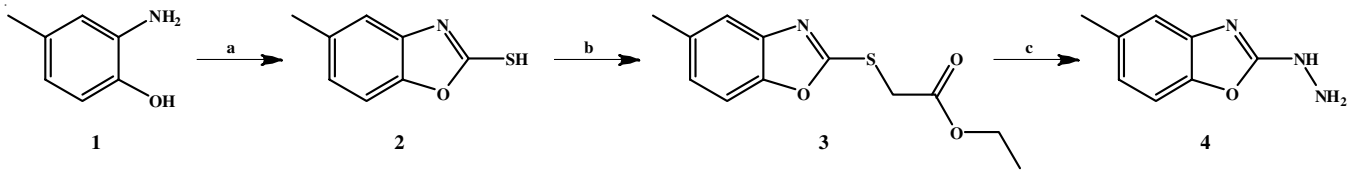
Synthesis of 5-methyl-1,3-benzoxazole-2-thiol (2): To 30 mL methanolic solution of KOH (0.014 mmol), CS_2 (0.014 mmol) was added dropwise with continuous stirring for 15 min at room temperature. To this solution, 4-methyl-2-aminophenol (0.010 mmol) was added in portions and refluxed for 6 h. The crude solid was collected by pouring the reaction mixture into crushed ice followed by neutralization with 10% acetic acid solution and recrystallized in ethyl acetate to get short thin needle like creamish crystals. Petroleum ether and ethyl acetate in the ratio 8:2 was used as eluent to monitor TLC. Yield: 92.4%. m.p.: 215-219 ºC. Elemental anal. of C_8H_7NOS calcd. (found) %: C 58.16 (58.20), H 8.48 (8.45), N 4.27 (4.21). IR (KBr, ν_{max}, cm^{-1}): 3066 (Ar-C-H), 2355 (-SH), 1668 (-C=N), 1470 ($-CH_3$), 1138.76-1105.98 (-C-O-); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.416 (s, 3H, Ar-CH_3), 7.020-7.259 (m, 3H, Ar-H), 10.756 (s, 1H, -SH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 21.19 (3C, Ar-CH_3), 109.56-146.8 (6C Ar-C), 181.05 (1C, C-SH); MS (LC-MS): *m/z* 165.02 (M^+).

Synthesis of ethyl-[(5-methyl-1,3-benzoxazol-2-yl)-sulfanyl]acetate (3): To K_2CO_3 solution (0.012 mmol) in acetone (25 mL) and compound **2** (0.010 mmol), ethylchloroacetate (0.012 mmol) was added dropwise and stirred for 20 min then refluxed at 60 ºC for about 5 h. The crude solid was collected by pouring the reaction mixture into crushed ice and vacuum dried. Finally, recrystallized in EtOH to obtain long needle like pale brown crystals. Petroleum ether and ethyl acetate in the ratio 7:3 was used as eluent to monitor TLC. Yield: 90.26%. m.p.: 85-87 ºC. Elemental anal. of $C_{12}H_{13}NO_3S$ calcd. (found) %: C 57.35 (57.29), H 5.57 (5.52), N 5.21 (5.27). IR (KBr, ν_{max}, cm^{-1}): 3064 (Ar-C-H, s), 2976, 2931 (C-H), 1741.41 (ester carbonyl C=O) 1626 (-C=N), 1454.06 (carbonyl C-O), 1313 ($-CH_2$, m); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 1.268-1.303 (t, 3H, CH_3), 2.436 (s, 3H, Ar-CH_3), 4.102 (s, 2H, -S-CH_2), 4.222-4.276 (q, 2H, $-CH_2$), 7.041-7.379 (m, 3H, Ar-H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ ppm: 14.06 (CH_3 carbon of C_2H_5), 21.39 (Ar-CH_3), 34.223 (CH_2 of S- CH_2 -COO), 60.13 (CH_2 carbon of C_2H_5), 109.29-150.32 (6C, Ar-C), 163.08 (N-C-O carbon of benzoxazole), 167.90 (C=O of ester); MS (LC-MS): *m/z* 251.06 (M^+).

Synthesis of 2-hydrazino-5-methyl-1,3-benzoxazole (4): Methanolic solution of compound **3** (0.01mmol) and hydrazine monohydrate (0.002 mmol) were stirred at room temperature for 3 h. The formed solid was filtered and washed with petroleum ether. Finally, recrystallized from ethyl acetate. Petroleum ether and ethyl acetate in the ratio 6:4 was used as eluent (**Scheme-I**). Yield: 93%. m.p.: 160-162 ºC. Elemental anal. of $C_8H_9N_3O$ calcd. (found) %: C 58.88 (58.92), H 25.75 (25.80), N 5.56 (5.60); IR (KBr, ν_{max}, cm^{-1}): 3316, 3301 (-NH-NH_2), 3046(Ar-C-H), 1643 (-C=N); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.405 (s, 3H, Ar-CH_3), 4.602 (s, 2H, -NH_2), 7.127-7.517 (m, 7H, Ar-H), 9.420 (bs, 1H, -NH); ^{13}C NMR ($CDCl_3$, 400 MHz) δ ppm: 20.89 (Ar-CH_3), 109.56-149.50 (6C Ar-C), 165.58 (N-C-O carbon of benzoxazole); MS (LC-MS): *m/z* 163.07 (M^+).

Synthesis hydrazinyl-benzoxazole Schiff's bases (5a-n): A mixture of compound **4** (0.01 mmol) and aryl aldehyde (0.01 mmol) along with the catalytic amount of glacial acetic acid in 20 mL of EtOH was refluxed for 4 h. The solid was collected by pouring the reacted contents onto crushed ice followed by the addition of brine solution and recrystallized in ethyl acetate. The colour of the hydrazino benzoxazole Schiff's bases were creamish to peachy.

5-Methyl-2-[-2-(2-nitrobenzylidene)hydrazinyl]-1,3-benzoxazole (5a): Yield: 85.37%. m.p.: 137-139 ºC. Elemental anal. of $C_{15}H_{12}N_4O_3$ calcd. (found) %: C 60.81 (60.80), H 4.08 (4.12), N 18.91 (18.95); IR (KBr, ν_{max}, cm^{-1}): 3304 (-NH, m), 3074 (Ar-C-H, s), 2966 (C-H), 1669 (-C=N-,), 1555 and 1345 ($-NO_2$), 1057 (-C-N); ^1H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.398 (s, 3H, Ar-CH_3), 7.06-8.01 (m, 7H, Ar-H), 8.21(s, 1H,



(a) CS_2, KOH, EtOH, (b) $ClCH_2COOC_2H_5$, K_2CO_3, acetone, (c) $NH_2NH_2.H_2O$, MeOH

Scheme-I: Synthesis of 2-hydrazinyl-5-methyl-1,3-benzoxazole (**4**)

-N=C-H), 11.47 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 22.69 (1C, Ar-CH_3), 143.9 (1C, N=CH azomethine carbon), 110.5-152.6 (13C, aromatic carbons); MS (LC-MS): *m/z* 296.09 (M^+).

5-Methyl-2-[-2-(4-nitrobenzylidene)hydrazinyl]-1,3-benzoxazole (5b): Yield: 88.61%. m.p.: 185-187 ºC. Elemental anal. of $C_{15}H_{12}N_4O_3$ calcd. (found) %: C 60.81 (60.79), H 4.08 (4.03), N 18.91 (18.87); IR (KBr, ν_{max}, cm^{-1}): 3309 (-NH, m), 3076 (Ar-C-H, s), 2970 (C-H), 1670 (-C=N-,), 1553 and 1344 (-NO_2), 1059 (-C-N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.398 (s, 3H, Ar-CH_3), 7.06-8.01 (m, 7H, Ar-H), 8.21(s, 1H, -N=C-H), 11.47 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 22.69 (1C, Ar-CH_3), 143.9 (1C, N=CH azomethine carbon), 110.5-152.6 (13C, aromatic carbons); MS (LC-MS): *m/z* 296.09 (M^+).

5-Methyl-2-[-2-(4-fluorobenzylidene)hydrazinyl]-1,3-benzoxazole (5c): Yield: 83.25%. m.p.: 178-180 ºC. Elemental anal. of $C_{15}H_{12}N_3OF$ calcd. (found) %: C 66.91 (66.85), H 4.49 (4.53), N 15.60 (15.58); IR (KBr, ν_{max}, cm^{-1}): 3290 (-NH, m), 3065 (Ar-C-H, s), 2953(C-H), 1668 (-C=N-), 1060 (-C-N), 1195 (C-F), ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.36 (s, 3H, Ar-CH_3), 7.0-7.61 (m, 7H, Ar-H), 8.17(s, 1H, -N=C-H), 11.20 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 23.50 (1C, Ar-CH_3), 145.02 (1C, N=CH azomethine carbon), 110.65-165.21 (13C, aromatic carbons); MS (LC-MS): *m/z* 269.10 (M^+).

5-Methyl-2-[-2-(4-chlorobenzylidene)hydrazinyl]-1,3-benzoxazole (5d): Yield: 86.16%. m.p.: 215-218 ºC. Elemental anal. of $C_{15}H_{12}ClN_3O$ calcd. (found) %: C 63.05 (63.02), H 4.23 (4.21), N 14.71 (14.65); IR (KBr, ν_{max}, cm^{-1}): 3303 (-NH, m), 3073 (Ar-C-H, s), 2968 (C-H), 1665 (-C=N-), 1059 (-C-N), 760 (C-Cl), ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.35 (s, 3H, Ar-CH_3), 7.04-7.81 (m, 7H, Ar-H), 8.20(s, 1H, -N=C-H), 11.35 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.36 (1C, Ar-CH_3), 143.01 (1C, N=CH azomethine carbon), 110.8-152.3 (13C, aromatic carbons); MS (LC-MS): *m/z* 285.07 (M^+), 287.06 (M+2) (3:1).

5-Methyl-2-[-2-(4-bromobenzylidene)hydrazinyl]-1,3-benzoxazole (5e): Yield: 78.48%. m.p.: 165-168 ºC. Elemental anal. of $C_{15}H_{12}N_3OBr$ calcd. (found) %: C 54.56 (54.58), H 3.66 (3.62), N 12.73 (12.78); IR (KBr, ν_{max}, cm^{-1}): 3279 (-NH, m), 3068 (Ar-C-H, s), 2972(C-H), 1666 (-C=N-), 1053 (-C-N), 612 (C-Br), ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.38 (s, 3H, Ar-CH_3), 7.06-7.51 (m, 7H, Ar-H), 8.17(s, 1H, -N=C-H), 11.41 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.30 (1C, Ar-CH_3), 144.20 (1C, N=CH azomethine carbon), 110.8-153.6 (13C, aromatic carbons); MS (LC-MS): *m/z* 329.82 (M^+), 331.01 (M+2) (1:1).

2-[-2-Benzylidenehydrazinyl]-5-methyl-1,3-benzoxazole (5f): Yield: 82.18%. m.p.: 98-101 ºC. Elemental anal. of $C_{15}H_{13}N_3O$ calcd. (found) %: C 71.72 (71.80), H 5.21 (5.16), N 16.72 (16.66); IR (KBr, ν_{max}, cm^{-1}): 3221 (-NH, m), 3068 (Ar-C-H, s), 1672 (-C=N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.370 (s, 3H, Ar-CH_3), 7.06-7.680 (m, 8H, Ar-H), 8.210 (s, 1H, -N=C-H), 10.95 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 23.91 (1C, Ar-CH_3), 144.2 (1C, N=CH azomethine carbon), 110.09-152.60 (13C, aromatic carbons); MS (LC-MS): *m/z* 251.11 (M^+).

5-Methyl-2-[-2-(3-methoxybenzylidene)hydrazinyl]-1,3-benzoxazole (5g): Yield: 87.02%. m.p.: 158-160 ºC. Elemental anal. of $C_{16}H_{15}N_3O_2$ calcd. (found) %: C 68.31 (68.35), H 5.37 (5.33), N 14.94 (14.90); IR (KBr, ν_{max}, cm^{-1}): 3305 (-NH, m), 3079 (Ar-C-H, s), 2970 (C-H), 1670 (-C=N-), 1055 (-C-N), 1028 (-C-O),^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.38 (s, 3H, Ar-CH_3), 3.88 (s, 3H, Ar-OCH_3), 6.85-7.20 (m, 7H, Ar-H), 8.25 (s, 1H, -N=C-H), 11.25 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 23.50 (1C, Ar-CH_3), 144.50 (1C, N=CH azomethine carbon), 112.21-160.81 (13C, aromatic carbons); MS (LC-MS): *m/z* 281.12 (M^+).

5-Methyl-2-[-2-(4-methoxybenzylidene)hydrazinyl]-1,3-benzoxazole (5h): Yield: 89.51%. m.p.: 119-121 ºC. Elemental anal. of $C_{16}H_{15}N_3O_2$ calcd. (found) %: C 68.31 (68.32), H 5.37 (5.40), N 14.94 (14.99); IR (KBr, ν_{max}, cm^{-1}): 3289 (-NH, m), 3074 (Ar-C-H, s), 2964(C-H), 1671 (-C=N-), 1052 (-C-N), 1015 (-C-O),^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.371 (s, 3H, Ar-CH_3), 3.851 (s, 3H, Ar-OCH_3), 6.80-7.51 (m, 7H, Ar-H), 8.23(s, 1H, -N=C-H), 11.32 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.38 (1C, Ar-CH_3), 144.12 (1C, N=CH azomethine carbon), 109.90-163.10 (13C, aromatic carbons); MS (LC-MS): *m/z* 281.12 (M^+).

5-Methyl-2-[-2-(4-methylbenzylidene)hydrazinyl]-1,3-benzoxazole (5i): Yield: 84.25%. m.p.: 171-173 ºC. Elemental anal. of $C_{16}H_{15}N_3O$ calcd. (found) %: C 72.43 (72.38), H 5.70 (5.68), N 15.84 (15.79); IR (KBr, ν_{max}, cm^{-1}): 3290 (-NH, m), 3072 (Ar-C-H, s), 2962(C-H), 1660 (-C=N-), 1053 (-C-N); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.378 (s, 3H, Ar-CH_3), 2.410 (s, 3H, Ar-CH_3), 7.05-7.510 (m, 7H, Ar-H), 8.210 (s, 1H, -N=C-H), d 10.91 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.41 (1C, Ar-CH_3), 144.15 (1C, N=CH azomethine carbon), 109.09-152.60 (13C, aromatic carbons); MS (LC-MS): *m/z* 265.12 (M^+).

***N,N*-Dimethyl-4-{-[2-(5-methyl-1,3-benzoxazol-2-yl)-hydrazinylidene]methyl}aniline (5j):** Yield: 79.05%. m.p.: 153-155 ºC. Elemental anal. of $C_{17}H_{18}N_4O$ calcd. (found) %: C 69.37 (69.33), H 6.16 (6.12), N 19.03 (19.10); IR (KBr, ν_{max}, cm^{-1}): 3304 (-NH, m), 3074 (Ar-C-H, s), 1668 (-C=N, s), 1056.8 (-C-N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.398 (s, 3H, Ar-CH_3), 2.964 (s, 6H, -N-CH_3), 6.708-7.921 (m, 7H, Ar-H), 8.801 (s, 1H, -N=C-H), 11.475 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 20.869 (1C, Ar-CH_3), 34.651-34.791 (2C, -N-CH_3), 144.82 (1C, N=CH azomethine carbon), 109.478-167.370 (13C, aromatic carbons); MS (LC-MS): *m/z* 294.08 (M^+).

2-{(-[2-(5-Methyl-1,3-benzoxazol-2-yl)hydrazinylidene]-methyl}phenol (5k): Yield: 83.85%. m.p.: 193-195 ºC. Elemental anal. of $C_{15}H_{13}N_3O_2$ calcd. (found) %: C 67.40 (67.42), H 4.90 (5.03), N 15.72 (15.70); IR (KBr, ν_{max}, cm^{-1}): 3444 (-OH, s), 3220 (-NH, m), 3073 (Ar-C-H, s), 1669 (-C=N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.396 (s, 3H, Ar-CH_3), 6.914-7.530 (m, 7H, Ar-H), 8.367 (s, 1H, -N=C-H), 10.954 (s, 1H, Ar-OH), 11.699 (bs, 1H, N-H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 20.86 (1C, Ar-CH_3), 144.81 (1C, N=CH azomethine carbon), 109.4-167.3 (13C, sp^2 aromatic carbons); MS (LC-MS): *m/z* 267.07 (M^+).

3-{(-[2-(5-Methyl-1,3-benzoxazol-2-yl)hydrazinylidene]methyl}phenol (5l): Yield: 81.29%. m.p.: 160-163 ºC.

Elemental anal. of $C_{15}H_{13}N_3O_2$ (%): C 67.40 (67.38), H 4.90 (5.05), N 15.72 (15.76); IR (KBr, ν_{max}, cm^{-1}): 3443 (-OH, s), 3220 (-NH, m), 3075 (Ar-C-H, s), 1667 (-C=N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.39 (s, 3H, Ar-CH_3), 6.94-7.53 (m, 7H, Ar-H), 8.35 (s, 1H, -N=C-H), 10.96 (s, 1H, Ar-OH), 11.65 (bs, 1H, N-H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 20.89 (1C, Ar-CH_3), 144.85 (1C, N=CH azomethine carbon), 109.4-168.3 (13C, sp^2 aromatic carbons); MS (LC-MS): *m/z* 267.07 (M^+).

2-Methoxy-4-{-[2-(5-methyl-1,3-benzoxazol-2-yl)-hydrazinylidene]methyl}phenol (5m): Yield: 82.69%. m.p.: 118-120 ºC. Elemental anal. of $C_{16}H_{15}N_3O_3$ (%): C 64.64 (64.58), H 5.09 (5.13), N 14.13 (14.18); IR (KBr, ν_{max}, cm^{-1}): 3451 (-OH, s), 3218 (-NH, m), 3076 (Ar-C-H, s), 1671 (-C=N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.415 (s, 3H, Ar-CH_3), 3.89 (s, 3H, Ar-OCH_3), 6.710-7.140 (m, 7H, Ar-H), 8.251 (s, 1H, -N=C-H), 10.971 (s, 1H, Ar-OH), 11.59 (bs, 1H, N-H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 23.86 (1C, Ar-CH_3), 57.1 (1C, Ar-OCH_3), 145.15 (1C, N=CH azomethine carbon), 110.1-151.5 (1C, sp^2 aromatic carbons); MS (LC-MS): *m/z* 297.11 (M^+).

2-Methoxy-6-{-[2-(5-methyl-1,3-benzoxazol-2-yl)-hydrazinylidene]methyl}phenol (5n): Yield: 85.47%. m.p.: 80-82 ºC. Elemental anal. of $C_{16}H_{15}N_3O_3$ (%): C 64.64 (64.68), H 5.09 (5.02), N 14.13 (14.15); IR (KBr, ν_{max}, cm^{-1}): 3445 (-OH, s), 3221 (-NH, m), 3074 (Ar-C-H, s), 1673 (-C=N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.391 (s, 3H, Ar-CH_3), 3.91 (s, 3H, Ar-OCH_3), 6.750-7.210 (m, 7H, Ar-H), 8.362 (s, 1H, -N=C-H), 10.981 (s, 1H, Ar-OH), 11.46 (bs, 1H, N-H); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.82 (1C, Ar-CH_3), 57.35 (1C, Ar-OCH_3), 144.15 (1C, N=CH azomethine carbon), 110.5-153.6 (1C, sp^2 aromatic carbons); MS (LC-MS): *m/z* 297.11 (M^+).

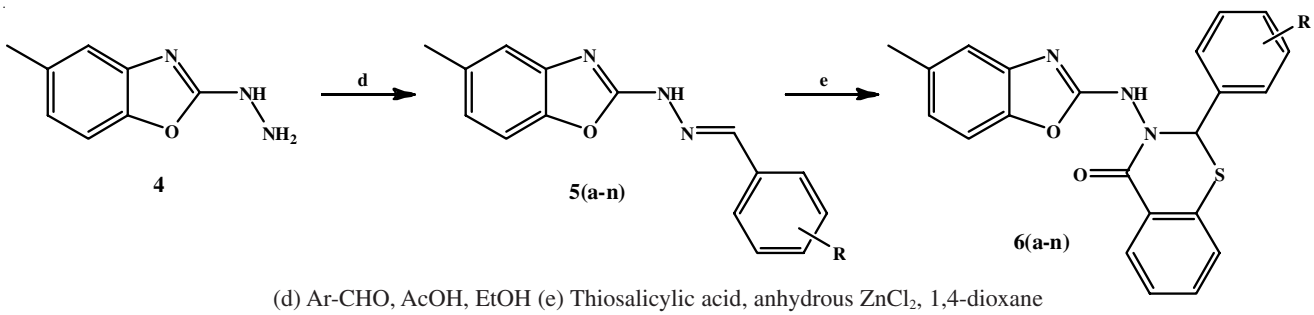
Synthesis of benzoxazole associated benzothiazine-4-one derivatives (6a-n): To a solution of anhydrous $ZnCl_2$ (0.001 mmol) in 1,4-dioxane, compound **5** (0.001 mmol) was added followed by thiosalicylic acid (TSA) (0.002 mmol). The reaction mixture then stirred for about 10 min at room temperature, then refluxed at 110-115 ºC for 14-16 h. The progress of the reaction was observed by the formation of pale yellow solid within the reaction mixture. The reaction contents were poured onto 150 mL of ice-cold water and excess of thiosalicylic acid was removed by neutralization with 3% sodium carbonate solution and the obtained solid was filtered and purified by column chromatography using petroleum ether and ethyl acetate in the ratio 8:2 as mobile phase. Petroleum ether and ethyl acetate in the ratio 7:3 was used as eluent in TLC (**Scheme-II**).

3-[(5-Methyl-1,3-benzoxazol-2-yl)amino]-2-(2-nitrophenyl)-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6a): Yield: 73.08%. m.p.: 210-212 ºC. Elemental anal. of $C_{22}H_{16}N_4O_4S$ calcd. (found) %: C 61.10 (61.05), H 3.73 (3.69), N 12.96 (12.92); IR (KBr, ν_{max}, cm^{-1}): 3221 (-NH, m), 3068 (Ar-C-H, s), 1710 (-C=O, s), 1550 and 1346 (-NO_2); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.372 (s, 3H, Ar-CH_3), 5.92 (s, 1H, S-CH-N), 7.06-8.07 (m, 11H, Ar-H), 11.47 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.69 (1C, Ar-CH_3), 54.80 (1C, N-C-S of benzothiazinone), 112.0-147.8 (18C, aromatic carbons), 159.0 (1C, carbonyl of benzothiazinone), 165.2 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 432.09 (M^+).

3-[(5-Methyl-1,3-benzoxazol-2-yl)amino]-2-(4-nitrophenyl)-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6b): Yield: 69.05%. m.p.: 277-279 ºC. Elemental anal. of $C_{22}H_{16}N_4O_4S$ calcd. (found) %: C 61.10 (61.12), H 3.73 (3.78), N 12.96 (13.03); IR (KBr, ν_{max}, cm^{-1}): 3436 (-NH, m), 3062 (Ar-C-H, s), 1712 (-C=O, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.398 (s, 3H, Ar-CH_3), 5.851 (s, 1H, S-CH-N), 7.173-8.03 (m, 11H, Ar-H), 12.024 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 21.48 (1C, Ar-CH_3), 61.15 (1C, N-C-S of benzothiazinone), 109.9-150.9 (18C,aromatic carbons), 158.4 (1C, carbonyl of benzothiazinone), 165.03 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 432.09 (M^+).

3-[(5-Methyl-1,3-benzoxazol-2-yl)amino]-2-(4-fluorophenyl)-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6c): Yield: 71.06%. m.p.: 280-282 ºC. Elemental anal. of $C_{22}H_{16}FN_3O_2S$ calcd. (found) %: C 65.17 (65.14), H 3.98 (4.01), N 10.36 (10.39); IR (KBr, ν_{max}, cm^{-1}): 3289 (-NH, m), 3076 (Ar-C-H, s), 1713 (-C=O, s), 1124 (C-F); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.365 (s, 3H, Ar-CH_3), 5.95 (s, 1H, S-CH-N), 6.85-7.86 (m, 11H, Ar-H), 11.49 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 25.1 (1C, Ar-CH_3), d 62.6 (1C, N-C-S of benzothiazinone), 108.9-161.3 (18C, aromatic carbons), 162.0 (1C, carbonyl of benzothiazinone), 165.5 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 405.09 (M^+).

3-[(5-Methyl-1,3-benzoxazol-2-yl)amino]-2-(4-chlorophenyl)-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6d): Yield: 78.36%. m.p.: 287-290 ºC. Elemental anal. of $C_{22}H_{16}ClN_3O_2S$



(d) Ar-CHO, AcOH, EtOH (e) Thiosalicylic acid, anhydrous $ZnCl_2$, 1,4-dioxane

R = **a**: 2-NO_2; **b**: 4-NO_2; **c**: 4-F; **d**: 4-Cl; **e**: 4-Br; **f**: H; **g**: 4-OCH_3; **h**: 3-OCH_3; **i**: 4-CH_3; **j**: 4-$N(CH_3)_2$; **k**: 2-OH; **l**: 3-OH; **m**: 3-OCH_3, 4-OH; **n**: 3-OCH_3, 2-OH

Scheme-II: Synthesis of 3-(5-methylbenzo[d]oxazol-2-ylamino)-2,3-dihydro-2-phenylbenzo[*e*][1,3]thiazin-4-one (**6a-n**)

calcd. (found) %: C 62.63 (62.59), H 3.82 (3.74), N 9.96 (10.02); IR (KBr, ν_{max}, cm^{-1}): 3251 (-NH, m), 3076 (Ar-C-H, s), 1714 (-C=O, s), 779 (C-Cl); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.380 (s, 3H, Ar-CH_3), 5.82 (s, 1H, S-CH-N), 7.0-7.85 (m, 11H, Ar-H), 11.49 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.2 (1C, Ar-CH_3), 62.9 (1C, N-C-S of benzothiazinone), 110.6-147.01 (18C, aromatic carbons), 159.02 (1C, carbonyl of benzothiazinone), 167.1 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 421.07 (M^+), 422.07 (M+1) [3:1].

3-[(5-Methyl-1,3-benzoxazol-2-yl)amino]-2-(4-bromophenyl)-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6e): Yield: 75.48%. m.p.: 220-224 ºC. Elemental anal. of $C_{22}H_{16}N_3O_2SBr$ calcd. (found) %: C 56.66 (56.63), H 3.46 (3.41), N 9.01 (9.18); IR (KBr, ν_{max}, cm^{-1}): 3285 (-NH, m), 3069 (Ar-C-H, s), 1712 (-C=O, s), 657 (C-Br); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.371 (s, 3H, Ar-CH_3), 5.90 (s, 1H, S-CH-N), 6.95-7.85 (m, 11H, Ar-H), 11.51 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.39 (1C, Ar-CH_3), 65.4 (1C, N-C-S of benzothiazinone), 110.8-147.0 (18C, aromatic carbons), 160.0 (1C, carbonyl of benzothiazinone), 166.2 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 465.01 (M^+), 467.04 (M+2) [1:1].

3-[(5-Methyl-1,3-benzoxazol-2-yl)amino]-2-phenyl-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6f): Yield: 79.38%. m.p.: 195-197 ºC. Elemental anal. of $C_{22}H_{17}N_3O_2S$ calcd. (found) %: C 68.20 (68.16), H 4.41 (4.46), N 10.85 (10.81); IR (KBr, ν_{max}, cm^{-1}): 3295 (-NH, m), 3073 (Ar-C-H, s), 1710 (-C=O, s), 1060 (-C-N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.352 (s, 3H, Ar-CH_3), 5.95 (s, 1H, S-CH-N), 7.06-7.90 (m, 12H, Ar-H), 11.40 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.4 (1C, Ar-CH_3), 62.7 (1C, N-C-S of benzothiazinone), 110.6 -147.0 (18C, aromatic carbons), 159.2 (1C, carbonyl of benzothiazinone), 165.3 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 387.10 (M^+).

2-(3-Methoxyphenyl)-3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6g): Yield: 72.15%. m.p.: 265-267 ºC. Elemental anal. of $C_{23}H_{19}N_3O_3S$ calcd. (found) %: C 66.17 (66.19), H 4.59 (4.63), N 10.07 (10.13); IR (KBr, ν_{max}, cm^{-1}): 3290 (-NH, m), 3070 (Ar-C-H, s), 1715 (-C=O, s), 1012 (-C-O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.355 (s, 3H, Ar-CH_3), 3.85 (s, 3H, Ar-OCH_3), 5.91 (s, 1H, S-CH-N), 6.57-7.86 (m, 11H, Ar-H), 11.42 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 25.1 (1C, Ar-CH_3), 55.9 (1C, Ar-OCH_3), 62.9 (1C, N-C-S of benzothiazinone), 109.9-160.7 (18C, aromatic carbons), 159.2 (1C, carbonyl of benzothiazinone), 167.6 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 417.11 (M^+).

2-(4-Nethoxyphenyl)-3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6h): Yield: 72.15%. m.p.: 238-240 ºC. Elemental anal. of $C_{23}H_{19}N_3O_3S$ calcd. (found) %: C 66.17 (66.20), H 4.59 (4.65), N 10.07 (10.02); IR (KBr, ν_{max}, cm^{-1}): 3305 (-NH, m), 3072 (Ar-C-H, s), 1715 (-C=O, s), 1010 (-C-O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.361 (s, 3H, Ar-CH_3), 3.87 (s, 3H, Ar-OCH_3), 5.87 (s, 1H, S-CH-N), 6.65-7.90 (m, 11H, Ar-H), 11.46 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 25.3 (1C, Ar-CH_3), 56.1 (1C, Ar-OCH_3), 63.1 (1C, N-C-S of benzothiazinone), 110.5-159.1 (18C, aromatic carbons), 160.0 (1C, carbonyl of benzothiazinone), 168.2 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 417.11 (M^+).

3-[(5-Methyl-1,3-benzoxazol-2-yl)amino]-2-(4-methylphenyl)-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6i): Yield: 74.19%. m.p.: 265-267 ºC. Elemental anal. of $C_{23}H_{19}N_3O_2S$ calcd. (found) %: C 68.81 (68.77), H 4.77 (4.72), N 10.47 (10.51); IR (KBr, ν_{max}, cm^{-1}): 3308 (-NH, m), 3072 (Ar-C-H, s), 1713 (-C=O, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.361 (s, 3H, Ar-CH_3), 2.350 (s, 3H, Ar-CH_3), 5.85 (s, 1H, S-CH-N), 6.86-7.90 (m, 11H, Ar-H), 11.43 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.6 (1C, Ar-CH_3), 25.2 (1C, Ar-CH_3), 63.0 (1C, N-C-S of benzothiazinone), 112.0 -147.0 (18C, aromatic carbons), 158.0 (1C, carbonyl of benzothiazinone), 164.3 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 401.12 (M^+).

2-[4-(Dimethylamino)phenyl]-3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6j): Yield: 68.27%. m.p.: 288-290 ºC. Elemental anal. of $C_{24}H_{22}N_4O_2S$ calcd. (found) %: C 66.96 (66.98), H 5.15 (5.13), N 13.01 (13.05); IR (KBr, ν_{max}, cm^{-1}): 3436 (-NH, m), 3050 (Ar-C-H, s), 1745 (-C=O, s), 1058 (-C-N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.398 (s, 3H, Ar-CH_3), 2.811 (s, 6H, Ar-NMe_2), 5.813 (s, 1H, S-CH-N), 7.102-7.989 (m, 11H, Ar-H), 11.902 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 23.056 (1C, Ar-CH_3), 42.015 (2C, Ar-NMe_2), 62.712 (1C, N-C-S of benzothiazinone), 109.6 -149.6 (18C, aromatic carbons), 158.5 (1C, carbonyl of benzothiazinone), 164.1 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 430.2 (M^+).

2-(2-Hydroxyphenyl)-3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6k): Yield: 69.28%. m.p.: 230-232 ºC. Elemental anal. of $C_{22}H_{17}N_3O_3S$ calcd. (found) %: C 65.49 (65.53), H 4.25 (4.19), N 10.42 (10.46); IR (KBr, ν_{max}, cm^{-1}): 3419 (-OH, s), 3238 (-NH, m), 3118 (Ar-C-H, s), 1761 (-C=O, s), 1049 (-C-N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.368 (s, 3H, Ar-CH_3), 5.89 (s, 1H, S-CH-N), 6.61-7.91 (m, 11H, Ar-H), 10.45 (s, 1H, Ar-OH), 11.47 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.36 (1C, Ar-CH_3), 52.4 (1C, N-C-S of benzothiazinone), 112.0 -154.9 (18C, aromatic carbons), 158.5 (1C, carbonyl of benzothiazinone), 166.0 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 403.10 (M^+).

2-(3-Hydroxyphenyl)-3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6l): Yield: 68.69%. m.p.: 245-247 ºC. Elemental anal. of $C_{22}H_{17}N_3O_3S$ calcd. (found) %: C 65.49 (65.46), H 4.25 (4.15), N 10.42 (10.37); IR (KBr, ν_{max}, cm^{-1}): 3445 (-OH, s), 3300 (-NH, m), 3070 (Ar-C-H, s), 1715 (-C=O, s), 1062 (-C-N, s); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.371 (s, 3H, Ar-CH_3), 5.93 (s, 1H, S-CH-N), 6.64-7.94 (m, 11H, Ar-H), 10.44 (s, 1H, Ar-OH), 11.45 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.42 (1C, Ar-CH_3), 52.7 (1C, N-C-S of benzothiazinone), 112.2 -155.3 (18C, aromatic carbons), 159.1 (1C, carbonyl of benzothiazinone), 165.8 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 403.10 (M^+).

2-(4-Hydroxy-3-methoxyphenyl)-3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6m): Yield: 69.23%. m.p.: 258-260 ºC. Elemental anal.

of $C_{23}H_{19}N_3O_4S$ calcd. (found) %: C 63.73 (63.68), H 4.42 (4.35), N 9.69 (9.65); IR (KBr, ν_{max}, cm^{-1}): 3390 (-OH, s), 3302 (-NH, m), 3069 (Ar-C-H, s), 1714 (-C=O, s), 1059 (-C-N, s), 1012 (-C-O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.349 (s, 3H, Ar-CH_3), 3.89 (s, 3H, Ar-OCH_3), 5.89 (s, 1H, S-CH-N), 6.40-7.85 (m, 10H, Ar-H), 10.49 (bs, 1H, Ar-OH), 11.48 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.7 (1C, Ar-CH_3), 56.3 (1C, Ar-OCH_3), 62.6 (1C, N-C-S of benzothiazinone), 109.8-160.2 (18C, aromatic carbons), 160.1 (1C, carbonyl of benzothiazinone), 165.2 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 433.11 (M^+).

2-(2-Hydroxy-3-methoxyphenyl)-3-[(5-methyl-1,3-benzoxazol-2-yl)amino]-2,3-dihydro-4*H*-1,3-benzothiazin-4-one (6n): Yield: 65.15%. m.p.: 239-241 ºC. Elemental anal. of $C_{23}H_{19}N_3O_4S$ calcd. (found) %: C 63.73 (63.65), H 4.42 (4.36), N 9.69 (9.64); IR (KBr, ν_{max}, cm^{-1}): 3430 (-OH, s), 3304 (-NH, m), 3069 (Ar-C-H, s), 1712 (-C=O, s), 1061 (-C-N, s), 1011 (-C-O); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.351 (s, 3H, Ar-CH_3), 3.87 (s, 3H, Ar-OCH_3), 5.91 (s, 1H, S-CH-N), 6.45-7.90 (m, 10H, Ar-H), 10.52 (bs, 1H, Ar-OH), 11.43 (bs, 1H, -NH); ^{13}C NMR (DMSO-d_6, 400 MHz) δ ppm: 24.9 (1C, Ar-CH_3), 56.6 (1C, Ar-OCH_3), 61.9 (1C, N-C-S of benzothiazinone), 109.6-160.1 (18C, aromatic carbons), 160.0 (1C, carbonyl of benzothiazinone), 164.9 (1C, O-C=N of benzoxazole); MS (LC-MS): *m/z* 433.11 (M^+).

Antimicrobial evaluation

Microorganisms: Different cultures of human pathogenic bacteria including Gram-positive bacteria namely *Bacillus subtilis* MTTC-2920, *Staphylococcus aureus* MTTC-5022, *Staphylococcus epidermidis* MTTC-12228 and Gram-negative bacteria namely *Escherichia coli* MTTC-5051, *Pseudomonas aeruginosa* MTTC-2945 were used for antibacterial screening. The fungal strains *viz. Aspergillus niger* MTTC-282, *Rhizopus oryzae* MTTC-9642, *Aspergillus flavus* MTTC-204304, *Candida albicans* MTTC-227 and *Saccharomyces cerevisiae* MTTC-9763 have been used for antifungal screening. The bacterial strains and fungal strains were repeatedly sub cultured on sterile nutrient agar media and potato dextrose agar (PDA) media to achieve the pure isolates. A loop full test organism was inoculated and incubated for 24 h at 37-38 ºC and utilized.

***in vitro* Antimicrobial activity:** The *in vitro* antimicrobial growth inhibition activity was carried for the newly synthesized compounds **6a-n** using agar well diffusion method [28]. Tetracycline and fluconazole were used as positive reference standards for the comparative evaluation of antibacterial activity and antifungal activity. Two-fold serial dilution method was employed to determine MIC [29] at the concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/mL.

Antioxidant activity by DPPH radical scavenging method: Free radical scavenging activity of the target moieties was analyzed by the reported diphenyl picryl hydrazyl (DPPH) assay [30,31]. Ascorbic acid was used as standard and methanolic DPPH solution (1 mL, 0.0003 mol) was used as control. The percentage scavenging activity was calculated by the following formula:

$$\text{Scavenging activity (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Molecular docking study

Ligand preparation: The structures of the newly synthesized molecules (ligands) were drawn in Chemdraw 11.0 (saved as mol files) [32] and the energies were minimized using Accelrys Discovery Studio (ADS). The minimized ligands were saved in (.sd) format for docking study.

Protein selection: The X-ray crystallographic structures of *S. aureus* Gyrase complex (PDB ID 2XCT) [33], *Candida albicans N*-myristoyltransferase (PDB ID 1IYL) [34] and Tyrosine Kinase (PDB ID 2HCK) [35] were repossessed from pdb data-base and saved as pdb format for further studies.

Docking study: Accelyrs Discovery Studio client version 3.5 software (Accelyrs Inc., http://www.accelrys.com) has been used to perform the molecular docking studies [36]. The X-ray crystallographic structures of all target proteins (PDB ID 2XCT-antibacterial, PDB ID 1IYL-antifungal and PDB ID 2HCK-antioxidant) bound with other inhibitors were attained from the protein data bank (PDB) at a resolution given in Table-1. The active site was characterized with a radius of 20 Å around the bound inhibitor which contained almost all the active site amino acids of the target protein molecule. Small molecules (ligands) were docked into the protein active site by a grid-based molecular docking method. The structure refinement [37] of designed molecular structures was carried by submitting them in CHARMm (Chemistry at HARvard Macromolecular Mechanics) force field. The water molecules, other hetero atoms and bound inhibitors were uninvolved from the macromolecule, polar hydrogen atoms have been supplemented. CHARMm force field was used to minimize the energy of all the compounds to get stable conformation of protein with the energy gradient of 0.01 kcal/mol/Å [38]. The last minimization of the ligand in the rigid receptor was performed using non-softened potential. The interaction energy and CHARMm energy (interaction energy with ligand strain) were calculated for every single pose. The poses had sorted by CHARMm energy and the top scoring poses (most negative implyinig favorable to binding) [39].

TABLE-1
PROTEIN RESOLUTION AND ITS STABLE CONFORMATIONAL ENERGY

PDB ID	Description	Resolution (Å)	Initial potential energy (kcal/mol)	Final potential energy (kcal/mol)
2XCT	The twinned 3.35A structure of *S. aureus* Gyrase complex with Ciprofloxacin and DNA	3.35	-15518.64477	-34205.18300
1IYL	Crystal Structure of *Candida albicans* N-myristoyltransferase with Non-peptidic Inhibitor	3.20	-13685.88737	-35362.55841
2HCK	SRC FAMILY KINASE HCK-QUERCETIN COMPLEX	3.00	-6158.16093	-30887.78720

The individual proteins (whose energy minimized) and the designed molecular structures with the binding site sphere radius were submitted to the docking software Lead IT job parameter. The lowest docking energy docked conformation was selected for analyzing the actual mode of binding pattern. The Discovery studio 4.0 visualizer was used to visualize the hydrogen bond, docking energy score and VDW interactions.

RESULTS AND DISCUSSION

The target compounds were synthesized *via* two-step strategy. Firstly, synthesis of 2-hydrazino-5-methyl-1,3-benzoxazole (**4**) was attained by a typical reaction of hydrazine hydrate with ethyl-2-(5-methylbenzo[*d*]oxazol-2-ylthio)acetate (**3**) . The reaction of ester derivative of thiols with hydrazine hydrate produce thioacetyl hydrazides [26] and formation of hydrazine derivative has been ensured by the rearrangement of thioacetyl hydrazides in a mild alkali [27]. It has been foreseen that the carbon linked to sulphur of ethyl-2-(5-methyl-benzo[*d*]oxazol-2-ylthio)acetate is electrophilic and hydrazine hydrate possibly will react at C-2 position and ejecting ethyl mercaptoacetate leading to the formation of compound **4** .

In the second step, Schiff's reaction was carried out by condensing hydrazino-5-methyl-1,3-benzoxazole (**4**) with differently substituted aromatic aldehydes in presence of the catalytic amount of acetic acid to accomplish the corresponding substituted benzylidene-1-(5-methylbenzo[*d*]oxazol-2-yl)hydrazine Schiff bases (**5a-n**), respectively. The azomethine group of compounds **5a-n** is the reacting site, at which the cyclization has been carried with thiosalicylic acid in the presence of anhydrous $ZnCl_2$ as catalyst to yield the analogous target molecules 3-(5-methylbenzo[*d*]oxazol-2-ylamino)-2,3-dihydro-2-phenylbenzo[*e*][1,3]thiazin-4-ones (**6a-n**). It has been assumed that anhydrous $ZnCl_2$ coordinates with azomethine group of Schiff's base (**5a-n**) and facilitates the cyclization in presence of thiosalicylic acid to benzothiazine-4-one derivatives (**6a-n**).

Structure of newly synthesized compounds was confirmed by FT-IR, ^{1}H & ^{13}C NMR, LC-MS and elemental analysis. The ^{1}H NMR analysis of compound **4** showed the presence of two broad singlets (D_2O exchangeable) at 4.602 ppm for two hydrogens of -NH_2 and 9.420 ppm for one hydrogen of -NH as well as the absence of singlet peak at 4.102 ppm for two hydrogens of -S-CH_2 which was present in the ^{1}H NMR spectrum of compound **3** has confirmed the formation of assigned molecule **4**. This was further supported by recording ^{13}C NMR spectrum and the signals appeared in the spectrum accounts for all the C-atoms present in compound **4**. In compounds **5a-n**, the observed elemental analysis data were altogether alike with the calculated elemental analysis. The IR peak position at 1670-1660 cm^{-1} in the spectra of compounds **5a-n** is assigned as the azomethine (-N=C-) band, in addition to this -NH- peak position of compound **4** showed red shift due to the formation of azomethine in compounds **5a-n**. The ^{1}H NMR spectrum of the compounds **5a-n** showed two singlets around 8.367 ppm and 11.699 ppm corresponding to the 1H of -N=C-H and 1H of N-H, respectively. The IR spectra of the compounds **6a-n** exhibited two bands at 1658 cm^{-1} for ν(C=O) and 1526 cm^{-1} for ν(N-O). The ^{1}H NMR spectra of the synthesized target compounds **6a-n** showed disappearance of the singlet at 8.367 ppm for -N=C-H peak, and instead appearance of singlet at 5.851 ppm for C-H proton adjacent to the nitrogen of benzothiazine-4-one ring supports the formation of target moieties. In addition to this, four aromatic protons of benzothiazine-4-one has appeared with their respective peak positions. Corresponding to the IR and ^{1}H NMR data, the actual structures of compounds **5a-n** and **6a-n** has been accomplished by their ^{13}C NMR and LC-MS analysis.

***in vitro* Antimicrobial activity:** The antibacterial screening was performed against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* (Gram-positive) and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative) pathogenic bacteria. The antifungal screening was performed using five fungal strains of *R. oryzae*, *A. niger*, *A. flavus*, *C. albicans*, and *S. cerevisiae*. To appraise the antimicrobial activity, the primary antimicrobial screening of compounds **6a-n** displayed considerable zone of growth inhibition at 100 µg/mL, the results of antibacterial and antifungal activity are given in Tables 2 and 3. Most of the synthesized compounds showed remarkable inhibition property against the strains used. Among the test samples, compounds **6b**, **6c**, **6e**, **6j**, **6m** and **6n** exposed comparatively phenomenal growth inhibition.

It was noticed that the R groups of the compounds with good inhibition activity are electron withdrawing and electron donating such as -NO_2, -Br, -NMe_2, -OH, -OMe and the compounds with the electron donating groups showed slightly less inhibition. The presence of electron withdrawing groups made target molecules more active along with the presence of potent core benzoxazole and benzothiazine ring structures. Based on the primary antimicrobial evaluation results of the target molecules, the minimum inhibition concentration (MIC) of the all the target compounds **6a-n** have been carried out. The compounds showed **6b**, **6c**, **6e**, **6j**, **6m** and **6n** have showed remarkable low MIC values for both bacterial and fungal organisms in comparison with standard drugs. The MIC results are tabulated in Table-4.

Antioxidant activity: All the newly synthesized compounds **6a-n** were assayed for free radical scavenging activity by DPPH method. Almost all the test compounds displayed significant free radical scavenging capacity in comparison with the standard ascorbic acid. In this series of synthesized compounds, **6b**, **6c**, **6e**, **6f**, **6k**, **6l**, **6m** and **6n** were found to hold maximum antioxidant property against standard ascorbic acid. The difference exhibited in DPPH scavenging capacity might be attributed due to the effect of different substitutions (Table-5). The inhibitory concentration (IC_{50}) values representing the concentration required to exhibit 50% antioxidant activity and have been shown in Table-5. The IC_{50} values of the newly synthesized compounds were derived from the linear regression plots of concentration versus % scavenging activity and are as nearly less as standard ascorbic acid indicating high antioxidant activity.

Molecular docking study: The results of docking protocol run have furnished the fundamental information concerned to the appropriate orientation of the compounds with in the active site of protein. In this study, the active site was defined

TABLE-2
in vitro ANTIBACTERIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **6a-n**

Compounds	Concentration (μg/mL)	Mean ± SD of zone of inhibition in mm at 100 μg/mL				
		Gram-positive organisms			Gram-negative organisms	
		B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*
6a	100	27.66 ± 0.12	28.30 ± 0.26	26.50 ± 0.26	22.20 ± 0.20	24.93 ± 0.11
6b	100	24.90 ± 0.17	27.16 ± 0.15	25.06 ± 0.15	24.20 ± 0.10	24.23 ± 0.25
6c	100	22.50 ± 0.24	22.93 ± 0.15	18.80 ± 0.26	19.73 ± 0.40	22.26 ± 0.20
6d	100	21.83 ± 0.26	24.43 ± 0.15	20.63 ± 0.15	21.43 ± 0.15	20.36 ± 0.47
6e	100	27.33 ± 0.28	28.46 ± 0.16	24.36 ± 0.37	22.36 ± 0.25	25.13 ± 0.32
6f	100	22.06 ± 0.11	21.36 ± 0.15	23.10 ± 0.10	17.13 ± 0.15	18.96 ± 0.06
6g	100	20.03 ± 0.15	21.10 ± 0.10	21.16 ± 0.15	21.03 ± 0.15	21.43 ± 0.51
6h	100	22.10 ± 0.16	25.20 ± 0.10	23.86 ± 0.35	20.66 ± 0.20	20.26 ± 0.25
6i	100	19.03 ± 0.15	22.96 ± 0.15	17.93 ± 0.15	17.23 ± 0.05	20.36 ± 0.15
6j	100	26.91 ± 0.26	27.66 ± 0.15	24.73 ± 0.25	23.86 ± 0.06	25.70 ± 0.17
6k	100	27.01 ± 0.24	26.90 ± 0.10	23.26 ± 0.20	22.50 ± 0.36	24.10 ± 0.30
6l	100	23.46 ± 0.15	25.16 ± 0.30	22.63 ± 0.15	22.40 ± 0.10	23.20 ± 0.26
6m	100	27.03 ± 0.20	27.33 ± 0.15	24.10 ± 0.36	22.90 ± 0.12	24.10 ± 0.28
6n	100	26.13 ± 0.21	27.63 ± 0.23	23.56 ± 0.20	22.70 ± 0.20	22.76 ± 0.23
DMSO	99.9%	–	–	–	–	–
Tetracycline	100	27.21 ± 0.14	28.55 ± 0.51	25.83 ± 0.20	24.56 ± 0.20	26.53 ± 0.15

TABLE-3
in vitro ANTIFUNGAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **6a-n**

Compounds	Concentration (μg/mL)	Mean ± SD of zone of inhibition in mm at 100 μg/mL				
		R. oryzae	*A. niger*	*A. flavus*	*C. albicans*	*S. cerevisiae*
6a	100	23.83 ± 0.28	25.16 ± 0.40	21.16 ± 0.16	18.11 ± 0.64	21.13 ± 0.73
6b	100	22.66 ± 0.58	23.86 ± 0.50	21.66 ± 0.52	21.66 ± 0.52	18.33 ± 0.21
6c	100	19.30 ± 0.60	19.66 ± 0.66	17.33 ± 0.52	16.33 ± 0.42	17.33 ± 0.15
6d	100	20.43 ± 0.60	21.56 ± 0.55	18.33 ± 0.53	16.66 ± 0.51	15.33 ± 0.52
6e	100	23.46 ± 0.55	25.16 ± 0.25	22.33 ± 0.26	19.20 ± 0.20	24.33 ± 0.51
6f	100	18.33 ± 0.57	19.66 ± 0.57	19.10 ± 0.34	16.33 ± 0.26	15.66 ± 0.57
6g	100	17.36 ± 0.60	17.16 ± 0.76	18.33 ± 0.57	18.33 ± 0.57	17.10 ± 0.46
6h	100	18.83 ± 0.76	21.03 ± 0.12	20.66 ± 0.52	18.12 ± 0.73	17.33 ± 0.15
6i	100	16.53 ± 0.56	16.66 ± 0.28	15.66 ± 0.76	13.08 ± 0.90	16.66 ± 0.15
6j	100	23.10 ± 0.10	19.86 ± 0.36	22.50 ± 0.50	18.66 ± 0.52	21.83 ± 0.51
6k	100	20.53 ± 0.50	21.83 ± 0.46	21.16 ± 0.25	17.33 ± 0.08	16.00 ± 0.73
6l	100	20.26 ± 0.64	18.36 ± 0.55	20.10 ± 0.10	18.33 ± 0.18	18.33 ± 0.57
6m	100	21.46 ± 0.50	22.70 ± 0.60	23.50 ± 0.32	19.33 ± 0.52	18.66 ± 0.51
6n	100	22.66 ± 0.57	23.40 ± 0.25	22.66 ± 0.83	18.66 ± 0.52	14.33 ± 0.57
DMSO	99.9%	–	–	–	–	–
Fluconazole	100	23.16 ± 0.76	24.66 ± 1.25	22.0 ± 1.0	20.33 ± 0.52	21.33 ± 0.52

TABLE-4
in vitro ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS **6a-n** (MIC, μg/mL)

Compounds	MIC (μg/mL) of bacteria and fungi									
	Gram-positive bacteria			Gram-negative bacteria		Fungi				
	B. subtilis	*S. aureus*	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*R. oryzae*	*A. niger*	*A. flavus*	*C. albicans*	*S. cerevisiae*
6a	6.25	6.25	6.25	6.25	6.25	12.5	6.25	3.125	3.125	6.25
6b	12.5	6.25	6.25	6.25	6.25	12.5	6.25	3.125	3.125	6.25
6c	12.5	6.25	12.5	25	25	25	12.5	25	12.5	12.5
6d	12.5	6.25	6.25	25	25	25	25	12.5	12.5	50
6e	3.125	1.56	3.125	6.25	12.5	6.25	3.125	6.125	6.25	3.125
6f	25	12.5	25	50	25	25	50	25	25	50
6g	25	12.5	12.5	12.5	25	25	50	25	25	50
6h	25	25	25	25	50	12.5	25	50	12.5	12.5
6i	25	25	25	12.5	12.5	25	12.5	50	12.5	12.5
6j	12.5	12.5	6.25	12.5	25	12.5	6.25	12.5	6.125	12.5
6k	12.5	25	25	25	25	12.5	25	12.5	12.5	12.5
6l	12.5	25	12.5	25	12.5	12.5	25	12.5	12.5	12.5
6m	3.125	3.125	3.125	3.125	12.5	6.25	3.125	6.25	6.125	3.125
6n	3.125	3.125	3.125	3.125	12.5	6.25	3.125	6.25	6.125	3.125
Tetracycline	1.56	1.56	3.125	3.125	50	–	–	–	–	–
Fluconazole	–	–	–	–	–	6.25	6.25	6.25	3.125	3.125

TABLE-5
DPPH RADICAL SCAVENGING ACTIVITY OF SYNTHESIZED COMPOUNDS **6a-n**

Entry	% Scavenging activity at different concentrations (µg/mL)					IC_{50} (µg/mL)
	25	50	100	250	500	
6a	13.16 ± 0.22	25.32 ± 0.32	53.85 ± 0.06	87.06 ± 0.42	98.34 ± 0.37	152.74
6b	12.81 ± 0.02	24.92 ± 0.17	51.62 ± 0.77	88.42 ± 0.04	99.06 ± 0.05	154.48
6c	13.91 ± 0.52	24.10 ± 0.51	57.81 ± 0.42	86.70 ± 0.08	97.43 ± 0.72	149.26
6d	10.71 ± 0.13	22.03 ± 0.19	47.23 ± 0.82	80.42 ± 0.25	86.81 ± 0.31	188.62
6e	13.36 ± 0.21	24.82 ± 0.20	51.02 ± 0.16	85.43 ± 0.38	98.08 ± 0.08	157.98
6f	9.26 ± 0.72	20.16 ± 0.58	45.29 ± 0.24	78.52 ± 0.61	88.61 ± 0.63	195.06
6g	11.91 ± 0.91	21.02 ± 0.74	48.67 ± 0.37	80.31 ± 0.27	89.79 ± 0.42	182.84
6h	10.13 ± 0.84	20.62 ± 0.76	44.03 ± 0.18	79.47 ± 0.17	92.05 ± 0.33	189.37
6i	11.56 ± 0.22	19.32 ± 0.32	46.85 ± 0.06	76.06 ± 0.42	88.24 ± 0.37	195.10
6j	12.41 ± 0.02	22.72 ± 0.17	44.62 ± 0.77	74.62 ± 0.04	91.06 ± 0.05	190.71
6k	13.92 ± 0.91	26.02 ± 0.74	55.57 ± 0.37	87.41 ± 0.27	97.79 ± 0.42	149.15
6l	12.97 ± 0.52	25.10 ± 0.51	52.81 ± 0.42	85.70 ± 0.98	96.43 ± 0.72	157.71
6m	14.51 ± 0.13	26.03 ± 0.19	53.23 ± 0.82	86.52 ± 0.25	98.51 ± 0.31	151.15
6n	13.61 ± 0.91	27.02 ± 0.74	54.37 ± 0.37	88.31 ± 0.27	97.89 ± 0.42	150.27
Ascorbic acid	13.40 ± 0.05	25.64 ± 0.06	52.50 ± 0.15	86.12 ± 0.51	96.33 ± 0.08	156.32

Each value represents mean ± SE; n = 3.

on the basis of bound inhibitor in the crystal structure of respective proteins. The best hit compound was selected by considering the significant criteria such as binding modes, good molecular interactions with the active site components and fitness scores. The compounds were docked with DNA Gyrase bacterial protein. The docking scores and interacting amino acids were depicted in Table-6. Substituted benzoxazole compounds **6a-n** were shown strong non-bonding interaction towards 2XCT protein. It is observed that compounds showing good binding energy in the range -24.469 to -30.525 kcal/mol with favorable binding pose as depicted in Fig. 1. The amino acid residue Glu109 from 2XCT protein showing hydrogen bonding with compounds **6a**, **6b** and **6m**. Apart from this the residues Tyr225, Asp412 and Val449 are involved in hydrogen bonding with compounds **6j** and **6k**. Among the reported compounds **6b** and **6j** exhibit strong interaction with active site amino acids with binding energy of -30.525 and -29.255 kcal/mol respectively towards 2XCT bacterial protein.

TABLE-6
INTERACTION ENERGY VALUES OF SYNTHESIZED COMPOUNDS (**6a-n**) WITH TARGET PROTEIN 2XCT

Compound	Docking energy	Ligand efficiency	RMS	No. of interactions	Interaction residues			No. of H bonds	Bond length (Å)
					Pi	Pi-Pi	H bonding		
6a	-28.577	-1.07	0.00968	7	Phe414	Val108, Leu415	Glu109	1	2.96922
6b	-30.525	-1.0	0.0096	06	Val108, Leu415	His227	Glu109	1	2.96922
6c	-24.469	-0.68	0.00999	13	Phe117, Leu394, Leu415	Phe240	His227, Gly413	2	2.3501 2.20162
6d	-26.029	-0.70	0.00872	10	Phe117, Leu394, Leu415, Val108, Leu415	Phe240	His227, Gly413, Asp412	3	2.01056 2.98679 2.25217
6e	-25.769	-0.69	0.0089	17	Phe240, Tyr354, Leu352, Val108	Phe115	Glu109, Asp110	2	2.02694 2.19763
6f	-26.906	-0.73	0.00998	16	Phe117, Leu415, Val108	Phe240	His227, Gly413	2	2.00744 2.17146
6g	-28.585	-0.63	0.00996	13	Leu415, Val108, Leu415	Phe115, Phe240	His227, Gly413, Asp412	3	3.98225 2.24777 2.02637
6h	-24.836	-0.66	0.00944	11	Phe408, Leu394, Leu415	Tyr225	Asp412	1	2.1987
6i	-24.971	-0.62	0.00994	12	Phe339, Tyr354, Leu350, Val108	Phe115	Glu109, Asp110	2	2.79366 2.40686
6j	-29.255	-0.58	0.00946	12	Cys223, Arg224, Leu415	Phe240, Tyr225	Tyr225, Asp412	2	1.69198 2.8167
6k	-26.889	-0.71	0.00844	9	Phe117, Phe339	-	Val449	1	2.01056
6l	-25.839	-0.46	0.00784	7	Val108, Ile111	Phe117	Glu109, Asp412	2	1.69203 2.25217
6m	-27.057	-0.49	0.00989	14	Val108, Leu415	-	Glu109	1	2.19763
6n	-25.235	-0.93	0.00866	12	Phe117, Phe240, Leu415	His227, Tyr225	–	–	–

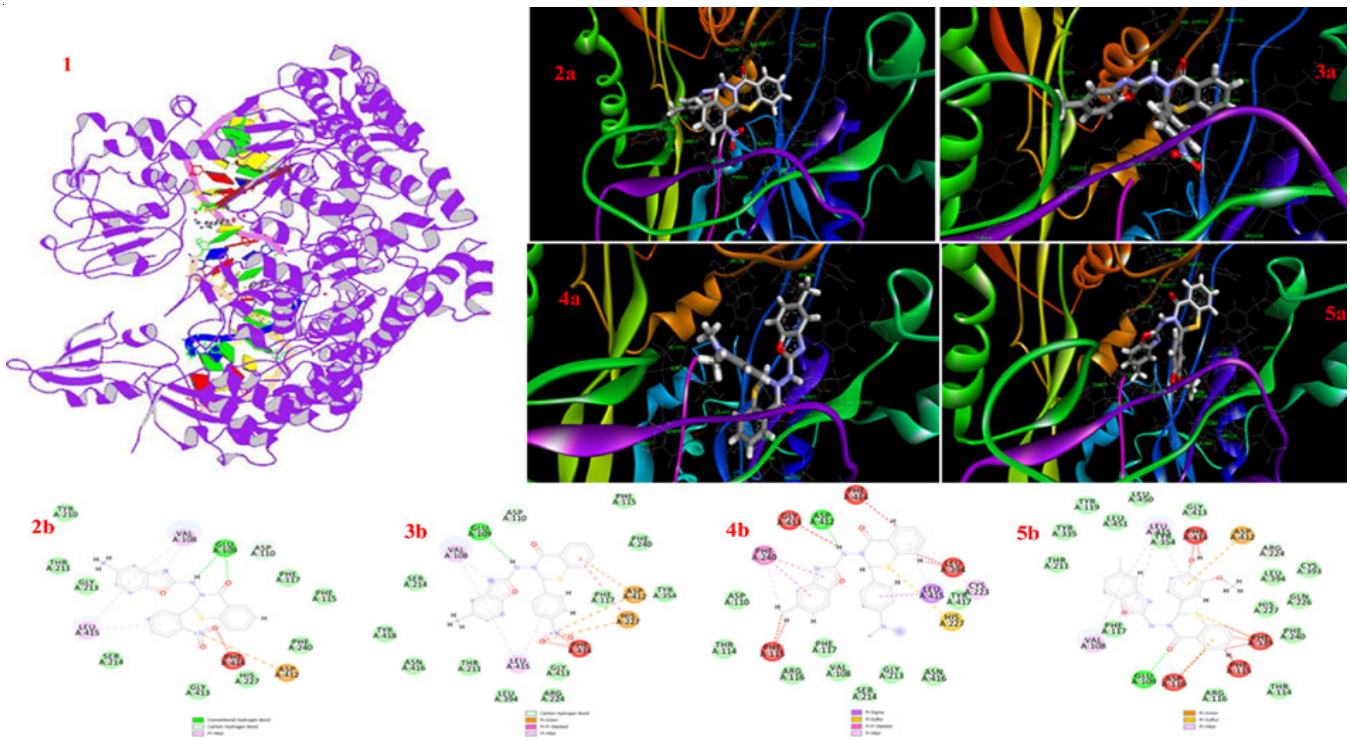


Fig. 1. Molecular docking on 2XCT (antibacterial) target protein, **1**) Crystal structure of 2XCT protein, **2a, 3a, 4a, 5a & 2b, 3b, 4b, 5b**) 3D and 2D view of binding pattern of compounds **6a**, **6b**, **6j**, **6m** with 2XCTprotein

The docking between fungal protein N-myristyltransferase 1IYL with compounds has been studied, the docking scores and interacting amino acids are depicted in Table-7. Few substituted benzoxazole compounds among the series **6a-n** showed significant docking energy with hydrogen bonding (Fig. 2). Compounds **6a**, **6e**, **6j**, **6m** and **6n** showed good binding energy in the range -8.109 to -16.223 kcal/mol. Compounds showing varying degree of hydrogen bonding with amino acid residue Glu109, His227, Gly413, Asp412 and Tyr225. Among the reported compounds, **6e**, **6j** and **6m** exhibit strong interaction

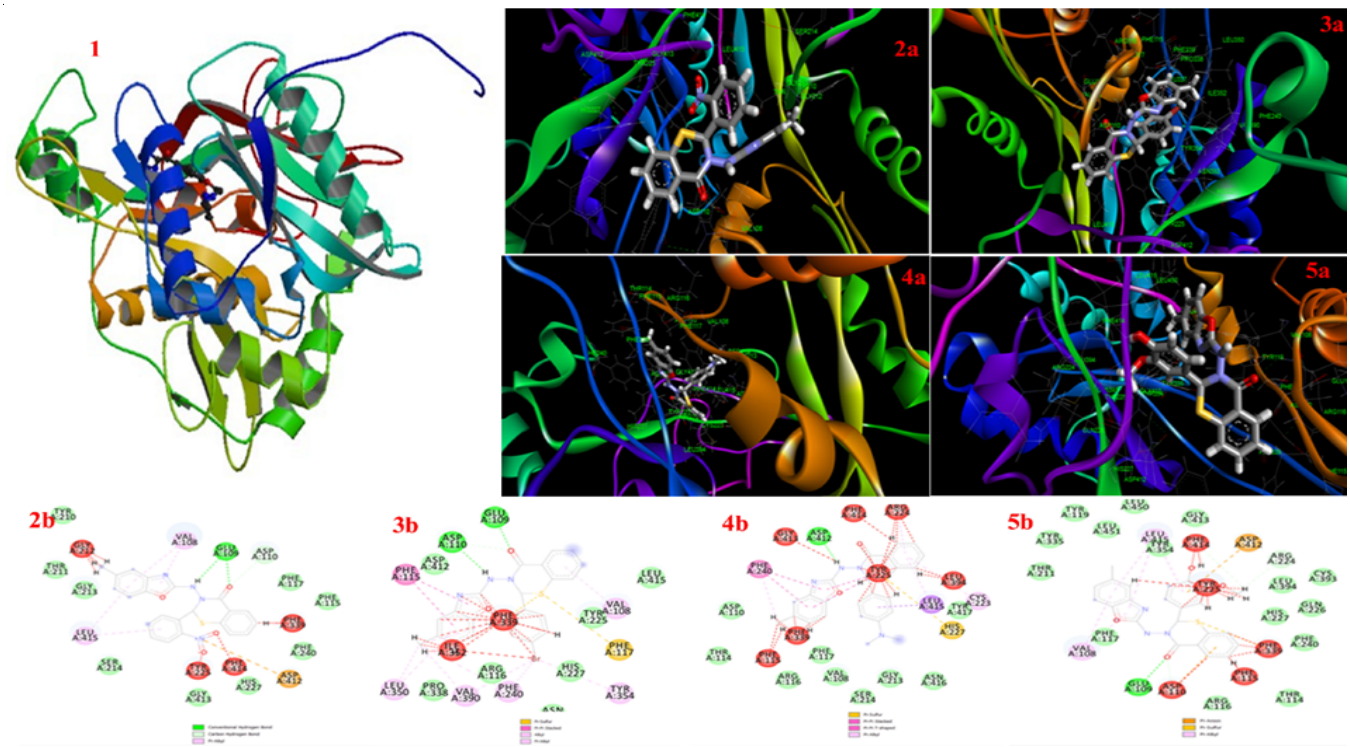


Fig. 2. Molecular docking on 1IYL (antifungal) target protein, **1**) Crystal structure of 1IYL protein, **2a, 3a, 4a, 5a & 2b, 3b, 4b, 5b**) 3D and 2D view of binding pattern of compounds **6a**, **6e**, **6j**, **6m** with 1IYL protein

TABLE-7
INTERACTION ENERGY VALUES OF SYNTHESIZED COMPOUNDS (**6a-n**) WITH TARGET PROTEIN 1IYL

Compound	Docking energy	Ligand efficiency	RMS	No. of interactions	Interaction residues			No. of H bonds	Bond length (Å)
					Pi	Pi-Pi	H bonding		
6a	-14.371	-1.07	0.00968	9	Val108, Leu415	-	Glu109	2	2.10206 2.53568
6b	-9.318	-1.0	0.0096	10	Val108, Leu415	His227	Glu109	1	2.56749
6c	-10.023	-0.68	0.00999	11	Phe117, Leu394, Leu415, Val108, Leu415	Phe240	His227, Gly413, Asp412	3	2.79504 2.40261 1.69203
6d	-9.862	-0.70	0.00872	12	Phe117, Leu394, Leu415, Val108, Leu415	Phe240	Gly413, Asp412	3	2.79366 2.40686 1.69198
6e	-16.223	-0.69	0.0089	17	Phe240, Phe339, Tyr354, Leu352, Val108	Phe115	Glu109, Asp110	2	2.05499 2.05715
6f	-12.567	-0.73	0.00998	11	Phe117, Leu394, Leu415, Val108	Phe240	His227, Gly413, Asp412	3	2.77183 2.51542 1.73406
6g	-8.109	-0.63	0.00996	11	Phe117, Leu415, Val108, Leu415	Phe115, Phe240	His227, Asp412	3	2.90616 2.06167 1.92042
6h	-12.21	-0.66	0.00944	15	Phe408, Leu394, Val108, Leu415	Tyr225, Phe339	Asp412	1	2.21861
6i	-8.93	-0.62	0.00994	18	Phe240, Phe339, Tyr354, Leu350, ILeu352, Val108	Phe115	Glu109, Asp110	2	2.05852 2.0695
6j	-15.928	-0.58	0.00946	12	Phe240, Cys223, Arg224, Leu415	Phe240, Tyr225	Tyr225, Asp412	2	1.85956 1.66715
6k	-6.628	-0.71	0.00844	7	Phe117, Phe339, Val449, Leu415	-	-	-	-
6l	-8.312	-0.46	0.00784	9	Val108, Ile111	Phe117	Glu109, Asp412	3	1.68741 1.95182 1.9035
6m	-15.662	-0.49	0.00989	12	Val108, Leu415	-	Glu109	1	2.17711
6n	-13.739	-0.93	0.00866	12	Phe117, Phe240, Leu415, Arg224	His227, Tyr225	-	-	-

with active site amino acids with binding energy of -16.223, -15.928 and -15.662 kcal/mol, respectively towards 1IYL fungal protein.

Tyrosine kinase (pdb id: 2HCK) was recognized as protein target for present antioxidant studies. Pdb file 2HCK was obtained from the protein data bank (rscb.org). The binding interactions of the actively docked conformations of the ligands and the target protein have been identified and marked. The identified interactions in all compounds include the hydrophobic interactions as well as hydrogen bonding. The binding interactions of all ligands have been analyzed and eight compounds were recognized as the most active among the set of given ligands **6a-e** and **6k-n**. These ligands showed strong hydrogen bonding and hydrophobic interactions with the target protein in which the binding energies ranging from -15.998 to -22.006 kcal/mol as shown in Table-8. The interactions shown in Fig. 3 focuses the main amino acids of the target protein pocket and the atoms in the ligand.

ADMET descriptors: Most of the failure of drugs during clinical trials was due to its deprived pharmacokinetic and toxicity properties. Hence, for the selection of successful drug moieties it is crucial to predict the ADMET properties before carrying expensive experimental procedures. In this work, *in silico* ADMET studies have been carried out by means of ADMET descriptors algorithm of Accelrys Discovery Studio (ADS). This protocol follows the six pharmacokinetic parameters namely aqueous solubility level, Blood-Brain-Barrier (BBB) penetration, hepatotoxicity levels, cytochrome P450 2D6 inhibition and plasma protein binding (PPB) in order to predict the molecular properties of selected ligands quantitatively. The ADMET data are given in Table-9.

Lipinski's rule of five factors: The pharmacokinetic properties predictions of compounds **6a-n** specified that all the compounds are endowed with the drug like properties. The molecular weight of compounds ranges from 387 to 466 a.m.u. The number of hydrogen bond donor is one for compounds **6a-j** and two for **6k-n** whereas the hydrogen bond acceptor values vary from 5 to 8. The results revealed that there is one rule violation in agreement with the rule of five *i.e.*, the partition coefficient values of all compounds are more than five (Table-10).

Conclusion

In this work, the synthesis and characterization of benzoxazole associated benzothiazine-4-one derivatives were reported. All the newly synthesized compounds were subjected to *in vitro* antimicrobial, antioxidant and molecular docking studies. The compounds showed remarkable antimicrobial and antioxidant

TABLE-8
INTERACTION ENERGY VALUES OF SYNTHESIZED COMPOUNDS (**6a-n**) WITH TARGET PROTEIN 2HCK

Compound	Docking energy	Ligand efficiency	RMS	No. of inter-actions	Interaction residues			No. of H bonds	Bond length (Å)
					Pi	Pi-Pi	H bonding		
6a	-17.012	-1.07	0.00968	10	Lys203	His201, His202, Gln528, Gln529	PTR527	1	2.39894
6b	-22.006	-1.0	0.0096	21	Tyr202, Lys203	-	Arg175, Ser177, Glu178, Thr179, Thr180, Gln528	6	2.60157, 2.88621, 2.31127, 2.14819, 2.79567, 2.91067
6c	-16.012	-0.68	0.00999	11	Arg155	-	Arg175, Glu178, Ser185	3	2.33305, 2.68952, 2.26613
6d	-11.09	-0.70	0.00872	7	Lys203, Arg205	Lys203	PTR527, Gln528	2	2.62877, 1.89993
6e	-16.207	-0.69	0.0089	11	PTR527, Arg155, Lys203	PTR527	Arg205, Gln526	2	1.96362, 1.87645
6f	-8.223	-0.73	0.00998		Arg155, Lys203,	-	Gln526	1	1.90038
6g	-15.932	-0.63	0.00996	6	Arg155	-	Ser185	1	2.11486
6h	-8.949	-0.66	0.00944	12	Lys203, Arg155	-	Thr179, Gln528	2	2.5683, 2.99713
6i	-5.491	-0.62	0.00994	8	PTR527, Arg155, Lys203	PTR527	Gln526	1	1.87645
6j	-1.594	-0.58	0.00946	6	Glu178, PTR527, Arg155	-	Gln526	1	2.20117
6k	-16.462	-0.71	0.00844	3	Lys203, PTR527	-	Gln528	1	2.11984
6l	-17.174	-0.46	0.00784	11	Arg155, Lys203, Arg155, Asp518	-	Thr179, PTR527	2	1.94924, 2.96467
6m	-16.815	-0.49	0.00989	12	Gln526, Lys203, PTR527, Glu524	-	Arg175, Ser185	3	2.02001, 2.34445, 1.96099
6n	-15.998	-0.93	0.00866	15	PTR527, Lys203, Arg155, Asp518	-	Arg155, Lys203, Thr179, Gln526	4	2.04328, 2.37792, 1.89982, 1.92377

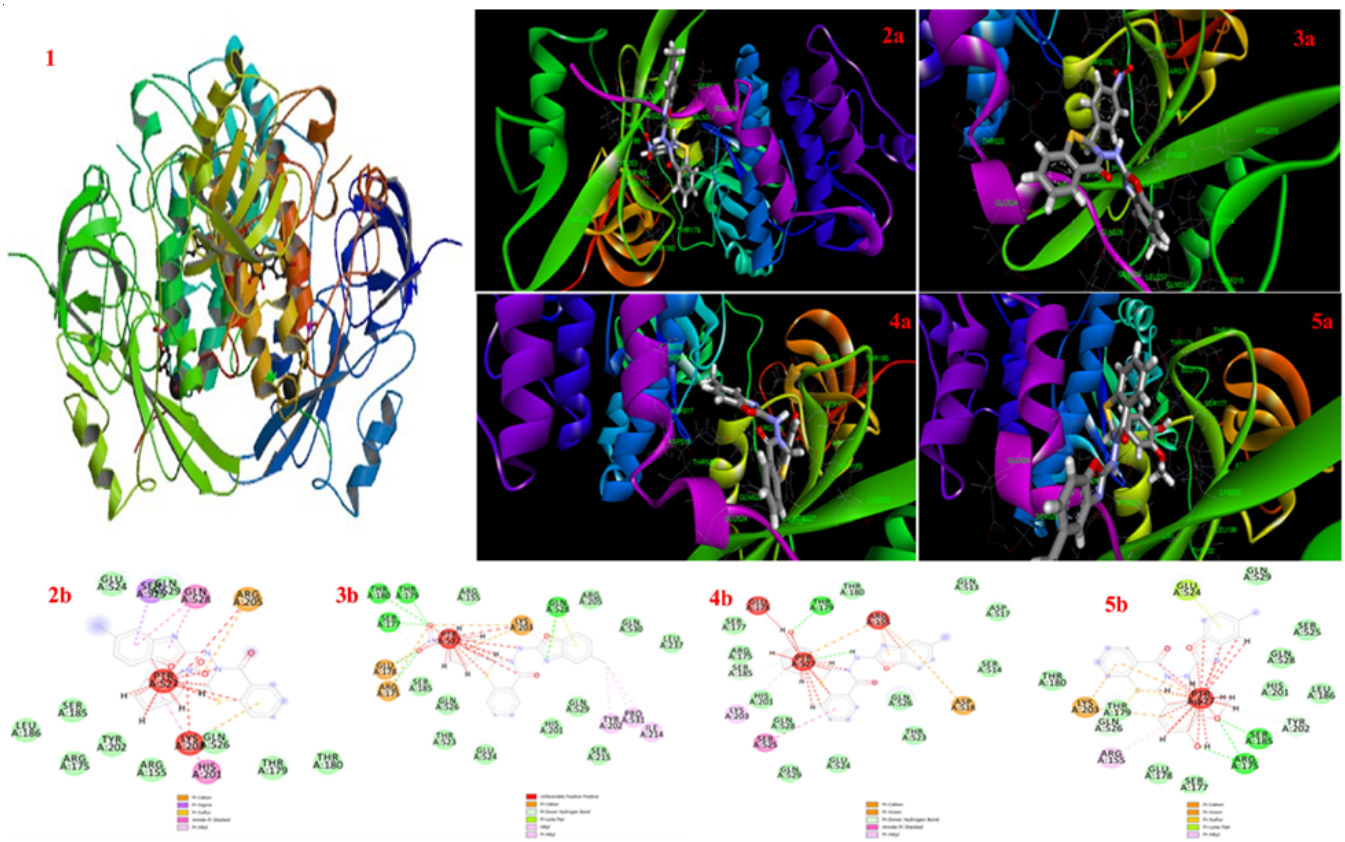


Fig. 3. Molecular docking on 2HCK (antioxidant) target protein, **1**) Crystal structure of 2HCK protein, **2a, 3a, 4a, 5a & 2b, 3b, 4b, 5b**) 3D and 2D view of binding pattern of compounds **6a**, **6b**, **6l**, **6m** with 2HCK protein

TABLE-9
PREDICTED ADMET PROPERTIES OF SYNTHESIZED COMPOUNDS **6a-n**

Compound	ADMET solubility level	ADMET BBB level	ADMET EXT CYP 2D6	ADMET EXT hepatotoxicity	ADMET EXT PPB	ADMET PSA-2D
6a	1	4	-5.74669	-0.0750535	7.7517	100.101
6b	1	4	-6.77948	3.59867	5.25475	100.101
6c	1	0	0.122935	4.229	8.24122	57.278
6d	1	4	0.0105519	1.55911	8.45104	57.278
6e	0	4	-2.74502	1.81844	4.43717	57.278
6f	1	1	-2.07017	-0.0685801	5.24571	57.278
6g	1	1	-4.58121	1.63781	5.4879	66.208
6h	1	1	-3.67586	1.34359	5.82922	66.208
6i	1	0	-1.88196	1.05422	6.05726	57.278
6j	1	1	-3.61782	3.60419	7.32416	60.631
6k	1	4	-3.22533	0.866357	6.1198	78.094
6l	1	4	-3.51946	1.7254	2.82356	78.094
6m	1	4	-4.98662	-0.670572	3.57414	87.024
6n	1	4	-4.0952	1.54752	5.80337	87.024

Note: ADMET solubility level: 0 - 2 highly soluble, ADMET BBB level: 2- medium penetration and 3 - Low penetration, ADMET EXT CYP 2D6: -ve - non-inhibitors & +ve –inhibition. ADMET EXT hepatotoxicity: 0-1: Non-toxic, ADMET EXT PPB: Greater the value greater the binding capacity. ADMET PSA-2D: Less than 200 are active

TABLE-10
LIPINSKI'S RULE OF FIVE FACTORS OF SYNTHESIZED COMPOUNDS **6a-n**

Compd.	m.w. (< 500)	Donor HB (< 5)	Accept HB (< 10)	Qp log Po/w (< 5)	Rule of five
6a	432.452	1	8	5.434	1
6b	432.452	1	8	5.434	1
6c	405.445	1	5	5.745	1
6d	421.899	1	5	6.204	1
6e	466.350	1	5	6.288	1
6f	387.454	1	5	5.539	1
6g	417.480	1	6	5.523	1
6h	417.480	1	6	5.523	1
6i	401.481	1	5	6.026	1
6j	430.522	1	6	5.702	1
6k	403.454	2	6	5.297	1
6l	403.454	2	6	5.297	1
6m	433.480	2	7	5.281	1
6n	433.480	2	7	5.281	1

activities in comparison to standard drugs used. The antioxidant IC_{50} values demonstrated the excellent antioxidant property. Docking of the analogues through Discovery studio docking suite has been performed so as to acquire the active conformations of the analogues. Based on the interactions, the analogues have been ranked for being the active ligands. The analogues in its docked conformations showed high number of binding interactions. Few compounds in the series **6a-n** emerged as 2XCT, 1IYL, 2HCK inhibitors. The data obtained from *in vitro* and *in silico* studies is compatible with one another and confirmed the potency of the compounds by ADMET properties prediction. This study highlighted that designed compounds are safe enough and potential to be considered as drug like molecules.

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CONFLICT OF INTEREST

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

REFERENCES

1. Y. Cetinkaya, P. Falk and C.G. Mayhall, *Clin. Microbiol. Rev.*, **13**, 686 (2000); https://doi.org/10.1128/cmr.13.4.686-707.2000
2. A.G. Atanasov, B. Waltenberger, E.M. Pferschy-Wenzig, T. Linder, C. Wawrosch, P. Uhrin, V. Temml, L. Wang, S. Schwaiger, E.H. Heiss, J.M. Rollinger, D. Schuster, J.M. Breuss, V. Bochkov, M.D. Mihovilovic, B. Kopp, R. Bauer, V.M. Dirsch and H. Stuppner, *Biotechnol. Adv.*, **33**, 1582 (2015); https://doi.org/10.1016/j.biotechadv.2015.08.001
3. D.M. Livermore, *Int. J. Antimicrob. Agents*, **16**, 3 (2000); https://doi.org/10.1016/S0924-8579(00)00299-5
4. N.C. Desai, H.V. Vaghani and P.N. Shihora, *J. Fluor. Chem.*, **153**, 39 (2013); https://doi.org/10.1016/j.jfluchem.2013.05.022
5. H. Müller, V. Gabrielli, O. Agoglitta and R. Holl, *Eur. J. Med. Chem.*, **110**, 340 (2016); https://doi.org/10.1016/j.ejmech.2016.01.032
6. R.V. Satyendra, K.A. Vishnumurthy, H.M. Vagdevi, B.L. Dhananjaya and A. Shruthi, *Med. Chem. Res.*, **24**, 1342 (2015); https://doi.org/10.1007/s00044-014-1207-6
7. N.D. Jayanna, H.M. Vagdevi, J.C. Dharshan, R. Raghavendra and S.B. Telkar, *Med. Chem. Res.*, **22**, 5814 (2013); https://doi.org/10.1007/s00044-013-0565-9
8. R.V. Satyendra, K.A. Vishnumurthy, H.M. Vagdevi, K.P. Rajesh, H. Manjunatha and A. Shruthi, *Eur. J. Med. Chem.*, **46**, 3078 (2011); https://doi.org/10.1016/j.ejmech.2011.03.017
9. T. Yuvaraj, P. Parameshwara Naik, T. Venkatesh, G. Krishnamurthy and T. Manjuraj, *J. Turk. Chem. Soc.*, **5A**, 845 (2018); https://doi.org/10.18596/jotcsa.341379
10. D.N. Ward, D.C. Talley, M. Tavag, S. Menji, P. Schaughency, A. Baier and P.J. Smith, *Bioorg. Med. Chem. Lett.*, **24**, 609 (2014); https://doi.org/10.1016/j.bmcl.2013.12.012
11. C.P. Kaushik and J. Sangwan, *Monatsh. Chem.*, **151**, 807 (2020); https://doi.org/10.1007/s00706-020-02604-7
12. H. Lage, E. Aki-Sener and I. Yalcin, *Int. J. Cancer*, **119**, 213 (2006); https://doi.org/10.1002/ijc.21792
13. O. Temiz-Arpaci, T. Coban, B. Tekiner-Gulbas, B. Can-Eke, I. Yildiz, E. Aki-Sener, I. Yalcin and M. Iscan, *Acta Biol. Hung.*, **57**, 201 (2006); https://doi.org/10.1556/ABiol.57.2006.2.7

14. Z. Zong, X. Wei, X. Yan and Y. Fan, *J. Mol. Struct.*, **1171**, 333 (2018); https://doi.org/10.1016/j.molstruc.2018.06.019
15. G. Khan, S. Sreenivasa, S. Govindaiah and V. Chandramohan, *Orient. J. Chem.*, **35**, 157 (2019); https://doi.org/10.13005/ojc/350117
16. S. Deshmukh, K. Dingore, V. Gaikwad and M. Jachak, *J. Chem. Sci.*, **128**, 1459 (2016); https://doi.org/10.1007/s12039-016-1141-x
17. G.M. Reddy, J.R. Garcia, V.H. Reddy, A.K. Kumari, G.V. Zyryanov and G. Yuvaraja, *J. Saudi Chem. Soc.*, **23**, 263 (2019); https://doi.org/10.1016/j.jscs.2018.07.003
18. A.R. Bhat, A. Tazeem, A. Azam, I. Choi and F. Athar, *Eur. J. Med. Chem.*, **46**, 3158 (2011); https://doi.org/10.1016/j.ejmech.2011.04.013
19. M.D. Naik and Y.D. Bodke, *Chem. Data Coll.*, **23**, 100261 (2019); https://doi.org/10.1016/j.cdc.2019.100261
20. R. Maheshwari, P. Chawla and S.A. Saraf, *Med. Chem. Res.*, **20**, 1650 (2011); https://doi.org/10.1007/s00044-010-9480-5
21. P.R. Shetty, G. Shivaraja, G. Krishnaswamy, K. Pruthviraj, V.C. Mohan and S. Sreenivasa, *Asian J. Chem.*, **32**, 1151 (2020); https://doi.org/10.14233/ajchem.2020.22583
22. C.T. Zeyrek, B. Boyacioglu, Ö. Temiz-Arpaci, H. Ünver and A. Elmali, *J. Mol. Struct.*, **1136**, 112 (2017); https://doi.org/10.1016/j.molstruc.2017.02.008
23. U.A. Ochsner, X. Sun, T. Jarvis, I. Critchley and N. Janjic, *Expert Opin. Investig. Drugs*, **16**, 573 (2007); https://doi.org/10.1517/13543784.16.5.573
24. B.D. Bax, P.F. Chan, D.S. Eggleston, A. Fosberry, D.R. Gentry, F. Gorrec, I. Giordano, M.M. Hann, A. Hennessy, M. Hibbs, J.Z. Huang, E. Jones, J. Jones, K.K. Brown, C.J. Lewis, E.W. May, M.R. Saunders, O. Singh, C.E. Spitzfaden, C. Shen, A. Shillings, A.J. Theobald, A. Wohlkonig, N.D. Pearson and M.N. Gwynn, *Nature*, **466**, 935 (2010); https://doi.org/10.1038/nature09197
25. D.J. Haydon, N.R. Stokes, R. Ure, G. Galbraith, J.M. Bennett, D.R. Brown, P.J. Baker, V.V. Barynin, D.W. Rice, S.E. Sedelnikova, J.R. Heal, J.M. Sheridan, S.T. Aiwale, P.K. Chauhan, A. Srivastava, A. Taneja, I. Collins, J. Errington and L.G. Czaplewski, *Science*, **321**, 1673 (2008); https://doi.org/10.1126/science.1159961
26. A. Hasan, N.F. Thomas and S. Gapil, *Molecules*, **16**, 1297 (2011); https://doi.org/10.3390/molecules16021297
27. C. Anbuselvan, *Asian J. Chem.*, **31**, 373 (2019); https://doi.org/10.14233/ajchem.2019.21654
28. N.R. Desai, K. Gurunathan, P.A. Suchetan, A.K.D. Basappa, S. Naveen, N.K. Lokanath and S. Sreenivasa, *Res. Chem. Intermed.*, **43**, 6131 (2017); https://doi.org/10.1007/s11164-017-2981-9
29. J.M. Madar, L.A. Shastri, S.L. Shastri, M. Holiyachi, N.S. Naik, F. Shaikh, V.M. Kumbar, K.G. Bhat, S.D. Joshi and V.A. Sungar, *ChemistrySelect*, **3**, 10738 (2018); https://doi.org/10.1002/slct.201802196
30. N. Kumar, S. Sreenivasa, B.S. Kalal, V. Kumar, B.S. Holla, V.R. Pai, N.R. Mohan and S. Govindaiah, *Lett. Drug Des. Discov.*, **16**, 994 (2019); https://doi.org/10.2174/1570180816666181220123924
31. B.K. Sarojini, B.G. Krishna, C.G. Darshanraj, B.R. Bharath and H. Manjunatha, *Eur. J. Med. Chem.*, **45**, 3490 (2010); https://doi.org/10.1016/j.ejmech.2010.03.039
32. K.S. Jithendra Kumara, G. Krishnamurthy, N. Sunil Kumar, N. Naik and T.M. Praveen, *J. Magn. Magn. Mater.*, **451**, 808 (2018); https://doi.org/10.1016/j.jmmm.2017.10.125
33. M.M. Patel and L.J. Patel, *Scientific World J.*, **2014**, 897187 (2014); https://doi.org/10.1155/2014/897187
34. Y.S. Mary, N.Z. Alzoman, V.V. Menon, E.S. Al-Abdullah, A.A. El-Emam, C.Y. Panicker, O. Temiz-Arpaci, S. Armakovic, S.J. Armakovic and C. Van Alsenoy, *J. Mol. Struct.*, **1128**, 694 (2017); https://doi.org/10.1016/j.molstruc.2016.09.024
35. A.H.M. Hussein, M.A.M. Gad-Elkareem, A.A.A.M. El-Adasy and I.M. Othman, *Phosphorus Sulfur Silicon Rel. Elem.*, **184**, 2263 (2009); https://doi.org/10.1080/10426500802453658
36. T. Manjuraj, G. Krishnamurthy, Y.D. Bodke and H.S. Bhojya Naik, *J. Mol. Struct.*, **1148**, 231 (2017); https://doi.org/10.1016/j.molstruc.2017.07.020
37. K.S. Jithendra Kumara, G. Krishnamurthy, B.E. Kumara Swamy, N.D. Shashi Kumar, S. Naik, B.S. Krishna and N. Naik, *Appl. Organomet. Chem.*, **31**, e3549 (2017); https://doi.org/10.1002/aoc.3549
38. D.I. Ugwu, U.C. Okoro, P.O. Ukoha, S. Okafor, A. Ibezim and N.M. Kumar, *Eur. J. Med. Chem.*, **135**, 349 (2017); https://doi.org/10.1016/j.ejmech.2017.04.029
39. N.R. Mohan, S. Sreenivasa, K.E. Manojkumar, T.M.C. Rao, B.S. Thippeswamy and P.A. Suchetan, *J. Braz. Chem. Soc.*, **25**, 1012 (2014); https://dx.doi.org/10.5935/0103-5053.20140073