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Synthesis, biological screening and docking studies of some indole derivatives as potential antioxidant

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Abstract: The present study envisioned some antioxidant candidates having 1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole (TRY), 3-(2-bromoethyl)indole (BEI) and 7-azindole (AI) nucleus. Derivatives of these indole molecules were synthesized and their scavenging activity for reactive oxygen species (ROS) investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. T3, T42 exhibited significant radical scavenging potential which is comparable to ascorbic acid (standard), while T36 appeared as most potent antioxidant by displaying better scavenging activity than standard molecule. Molecular docking study revealed good binding score and interactions of T36 with target human antioxidant enzyme (PDB code: 3MNG) validating the results of biological activity.

Keywords: 7-azaindole, 3-(2-bromoethyl)indole, tryptamine, 5-hydroxytryptamine, reactive oxygen species.

INTRODUCTION

Free radicals either reactive oxygen species (ROS) (e.g. superoxide, hydroxyl, alkoxyl, peroxyl and hydroperoxyl) or reactive nitrogen specie (RNS) (e.g. nitric oxide, nitrogen dioxide, per oxynitrite) causing oxidative stress and/ or nitrosative stress due to excessive free radical production and system's inability to eliminate them resulting in damage of membranes, proteins, RNA and DNA (Adwas *et al*., 2019, He *et al*., 2017), thus causing many diseases like cancer (Radwan *et al*., 2021), rheumatoid arthritis, atherosclerosis (Phull *et al*., 2018), diabetes (Zhu *et al*., 2021), cardiovascular diseases, inflammation, stroke (Papaconstantinou, 2019) and neurodegenerative disorders (Rosales *et al*., 2020, Kumari and Singh, 2019). These could be prevented by using antioxidants which ameliorate the oxidative damage through different mechanisms via scavenging ROS and RNS, chelating transition metals and up regulating antioxidant defense system of body (Shirinzadeh *et al*., 2020, Pizzino *et al*., 2017), therefore, use of antioxidants is intensively studied in medicinal chemistry for treating different ailments (Jasiewicz *et al*., 2021, Youssif *et al*., 2018).

The indole alkaloids found in microorganisms, plants and animals, possessing therapeutic potential as anticancer, antirheumatoidal, antihypertensive, anti-arrhythmic, antimicrobial, antiasthmatic, antiemetics and analgesic (Kanwal *et al*., 2021, Casaril *et al*., 2020) but one of the most promising is their antioxidant potential, due to their ability to scavenge ROS and RNS (Kanwal *et al*., 2021, Casaril *et al*., 2020). The delocalization of lone pair of electrons in aromatic ring system of indole derivatives made them potent antioxidant (Kumar, 2020). Either a single electron is transferred from the nitrogen atom and a radical cation is formed or possibly a hydrogen atom could also be transferred (in case of -NH group) from the antioxidant molecule to DPPH and form a resonance-stabilized indolyl radical (Kanwal *et al*., 2021, Casaril *et al*., 2020).

Indole containing molecules (fig. 1) like melatonin, indole-3-propionic acid, indomethacin, tryptophan, serotonin, 6-chloromelatonin possess strong scavenging effect against $^1O_2$, $O_2^{\bullet-}$, H_2O_2, hydroxyl radical ($HO^{\bullet}$) and $ROO^{\bullet}$ (Estevão *et al*., 2010). The present work aimed to synthesize substituted indole derivatives (AI, BEI, TRY) with structural components (fig. 2), based on the indomethacin and melatonin templates by altering indole nucleus (blue), linking (green) group and terminal lipophilic/ neutral group (red) of these medicinally active molecules (fig. 1) reassuring the free radical scavenging potential of synthesized analogues.

MATERIALS AND METHODS

Chemicals, reagents and instruments

All chemicals and reagents including analytical grade 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid and methanol were purchased from Merck and Sigma Aldrich. Progress of the reaction were monitored by using TLC plates (0.25mm thickness, silica-gel $60GF_{254}$) and visualized by Iodine vapors and UV/Visible lamp,

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HPUVIS Desaga (Heidelberg) at 254 and 365nm by using ethanol: chloroform (4:1) and hexane: ethanol (4:1) with few drops of ethyl acetate as mobile phase. Melting points were taken on STUART meting point apparatus (U.S.A) and uncorrected. Calibrated analytical balance (OHAUS Corporation, U.S.A) was used for weighing, while mixing and stirring of each reaction was done on hot plate-Stirrer (Bibby-Sterilin, UK). Spectroscopic analysis was done by employing UV-Visible spectrophotometer (Shimadzu-1601, Japan), Infra-red technique (Nicolet Avatar 300-FTIR), Mass spectrometer (JEOL 600H-2, U.S.A) and ^{1}H-NMR (Bruker Advance, France) using d_6-DMSO, deutrated water (D_2O) and methanol (MeOD).

General procedure for the synthesis of compounds

Mixture of parents (AI, BEI, TRY) and reactants were dissolved in tetrahydrofuran (THF) at basic pH, stirred magnetically at room temperature (for 70-75 hours) and refluxed (for 5-60 hour) at 50°C. Final product was filtered by vacuum filtration, washing (solvent THF) and vacuum drying over anhydrous silica beads in the desiccator. General synthetic scheme of substituted indole derivatives outlined in fig. 3 to 5.

In-vitro antioxidant activity using DPPH assay

The free radical scavenging activity of synthesized derivatives was tested with DPPH with slight modification (Jasiewicz *et al*., 2021). Different concentrations of test compounds (0 to 200µM) in solvent methanol were combined with DPPH (1:1 ratio) and kept in the dark for 30 minutes (ambient temperature). Absorbance was measured at 517nm by using spectrophotometer UV-1601, Shimadzu. Solution of DPPH (0.1mM) and ascorbic acid a natural anti-oxidant (Nobushi and Uchikura, 2010, Tan *et al*., 2014) were used as control and standard, respectively. All *in-vitro* tests were performed in triplicate in order to calculate IC_{50} value along with SEM (standard error mean).

STATISTICAL ANALYSIS

Percentage inhibition (% inhibition) was calculated by: % inhibition=(Absorbance of control-Absorbance of sample) (absorbance of control)x100. The IC_{50} value of each sample was determined by plotting the concentration of test compounds and % scavenging through online software (https://www.graphpad.com/quickcalcs/) (Bhat *et al*., 2020, Jasiewicz *et al*., 2021).

Molecular docking

T36 and standard were docked against human antioxidant enzyme (PDB code: 3MNG), to evaluate their interactions with target protein by using PyRx 0.9.2 (AutoDock Vina). The structure of human antioxidant enzyme (resolution: 1.45˚A) were fetched from protein databank (rcsb.org/pdb/home/home.do). Except co-crystallized inhibitor i.e. (4s, 5s)-1,2-dithiane-4,5-diol (D1D) all other molecules were removed, D1D and protein saved separately after minimization up to 10000 steepest descents using the Chimera program. Ascorbic Acid was downloaded from PubChem while compound T36 was sketched with the help of ChemDraw Ultra 8.0 software and were optimized by using Open Babel (Force Field: mmff94, Optimization Algorithm: Steepest Descent: 500) and saved in PDB format. Active binding pocket was adjusted according to the active side residues reported in literature ((Roman, 2015, Priya *et al*., 2018)). The Grid center size (Å) center X=13.854, Y=41.761, Z=18.994; dimension X=27.139, Y=27.122, Z=29.805 were used in docking study. Docking protocol was validated by re-docking of co-crystalized ligand (D1D) into respective enzyme with root mean square deviation (RMSD) of <2.0˚A. Post-docking results were analyzed by UCSF Chimera (v.1.10.2).

RESULTS

N-substituted fourteen derivatives of substituted indole molecules (AI, BEI, TRY) have been synthesized and their potential antioxidant effect have been explored results are represented in table 1. All molecules have three distinct fragments (fig. 2) influencing the biological activity. The obtained results revealed promising antioxidant activity of all synthesized compounds with the IC_{50} values ranging from 0.0001-0.058mM indicating their potential as antioxidants. Among all T36 was found best molecule with better activity than standard (ascorbic acid) (fig. 6). Docking results of T36 against human antioxidant enzyme (PDB code: 3MNG) revealed good fit in to the protein and presented better docking score and interactions than standard verifying the results of *in-vitro* antioxidant activity.

Spectral characterization 7-(3-oxo-3-phenylpropyl)-1H-pyrrolo[2,3-b]-7-pyridinium chloride (A20)

White crystalline powder; Yield 31.15%; m.p. 110-113°C; UV (Ɛ): 9391.813 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3340.31(NH), 1672.20(C=O), 1613.62(C=C aromatic), 1442.00(C-C aromatic), 1303.06(C-N amine), 786.37(C-H aromatic), FAB-MS(m/z): 252 [M+H]^+,H-NMR (D20), δ (ppm): (d, *J*=8.00Hz, 1H, H6), 8.501 (d, *J*=8.00Hz, 1H, H4), 7.808 (d, *J*=7.60Hz, 2H, H2', H4'), 7.576-7.519 (m, 1H, H5), 7.449-7.373 (m, 3H, H1', H5', H6'), 5.059 (t, *J*=12.40Hz, 2H, H2''), 4.893-4.824 (m, 1H, H1), 3.868 (t. *J*=12.40Hz, 2H, H1''), 2.866-2.794 (m, 1H, H2), 2.734-2.675 (m, 1H, H3).

7-[(naphthalen-2-yl)carbonyl]-pyrrolo[2,3-b]-7-pyridinium (A25)

White crystalline powder; Yield 44.93%; m.p. 184-186°C; UV (Ɛ): 27064.507 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3089.08(NH), 1683.09(C=O), 1625.88(C=C aromatic), 1466.62(C-C aromatic), 1360.27(C-N amine), 824.97 (C-H aromatic), FAB-MS(m/z): 274[M+H]^+,H-NMR (d6-DMSO), δ (ppm): 8.596 (s, 1H, H-1'), 8.115 (d, *J*=8.00Hz, 1H, H-6), 8.015-7.954 (m, 4H, H-5', H-6', H-7', H-8'), 7.668-7.576 (m, 5H, H-3', H-4', H-4, H-5, H-6), 3.314 (s, 2H, H-2, H-3).

7-[(4-methylphenyl)carbonyl]-1H-pyrrolo[2,3-b]-7-pyridinium (A29)

White crystalline shiny powder; Yield 97.00%; m.p. 170-175°C; UV (Ɛ): 16265.266 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 1666.74(C=O), 1605.45(C=C aromatic), 1576.84(phenyl ring), 1409.31(CH_3), 1115.10(C-N amine), 833.14 and 743.25(C-H aromatic), FAB-MS(m/z): 238 [M+H]^+,H-NMR (d6-DMSO), δ (ppm): 7.832 (d, *J*=8.40Hz, 4H, H-2', H-6', H-6, H-4), 7.298 (d, *J*=8.00Hz, 3H, H-5, H-3', H-5'), 3.314 (s, 2H, H-2, H-3), 2.358 (s, 3H, H-7').

4-carbamoyl-1-[2-(1H-indol-3-yl)ethyl]pyridinium bromide (B46)

Yellow amorphous powder; Yield 46.55%; m.p. 224°C; UV (Ɛ): 24927.337 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3170.49(primary amide), 1683.09(C=O), 1629.97(C=C aromatic), 1564.59(C-C aromatic), 1462.43(CH_2 alkane), 1237.68(C-N amine), 874.01 and 788.20(C-H aromatic), FAB-MS(m/z): 267[M+H]^+,H-NMR (D2O), δ (ppm): 8.499 (d, *J*=6.00Hz, 2H, H-2, H-6), 8.009 (d, *J*=6.00Hz, 2H, H-3, H-5), 7.431 (d, *J*=8.00Hz, 2H, H-4', H-7'), 7.296 (d, *J*=8.00Hz, 1H, H-1'), 7.182 (t, *J* = 14.80 Hz, 2H, H-5', H-6'), 7.030 (t, *J*=14.80Hz, 1H, H-2'), 6.936 (s, 2H, H-8), 4.884 (t, *J* = 12.00 Hz, 2H, H-8'), 3.441 (t, *J*=12.00Hz, 2H, H-9').

1-[2-(1H-indol-3-yl)ethyl]-4-({[2-(1H-indol-3-yl)ethyl] azaniumyl}methyl)-piperidin-1-ium dibromide (B52)

Dark brown shiny crystals; Yield 98.00%; m.p. 146°C; UV (Ɛ): 28462.053 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3497.39 and 3452.44(NH amine), 3391.15(C-H aromatic), 1679.00(C=C aromatic), 1552.33 and 1503.29(C-C aromatic), 1094.67(C-N amine), 735.07(C-H aromatic), FAB-MS(m/z): 403[M+H]^+,H-NMR (d6-DMSO), δ (ppm): 7.597-7.556 (m, 2H, H-2', H-2"), 7.386-7.268 (m, 4H, H-4', H-7', H-4", H-7"), 7.116-7.007 (m, 4H, H-5', H-6', H-5", H-6"), 3.381-3.318 (m, 2H, H-7), 3.113-3.058 (m, 5H, H-3, H-4, H-5), 1.246 (t, *J*=12.80Hz, 4H, H-2, H-6), 1.189 (t, *J* =14.40Hz, 4H, H-9', H-9"), 1.079 (m, 4H, H-8', H-8").

1-[2-(1H-indol-3-yl)ethyl]-4-({[2-(1H-indol-3-yl)ethyl] azaniumyl}methyl)-piperidin-1-ium dibromide (T3)

Greenish white amorphous powder; Yield 80.85%; m.p. 210°C; UV (Ɛ): 18483.384 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3378.89(NH), 1723.95(C=O), 1670.83(phenyl ring), 1642.22(C=C aromatic), 1601.36 and 1392.96(C-C aromatic), 1356.19 and 1033.37(C-N amine), 845.40, 763.68, 739.16, 702.38 and 653.35(C-H aromatic), FAB-MS(m/z): 368[M+H]^+,H-NMR (d6-DMSO), δ (ppm): 8.136 (d, *J*=8.40Hz, 2H, H-2', H-6'), 7.828 (d, *J*=8.40Hz, 2H, H-3', H-5'), 7.747 (d, *J*=8.40Hz, 2H, H-7', H-11'), 7.512 (t, *J*=14.80Hz, 3H, H-8', H-9', H-10'), 7.363 (d, *J*=7.60Hz, 1H, H-8), 7.270 (d, *J*=8.00Hz, 1H, H-5), 7.016 (t, *J*=1.20Hz, 2H, H-6, H-7), 4.150 (s, 2H, H-4), 3.790 (s, 2H, H-2), 3.107 (m, 2H, H-1"), 2.499-2.481 (m, 2H, H-3).

2-[2-(3-nitrophenyl)-2-oxoethyl]-1H,2H,3H,4H,9H-pyrido[3,4-b]indol-2-ium bromide (T5)

Brown amorphous shiny powder; Yield 34.45%; m.p. 245°C; UV (Ɛ): 24319.153 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3427.92, 3411.58 and 3248.13(NH), 3092.85(C-H aromatic), 1736.21(phenyl ring), 1687.17(C=O), 1535.98(C=C aromatic), 1499.21(C-C aromatic), 1372.53(NO_2), 1196.82, 1155.96 and 1041.54(C-N amine), 833.14, 747.33, 686.04 and 665.61(C-H aromatic), FAB-MS(m/z): 337[M+H]^+,H-NMR (D2O), δ (ppm): 8.520 (s, 1H, H-2'), 7.551 (d, *J*=8.00Hz, 2H, H-4', H-6'), 7.443 (d, *J*=8.40Hz, 2H, H-5, H-8), 7.231 (t, *J*=14.80Hz, 1H, H-1), 7.143 (t, *J*=15.20Hz, 3H, H-5', H-6, H-7), 4.380 (s, 2H, H-1"), 3.530 (t, *J*=12.40Hz, 4H, H-2, H-4), 3.021 (t, *J*=12.00Hz, 2H, H-3).

2-[2-(4-nitrophenyl)-2-oxoethyl]-1H,2H,3H,4H,9H-pyrido[3,4-b]indol-2-ium bromide (T6)

Golden brown amorphous shiny powder; Yield 37.42%; m.p. 261°C; UV (Ɛ): 40666.468 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3350.00(NH), 1666.74(C=O), 1585.02(C=C aromatic), 1392.96(CH_2), 1335.75(NO_2), 918.96, 698.30 and 649.26(C-H aromatic), FAB-MS(m/z): 337 [M+H]^+,H-NMR (D2O), δ (ppm): 7.552 (d, *J*=8.00Hz, 2H, H-3', H-5'), 7.443 (d, *J*=8.00Hz, 4H, H-2', H-6', H-5, H-8), 7.231 (t, *J*=14.80Hz, 2H, H-6, H-7), 4.383 (s, 2H, H-1"), 3.533 (t, *J*=12.00Hz, 4H H-2, H-4), 3.023 (t, *J*=11.60Hz, 2H, H-3).

2-[2-(4-fluorophenyl)-2-oxoethyl]-1H,2H,3H,4H,9H-pyrido[3,4-b]indol-2-ium bromide (T8)

Light brown amorphous shiny powder; Yield 28.41%; m.p. 271°C; UV (Ɛ): 56859.890 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3219.52(NH), 2921.23 and 2774.12(C-H aromatic), 1625.88(C=O), 1572.76(C=C aromatic), 1478.77(CH_2), 1446.08(phenyl ring), 1335.75 and 1298.98(C-N amine), 1209.08(C-F), 874.01, 730.99 and 681.95(C-H aromatic), FAB-MS(m/z): 296[M+H]^+,H-NMR (D2O), δ (ppm): 7.558 (d, *J*=8.00Hz, 4H, H-2', H-3', H-5', H-6'), 7.449 (d, *J*=8.00Hz, 2H, H-5, H-8), 7.237 (t, *J*=15.20Hz, 2H, H-6, H-7), 4.388 (s, 2H, H-1"), 3.539 (t, *J*=12.40Hz, 4H, H-2, H-4), 3.030 (t, *J*=12.40Hz, 2H, H-3).

2-[2-(2,4-difluorophenyl)-2-oxoethyl]-1H, 2H, 3H, 4H, 9H-pyrido[3,4-b]indol-2-ium chloride (T9)

Pinkish amorphous shiny powder; Yield 23.28%; m.p. 274°C; UV (Ɛ): 45632.297 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3199.09(NH), 2794.55(C-H aromatic), 1703.52(C=O), 1576.84(C=C aromatic), 1564.59 and 1503.29(phenyl ring), 1437.91(CH_2), 1249.94 and 1200.91(C-F), 1343.93 and 1160.05(C-N amine) 874.01, 820.89 and 739.16(C-H), FAB-MS(m/z): 328[M+H]^+,H-NMR (D2O), δ (ppm): 7.562 (d, *J*=8.00Hz, 3H, H-3', H-5', H-6'), 7.451 (d, *J*=8.00Hz, 2H, H-5, H-8), 7.237 (t, *J*=15.20Hz, 2H, H-6, H-7), 4.403 (s, 2H, H-1"), 3.551 (t, *J*=12.00Hz, 4H, H-2, H-4), 3.039 (t, *J*=12.00Hz, 2H, H-3).

Table 1: Antioxidant activity (IC_{50} ± SEM (mM)) of synthesized derivatives, parents (AI, BEI, TRY) and standard (ascorbic acid).

S. No.	Compounds	IC_{50} ± SEM (mM)	S. No.	Compounds	IC_{50} ± SEM (mM)
1	Ascorbic Acid	0.001 ± 0.006	10	T3	0.001 ± 0.0001
2	AI	0.144 ± 0.667	11	T5	0.015 ± 0.001
3	A20	0.006 ± 0.001	12	T6	0.016 ± 0.002
4	A25	0.018 ± 0.008	13	T8	0.026 ± 0.001
5	A29	0.058 ± 0.039	14	T9	0.022 ± 0.005
6	BEI	0.073 ± 0.002	15	T19	0.034 ± 0.002
7	B46	0.004 ± 0.001	16	T35	0.016 ± 0.001
8	B52	0.01 ± 0.001	17	T36	0.0001 ± 0.002
9	TRY	0.047 ± 0.005	18	T42	0.001 ± 0.055

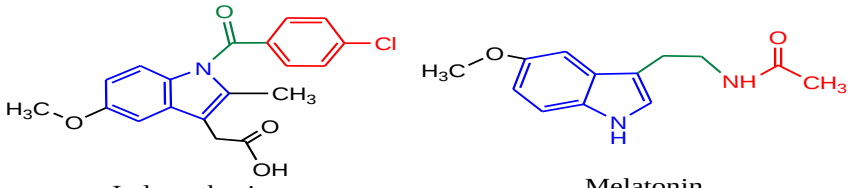


Fig. 1: Structure components of indole containing medicinal compounds having antioxidant activity.

Fig. 2: structure components of synthesized indole derivatives

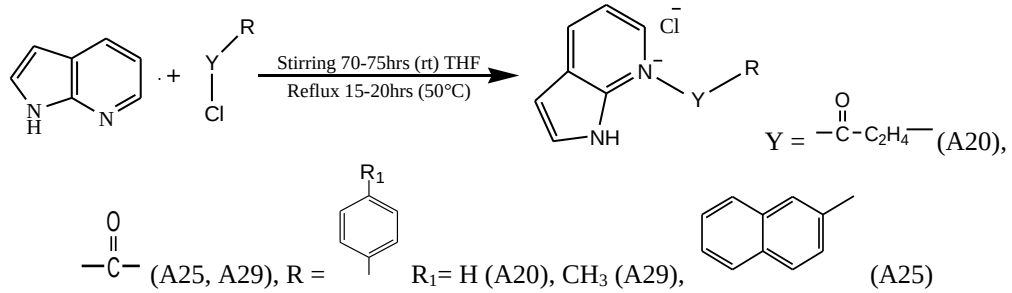
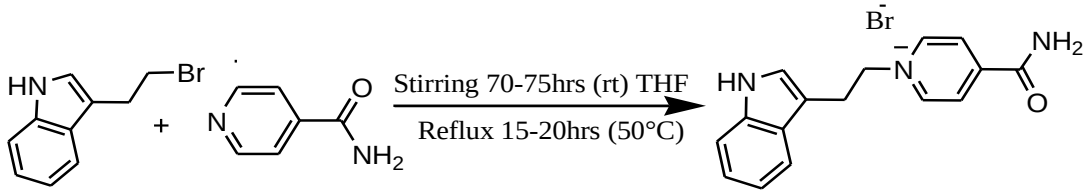
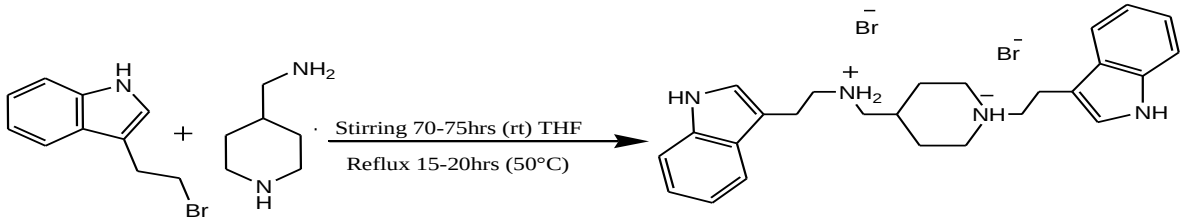


Fig. 3: General synthetic scheme for 7-azaindole (AI) derivatives



(a) Synthesis of 4-carbamoyl-1-[2-(1H-indol-3-yl) ethyl] pyridinium bromide (B46)



(b) Synthesis of 1-[2-(1H-indol-3-yl) ethyl]-4-({[2-(1H-indol-3-yl) ethyl] azaniumyl} methyl)-piperidin-1-ium dibromide (B52)

Fig. 4: Synthetic scheme for 3-(2-bromoethyl) indole (BEI) derivatives

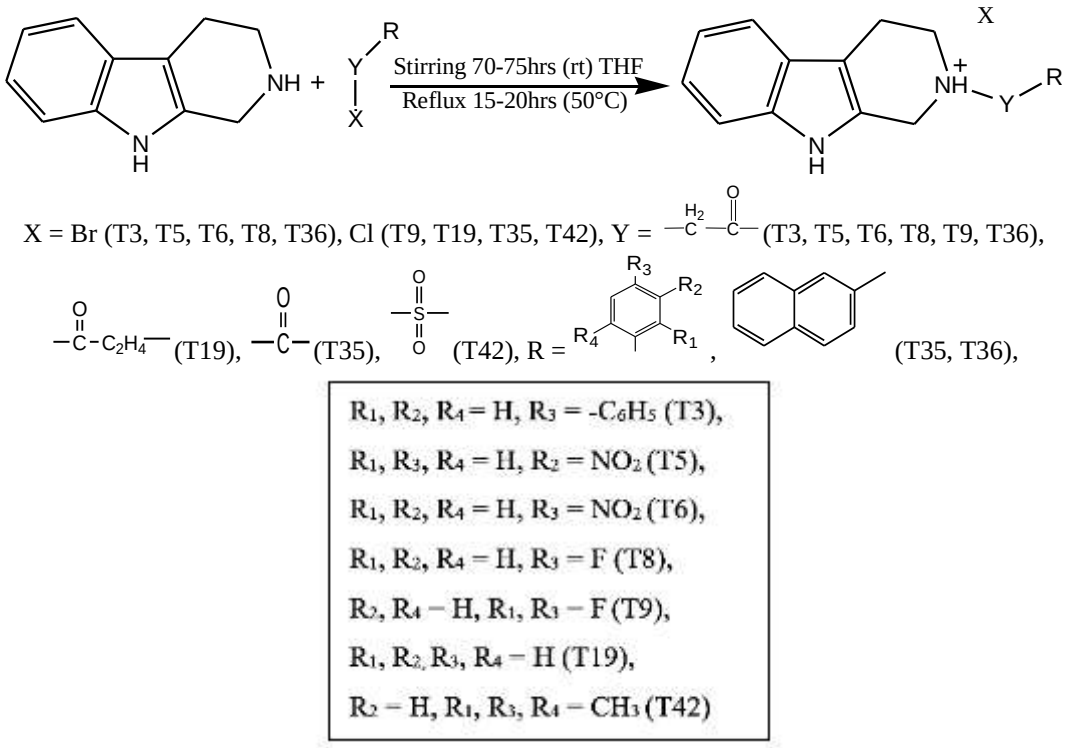
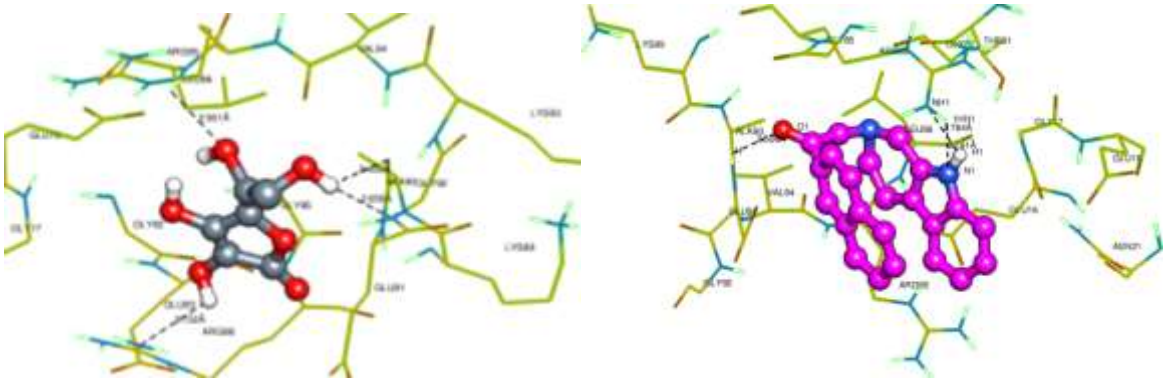


Fig. 5: General synthetic scheme for 1, 2, 3, 4-tetrahydro-9H-pyrido [3, 4-b] indole (TRY)



A B

Fig. 6: 3D interaction of ascorbic acid (A) and T36 (B) against 3MNG protein.

2-(3-oxo-3-phenylpropyl)-1H,2H,3H,4H,9H-pyrido[3,4-b]indol-2-ium chloride (T19)

Light yellow amorphous powder; Yield 90.38%; m.p. 218°C; UV (Ɛ): 31486.069 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3196.50(C-H aromatic), 1681.93(C=O), 1456.26, 1444.68 and 1427.32(C-C aromatic), 1321.24, 1307.74 and 1249.87(C-N amine), 894.97, 756.10 and 721.38(C-H aromatic), 711.73 and 671.239(N-H wag amine), FAB-MS(m/z): 306[M+H]^+,H-NMR (D2O), δ (ppm): 7.969 (d, *J*=7.60Hz, 2H, H-2', H-6'), 7.664 (t, *J*=14.80Hz, 3H, H-3', H-4', H-5'), 7.218-7.170 (m, 2H, H-5, H-8), 7.128-7.088 (m, 2H, H-6, H-7), 4.390 (s, 2H, H-4), 3.537 (t, *J*=12.40Hz, 2H, H-2), 3.108 (t, *J*=11.60Hz, 2H, H-3), 3.020 (t, *J*=12.00Hz, 2H, H-2"), 1.802-1.778 (m, 2H, H-1").

2-[(naphthalen-2-yl) carbonyl]-1H, 2H, 3H, 4H, 9H-pyrido [3, 4-b] indole (T35)

Off white amorphous powder; Yield 77.99%; m.p. 241°C; UV (Ɛ): 10542.432 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3182.90(NH), 3056.02(C-H aromatic), 1627.92, 1597.06 and 1581.63(C=C aromatic), 1487.12(CH_2), 1452.40 and 1429.25(C-C aromatic), 1323.17 and 1238.30(C-N amine), 871.82, 829.39 and 794.67(C-H aromatic), 779.24 and 763.81(C-H phenyl ring), FAB-MS(m/z): 327[M+H]^+,H-NMR (d6-DMSO), δ (ppm): 8.050-7.908 (m, 2H, H-3', H-4'), 7.330 (d, *J*=7.60Hz, 2H, H-5', H-8'), 7.294 (d, *J*=8.00,Hz, 2H, H-5, H-8), 7.034 (t, *J*=14.80Hz, 2H, H-6', H-7'), 6.958 (t, *J*=14.40Hz, 2H, H-6, H-7), 4.042 (s, 2H, H-4), 3.160 (t, *J*=11.60Hz, 2H, H-2), 2.726 (t, *J*=10.80Hz, 2H, H-3).

2-[2-(naphthalen-2-yl)-2-oxoethyl]-1H, 2H, 3H, 4H, 9H-pyrido [3, 4-b] indol-2-ium bromide (T36)

Light green amorphous powder; Yield 49.22%; m.p. 235°C; UV (Ɛ): 28372.467 $mol^{-1}cm^{-}1$, IR (ν_{max}) cm^{-1}: 3272.64(NH), 2917.14(C-H aromatic), 1674.91, 1634.05 and 1601.36(C=O), 1470.60(CH_2), 1450.17 and 1437.91(phenyl ring), 1388.88 and 1323.50(C-N amine), 882.18, 857.66, 812.71 and 739.16(C-H aromatic), FAB-MS(m/z): 342[M+H]^+,H-NMR (d6-DMSO), δ (ppm): 8.757 (s, 1H, H-2'), 8.132 (d, *J*=3.60Hz, 1H, H-8'), 8.054-8.028 (m, 1H, H-7'), 7.523 (d, *J*=8.00Hz, 2H, H-3', H-6'), 7.394 (d, *J*=8.00Hz, 2H, H-5, H-8), 7.146 (t, *J*=14.40Hz, 2H, H-4', H-5'), 7.064 (t, *J*=15.20Hz, 2H, H-6, H-7), 5.336 (s, 2H, H-1''), 4.615 (s, 2H, H-4), 3.157-3.057 (m, 2H, H-2), 1.191 (t, *J*=14.40Hz, 2H, H-3).

2-[(2, 4, 6-trimethylphenyl) sulfonyl]-1, 2, 3, 4-tetrahydro-9H-pyrido [3, 4-b] indole (T42)

Off white amorphous powder; Yield 40.15%; m.p. 241°C; UV (Ɛ): 53125.823 $mol^{-1}cm^{-1}$, IR (ν_{max}) cm^{-1}: 3558.68(NH), 3178.66 and 2798.64(C-H aromatic), 1609.53 and 1556.41(C=C aromatic), 1482.86 and 1446.08(C-C aromatic), 1339.84(C-N amine), 1298.98 and 1241.77(O=S=O), 1155.96, 1049.72 and 1004.77 (sulfonamide), 882.18, 845.40, 820.89 and 743.25 (C-H aromatic), FAB-MS(m/z): 355[M+H]^+,H-NMR (D2O), δ (ppm): 7.447 (d, *J*=8.00Hz, 2H, H-5, H-8), 7.234 (t, *J*=14.80Hz, 2H, H-6, H-7), 7.146 (t, *J*=14.80Hz, 2H, H-3', H-5'), 4.392 (s, 2H, H-4), 3.541 (t, *J*=12.00Hz, 2H, H-2), 3.031 (t, *J*=12.40Hz, 2H, H-3), 2.474 (s, 9H, H-7', H-8', H-9').

DISCUSSION

Well known free radical scavenging properties of indole molecules (Casaril, Domingues *et al.* 2020; Kanwal, Khan *et al*. 2021) persuaded to explore the antioxidant potential of synthesized indole derivatives (AI, BEI, TRY) (Mohammadi-Farani *et al*., 2013).

The effect of antioxidants on DPPH (a stable free radical) is because of its ability to donate hydrogen atom, upon which color changes from purple to yellow (Hall *et al*., 2010), indicated via decrease in its absorbance at 517nm. The scavenging reaction between DPPH and antioxidant (H-A) are written as:

$$DPPH + (H — A) \rightarrow \underset{(Purple)}{DPPH} — \underset{(Yellow)}{H} + A$$

The concentration of antioxidant which is required to reduce 50% (IC_{50}) of DPPH is an extensively used parameter to quantify the activity (Casaril *et al*., 2020, Kanwal *et al*., 2021).

Free radical scavenging effect of newly synthesized derivatives showed that all the synthesized derivatives possessed better antioxidant effect than the parent molecules (AI, BEI, TRY). The antioxidant effect of AI derivatives were in the order of A20>A25>A29. Among them, A20 with unsubstituted phenyl ring exhibited potent antioxidant effect with significant IC_{50} value reflecting that the linker (ethyl carbonyl) between unsubstituted phenyl and indole ring enhancing the free radical scavenging effect (table 1). Similarly, B46 showed better antioxidant potential than B52 because of the electron withdrawing group (-$CONH_2$) at *para* position of pyridine ring (table 1). T3 having two carbon as a linker between indole and *para*-phenyl benzene ring showed significant antioxidant potential same as standard (ascorbic acid).

Among the two naphthyl derivatives of TRY, T36 where acetyl group bridging tryptoline to naphthyl ring emerged out as a most effective antioxidant showing better activity than the standard (table 1). T42 with sulphonyl moiety as a linker between tryptoline and 2,4,6-tri-methyl phenyl presented significant antioxidant activity which is similar to the standard. Antioxidant potential of tryptoline derivatives was in the order of T36>T3>T42>T5>T6>T35 >T9>T8>T19>TRY (table 1). TRY derivatives showed IC_{50} values in the range of 0.0001-0.047mM, AI analogues produced IC_{50} values 0.006 to 0.144mM, while BEI derivatives presented IC_{50} values from 0.004 to 0.073 mM (table 1). This showed that TRY nucleus influenced for imparting significant antioxidant potential along with linkers and terminal lipophilic groups as compared to other molecules. Overall, the SAR study revealed that not only the indole nucleus, but the linker as well as the nature of substitution on terminal ring is affecting the level of antioxidant potential of the molecules.

T36 was the compound presented better *in-vitro* results than the standard, therefore T36 along with standard were docked and evaluated for their mode of binding against human antioxidant enzymes (PDB ID: 3MNG). T36 showed better binding scores (-6.7kcal/mol) than the standard (-4.9kcal/mol). Standard was involved in hydrogen bonding with Arg86, Ala90, Gly92, Leu96 and hydrophobic interactions with Glu16, Gly17, Gly82, Glu83, Gly85, Arg86, Lys89, Ala90, Glu91, Gly92, Lys93, Val94, Arg95 and Leu96 (fig 6). T36 settled in the same cavity where standard was bound and attracted by same amino acids along with additional binding residues. Interestingly, the conformation of molecule folded inside the active site of enzyme cavity. TRY ring of T36 glide into the enzyme where its terminal naphthyl ring turned back to face the TRY while, the NH of pyrrole ring stabilizing the molecule (T36) by engaging with Arg86 via hydrogen bonding (fig 6) and Arg86 has been reported for hydrogen bonding (Sivala *et al.* 2021, Vadabingi *et al*. 2020). Meanwhile, carbonyl of acetyl linker established additional hydrogen bond with Glu91 which also has importance in binding interaction (Sravya *et al.* 2020, Sudhamani *et al.* 2019). Molecule is surrounded by

hydrophobic residues of the enzyme pocket including, Glu16, Gly17, Glu18, Asn21, Thr81, Gly82, Gly85, Arg86, Lys89, Ala90, Glu91, Gly92, Val94, Arg95 and Leu96 (fig 6). The binding energy and interactions of T36 are in accordance with *in-vitro* antioxidant activity (table 1) and these findings may be justified because of its larger size and lipophilic structural features creating distinct conformation and interactions with key residues inside the cavity and responsible for showing better scavenging potential than the standard.

CONCLUSION

The obtained results revealed that almost all synthesized indole derivatives are good antioxidant agents and should be employed further in drug development studies. Among all, tryptoline derivatives revealed potential activity. T36 with terminal naphthyl group emerged out as a most effective free radical scavenger, in addition linker group (methyl carbonyl) incorporating required length and flexibility in the structure, influencing the antioxidant potential of molecule. Docking of T36 against the target protein discovered the contribution of structural features in binding with enzyme and validated the good performance of molecule in biological assay.

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