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Research Article

DESIGN, SYNTHESIS AND 3D-QSAR STUDIES OF NEW BIS (INDOLYL) METHANES AS ANTIOXIDANT AGENTS

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ABSTRACT

The reaction of indoles with aromatic aldehydes in the presence of 5 mol% p-toluenesulfonic acid as a catalyst in acetonitrile media afforded high yields of bis(indolyl)methane via conventional and ultrasonication methodologies. It was observed that the ultrasonication method produced a higher yield of products in shorter reaction times. All synthesized compounds were evaluated for their antioxidant activities. Among them, compounds 3a, 3f, 3g, 3j, 3k, 3p, 3q and 3t showed moderate to good antioxidant activity. 3D-QSAR analysis was performed to the obtained compounds and investigated using comparative molecular field analysis (CoMFA) method, helping to better understand the structural requirements of lead compounds with high antioxidant activity and biological compatibility.

Keywords: 3D-QSAR studies, Bis (indolyl) methane, Antioxidant, Ultrasonication

1. INTRODUCTION

In recent decades, indole bearing compounds as valuable *N*-heterocyclic precursors have great importance for researchers. These compounds are widely found in pharmaceuticals, materials, biomedicals and agro-chemicals [1-3]. Bis (indolyl) alkanes possess all-purpose of biological studies such as antimicrobial [4-7], antitumor [8, 9] Cytotoxic [10-12], analgesic, anti-inflammatory [13, 14] and antioxidant [15-17]. One of the very renowned derivatives of indole is bis(indolyl) methane (BIM) which helped to explore various physical and biological features. BIMs are frequently studied in various research [18, 19] BIMs are very active cruciferous material that helps in exploring the importance of estrogen metabolism and covers apoptosis in cancer cells in human beings [20]. Some of the important drugs involving indole as a functional moiety are indomethacin, sumatriptan, frovatriptan [21, 22]. Due to the special pharmacological activities and the dispersion of indole moiety in several natural products, a considerable interest has been focused on the evaluation of efficient synthetic procedure for the synthesis of bis (indolyl) alkanes. A number of synthetic prescripts for their synthesis have been studied [23-27]. However, the utilization of toxic reagents, high temperature, and alterable organic solvents are among the drawbacks of most of this procedure [28-33]. Hence there is a requirement for a novel, efficient, and reasonable synthetic methodologies depend on green chemistry procedures in organic synthesis. Ultrasound irradiation as a noteworthy technique has been broadly used in several research areas such as biomedical, organic, inorganic and materials chemistry [34]. Synthesis of organic compounds led to shorter reaction time, higher yields and benign conditions under ultrasound irradiation [35, 36]. In the present work, we studied the use of *p*-toluenesulfonic acid as a catalyst for the synthesis of bis (indolyl) methane derivatives under conventional and ultrasonic irradiation process (Scheme 1) and studied their antioxidant activity. Additionally, we have performed the 3D-QSAR on our synthesized molecules using the CoMFA module through researching structure-activity relationships, which may be used further in designing and predicting the antioxidant activity of novel molecules.

2. MATERIAL AND METHODS

2.1. Chemicals

All the chemicals purchased from Sigma-Aldrich and Merck India and used without purification to carry out this work. Melting points were measured by open capillary tube. The purity of the compounds was examined through thin-layer chromatography (silica gel 60 F254), using hexane/ ethyl acetate in the ratio of 7:3, on F254 silica-gel pre-coated sheets (Merck).

2.2. Instruments

The infrared (IR) spectra were recorded on a FTIR Bruker Alpha II ECO-ATR spectrometer. The ^{1}H-NMR spectra were studied on a JEOL 500 MHz spectrometer in DMSO-d_6 using TMS as the internal standard and ^{13}C-NMR spectra were recorded on JEOL 125MHz using DMSO-d_6 as solvent. Mass spectra were recorded with Electronspray ionization-MS-spectrometer (WATER-Q-TOF, Premier-HAB213). The antioxidant activity was carried out by DPPH assay method, and absorbance of standard and test solution were observed using Systronics-2201 UV/Vis Double Beam spectrophotometer.

2.3. Synthesis of bis(indolyl) methane derivatives under conventional method (3a-v)

Electrophilic substitution reaction was performed to a vigorously stirred solution of substituted indole (2.0 mmol) and variety of aromatic aldehydes (1.0 mmol) in CH_3CN (5 mL). *P*-TSA (0.05 mmol) was mixed followed by stirring to excellent yields of bis(indolyl)methanes at room temperature for 1-3 h. Finally, the reaction was monitored by TLC, then the reaction mixture was removed and washed with DCM. The organic material layer was washed with salt solution (NaCl+H_2O), dried over Na_2SO_4 and isolated by filtration. The solvent was separated under reduced vacuum pressure. The finally ultimate crude product was purified by column chromatography (silica gel, 100-200 mesh) using *n*-hexane- EtOAC (7:3) as an eluent.

2.4. Synthesis of bis(indolyl)methane derivatives under ultrasound irradiation method (3a-v)

To a solution of substituted indole (1) (2.0 mmol) and variety of aromatic aldehydes (2) (1.0 mmol) in CH_3CN (4 mL), *p*-TSA (0.05 mmol) was added and subjected to ultrasound irradiation (Model No. KS-750F) at a frequency of 20 KHz at room temperature for 10-30 min. Finally, the reaction was monitored by TLC, the reaction mixture was then removed with DCM. The organic material layer was washed with salt solution (NaCl+H_2O), dried over Na_2SO_4 and isolated by filtration. The solvent was separated under vacuum, and finally ultimate crude product was purified by column chromatography (silica gel, 100-200 mesh) using *n*-hexane- EtOAC (7:3) as eluent.

2.5. Antioxidant Activity

Free radical scavenging activity (RSA) of bis(indolyl) methane derivatives was measured by in vitro, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [37,38]. Approximately 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of methanol and stored at 4°C until required. Approximately 3 mL of this stock solution was added to 2 mL of methanolic solution containing test sample of different concentrations (20, 40, 60, 80 and 100 µg/mL). The solutions were incubated for 30 min in dark and absorbance was measured at 517 nm. Ascorbic acid was used as standard control. This radical scavenging activity (RSA) was calculated using the following equation.

$$\%RSA = [(AC-AS)/AC] \times 100$$

Where AC is the absorbance of the control and AS is the absorbance of the tested compound.

2.6. 3DQSAR studies

The QSAR studies using the SYBYL-X 2.1.1 package (Tripos, Inc., USA). The biological activity of synthesized compounds was reported in IC50, which were converted to the corresponding negative logarithm of IC50 (pIC_{50}= -logIC_{50}) (Table 4). Initially, the energy of the molecules was optimized by Tripos force field and Gasteiger-Huckel charge [39].

2.7. Spectral and physical data for compounds

2.7.1. 3,3'-(Phenylmethylene)bis(2-Phenyl-1H-indole) (3a, $C_{35}H_{26}N_2$)

Olive solid; M.p.: 258-260°C; FT-IR (KBr): ν= 3574 (NH), 1520 (C=C), 3500 (=C-H), 2919 (C-H) cm^{1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.46 (s, 1H, Ar-CH), 7.02-7.89 (m, 23H, Ar-H), 10.60 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 37.7 (Ar-CH), 111.2, 119.4, 120.3, 122.3, 126.6, 125.7, 127.4, 128.2, 129.1, 128.6, 128.9, 129.2, 130.1, 135.6, 136.3, 142.4, ppm; MS: m/z = 475.20 (M^+).

2.7.2. 3,3'-((4-fluorophenyl)methylene)bis(2-Phenyl-1H-indole) (3b, $C_{35}H_{25}FN_2$)

Purple solid; M.p.: 240-241°C; FT-IR (KBr): ν= 3364 (NH), 1620 (C=C), 2910 (=C-H), 1200 (C-F) cm^{1}; ^{1}H

NMR (500 MHz, DMSO-d_6): δ = 5.35 (s, 1H, Ar-CH), 6.69-7.82 (m, 22H, Ar-H), 7.85 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 40.3 (Ar-CH), 112.4, 118.4, 121.3, 121.7, 123.6, 124.7, 125.4, 126.2, 128.2, 129.4, 130.5, 133.4, 135.6, 136.2, 139.7, 143.5 ppm; MS: m/z = 492.20 (M^+).

2.7.3. 3,3'-((2-Chlorophenyl)methylene)bis(2-Phenyl-1H-indole) (3c, $C_{35}H_{25}ClN_2$)

Light gray solid; M.p.: 239-242°C; FT-IR (KBr): ν = 3392 (NH), 1625 (C=C), 3060 (=CH), 2920 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.48 (s, 1H, Ar-CH), 6.99-7.89 (m, 22H, Ar-H), 7.97 (bs, 2H, NH) ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ = 39.2 (Ar-CH), 111.4, 119.4, 120.3, 121.7, 123.5, 126.6, 125.7, 127.4, 128.2, 130.4, 131.2, 131.9, 129.2, 132.1, 135.6, 139.2, 145.4 ppm; MS: m/z = 509.11 $(M+1)^+$.

2.7.4. 3,3'-((4-Chlorophenyl)methylene)bis(2-Phenyl-1H-indole) (3d, $C_{35}H_{25}ClN_2$)

Dark gray solid; M.p.: 240-241°C; FT-IR (KBr): ν = 3360 (NH), 1619 (C=C), 3056 (=C-H), 2925 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=5.35 (s, 1H, Ar-CH), 6.69-7.59 (m, 12H, Ar-H), 10.40 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ=40.5 (Ar-CH), 111.4, 117.4, 119.3, 121.7, 126.6, 125.7, 127.4, 128.2, 129.6, 130.2, 131.9, 131.3, 133.1, 135.6, 139.2, 145.4 ppm; MS: m/z=508.17 (M^+), 509.11 $(M+1)^+$.

2.7.5. 3,3'-((4-bromophenyl)methylene)bis(2-Phenyl-1H-indole) (3e, $C_{35}H_{25}BrN_2$)

Yellow solid; M.p.: 260-261°C, FT-IR (KBr): ν = 3425 (NH), 1620, 1520, 1456 (C=C), 3050 (=C-H), 550 (C-Br) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.45 (s, 1H, Ar-CH), 7.02-7.59 (m, 22H, Ar-H), 10.15 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 39.8 (Ar-CH), 112.4, 119.4, 120.0, 122.7, 123.6, 126.7, 126.4, 127.4, 128.4, 129.5, 131.4, 132.2, 133.6, 135.7, 138.2, 140.7 ppm; MS: m/z = 552.17 (M^+).

2.7.6. 3,3'-(p-tolylmethylene)bis(2-Phenyl-1H-indole) (3f, $C_{36}H_{28}N_2$)

Yellow solid; M.p.: 246-248°C; FT-IR (KBr): ν = 3494 (NH), 1640, 1454 (C=C), 2923 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 2.28 (s, 3H, Ar-CH_3), 5.41 (s, 1H, Ar-CH), 6.95-7.89 (m, 22H, Ar-H), 10.65 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 33.2 (Ar-CH_3), 40.8 (Ar-CH), 111.2, 116.7, 119.4, 122.9, 124.2, 126.9, 127.5, 129.3, 130.2, 132.9, 136.3, 137.6, 139.2, 140.2, 142.5, 144.4 ppm; MS: m/z = 488.23 (M^+).

2.7.7. 3,3'-((4-Methoxyphenyl)methylene)bis(2-Phenyl-1H-indole) (3g, $C_{36}H_{28}N_2O$)

Greenish gray solid; M.p.: 258-260°C; FT-IR (KBr): ν = 3389 (NH), 1635 (C=C), 3050 (=C-H), 2950 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.64 (s, 3H, OCH_3), 5.72 (s, 1H, Ar-CH), 6.87-7.75 (m, 2H, Ar-H), 10.71 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 41.2 (Ar-CH), 55.5 (Ar-OCH_3), 105.4, 115.4, 117.3, 121.7, 124.6, 125.7, 126.4, 127.2, 128.6, 128.9, 129.5, 130.8, 133.1, 136.6, 139.2, 140.2 ppm; MS: m/z = 504.86 $(M+1)^+$.

2.7.8. 3,3'-((4-nitrophenyl)methylene)bis(2-Phenyl-1H-indole) (3h, $C_{35}H_{25}N_3O_2$)

Yellow solid; M.p.: 245-246°C; FT-IR (KBr): ν = 3364 (NH), 1625 (C=C), 2925 (C-H) cm^{-1}, ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.68 (s, 1H, Ar-CH), 6.74-8.19 (m, 22H, Ar-H), 10.29 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ= 40.8 (Ar-CH), 111.5, 117.6, 118.3, 119.8, 122.1, 123.4, 125.6, 127.5, 128.5, 129.2, 129.7,132.4, 134.2, 134.8, 136.6, 142.3, 145.3 ppm; MS: m/z = 518.2 $(M-1)^+$

2.7.9. 3,3'-((3-nitrophenyl)methylene)bis(2-Phenyl-1H-indole) (3i, $C_{35}H_{25}N_3O_2$)

Brick red solid; M.p.: 190-191°C, FT-IR (KBr): ν = 3338 (NH), 1540, 1326 (C=C), 2919 (C-H) cm^{-1}, ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.78 (s, 1H, Ar-CH), 6.64-8.29 (m, 22H, Ar-H), 7.89 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 39.8 (Ar-CH), 111.2, 118.2, 119.4, 121.3, 122.1, 123.4, 123.6, 126.5, 128.5, 129.2, 129.7,132.4, 134.2, 134.8, 136.6, 142.3, 144.3 ppm; MS: m/z = 518.2 $(M-1)^+$.

2.7.10. 2-(bis(2-phenyl-1H-indol-3-yl)methyl)-5-methoxyphenol (3j, $C_{36}H_{28}N_2O_2$)

Light yellow solid; M.p.: 190-191°C; FT-IR (KBr): ν= 3392 (NH), 1610, 1508 (C=C), 3057(=C-H), 3525 (OH) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.73 (s, 3H, OCH_3), 5.52 (s, 1H, Ar-CH), 5.31 (s, 1H, Ar-OH), 6.27-7.85 (m, 21H, Ar-H), 10.73 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ=41.3 (Ar-CH), 55.6 (Ar-OCH_3), 105.4, 116.4, 118.3, 121.3, 124.6, 125.7, 127.4, 127.2, 128.6, 128.9, 129.5, 129.8, 130.1, 136.6, 139.2, 140.2 ppm; MS: m/z = 520.01 $(M+1)^+$.

2.7.11. 3,3'-(Phenylmethylene)bis(2-Methyl-1H-indole) (3k, $C_{25}H_{22}N_2$)

Reddish pink solid; M.p.: 182-183°C; FT-IR (KBr): ν = 3364 (NH), 1625 (C=C), 2980 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.58 (s, 1H, Ar-CH), 2.29 (s, 6H, $2CH_3$), 6.68-7.59 (m, 13H, Ar-H), 11.14 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 40.2 (Ar-CH), 12.7 (Ar-CH_3), 110.4, 116.4, 120.3, 121.7, 123.6, 124.7, 125.4, 126.2, 130.4, 135.6, 136.2, 139.7 ppm; MS: m/z = 349.16 $(M-1)^+$.

2.7.12. 3,3'-((4-fluorophenyl)methylene)bis(2-methyl-1H-indole) (3l, $C_{25}H_{21}FN_2$)

Purple solid; M.p.: 130-131°C; FT-IR (KBr): ν = 3366 (NH), 1620, 1508 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.62 (s, 1H, Ar-CH), 2.49 (s, 6H, 2CH3), 6.99-7.69 (m, 12H, Ar-H), 10.72 (bs, 2H, NH) ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ = 43.2 (Ar-CH), 12.7 (Ar-CH_3), 111.7, 112.4, 119.3, 121.7, 123.6, 124.7, 125.4, 126.2, 132.4, 134.6, 136.2, 146.7 ppm; MS: m/z = 368.54 $(M+1)^+$.

2.7.13. 3,3'-((2-chlorophenyl)methylene)bis(2-Methyl-1H-indole) (3m, $C_{25}H_{21}ClN_2$)

Brownish red solid; M.p.: 150-151°C; FT-IR (KBr): ν = 3376 (NH), 1624 (C=C), 2937(C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ=5.42 (s, 1H, Ar-CH), 2.29 (s, 6H, 2CH3), 6.99-7.79 (m, 12H, Ar-H), 10.54 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 45.2 (Ar-CH), 12.6 (Ar-CH_3), 110.7, 113.4, 118.3, 119.7, 120.6, 121.7, 123.4, 126.2, 127.2, 129.2, 131.4, 134.6, 137.2, 141.9 ppm; MS: m/z = 384.64 $(M+1)^+$.

2.7.14. 3,3'-((4-chlorophenyl)methylene)bis(2 Methyl-1H-indole) (3n, $C_{25}H_{21}ClN_2$)

Brownish red solid; M.p.: 180-181°C; FT-IR (KBr): ν = 3380 (NH), 1626 (C=C), 2940(C-H), 600 (C-Cl) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.52 (s, 1H, Ar-CH), 2.28 (s, 6H, 2CH3), 7.22-7.99 (m, 12H, Ar-H), 10.84 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 46.2 (Ar-CH), 12.5 (Ar-CH_3), 111.7, 114.4, 118.3, 119.7, 120.6, 121.7, 126.2, 127.2, 129.2, 130.4, 134.6, 141.2 ppm; MS: m/z = 384.64 $(M+1)^+$.

2.7.15. 3,3'-((4-bromophenyl)methylene)bis(2-Methyl-1H-indole) (3o, $C_{25}H_{21}BrN_2$)

Brick red solid; M.p.: 175-176°C; FT-IR (KBr): ν = 3420 (NH), 1619, 1508, 1450 (C=C), 2982 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.56 (s, 1H, Ar-CH), 2.28 (s, 6H, 2CH3), 6.86-7.89 (m, 12H, Ar-H), δ 10.90 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 43.9 (Ar-CH), 12.3 (Ar-CH_3), 111.4, 117.4, 118.1,120.8, 121.5, 124.2, 126.4, 127.8, 130.0, 133.4, 136.5, 138.9, 12.4 ppm; MS: m/z = 429.1 $(M+1)^+$.

2.7.16. 3,3'-((4-methoxyphenyl)methylene)bis(2-methyl-1H-indole) (3p, $C_{26}H_{24}N_2O$)

Wine red solid; M.p.: 140-143°C; FT-IR (KBr): ν = 3364 (NH), 1625 (C=C), 2958 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.72 (s, 3H, -OCH3), 5.42 (s, 1H, Ar-CH), 2.2 (s, 6H, $2CH_3$), 6.97-7.59 (m, 12H, Ar-H), 10.78 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 45.4 (Ar-CH), 12.4 (indolyl-CH_3), 55.5 (Ar-OCH_3), 111.4, 112.4, 114.3, 118.7, 119.6, 121.7, 125.4, 126.2, 130.4, 133.6, 136.2, 146.7 ppm; MS: m/z = 381.09 $(M+1)^+$.

2.7.17. 3,3'-((3-methoxyphenyl)methylene)bis(2-methyl-1H-indole) (3q, $C_{26}H_{24}N_2O$)

Gray solid; M.p: 130-131°C, FT-IR (KBr): ν= 3364 (NH), 1626 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 3.72 (s,3H, -OCH3), 5.54 (s, 1H, Ar-CH), 2.5 (s, 6H, $2CH_3$), 6.76-7.79 (m, 12H, Ar-H), 10.78 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ 46.4 (Ar-CH), 12.4 (indolyl-CH_3), 55.8 (Ar-OCH_3), 111.4, 112.4, 114.3, 118.7, 119.6, 121.7, 125.4, 126.2, 130.4, 131.2, 133.6, 136.2, 139.5, 148.7 ppm; MS: m/z = 381.09 $(M+1)^+$.

2.7.18. 3, 3''-((4-nitrophenyl) methylene) bis (2-methyl-1H-indole) (3r, $C_{25}H_{21}N_3O_2$)

Light orange solid; M.p: 217-219°C; FT-IR (KBr): ν = 3368 (NH), 1628 (C=C), 2940 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.58 (s, 1H, Ar-CH), 2.5 (s, 6H, $2CH_3$), 6.72-7.92 (m, 12H, Ar-H), 10.90 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 41.3 (Ar-CH), 12.4 (indolyl-CH_3), 111.5, 112.8, 118.6, 119.3, 120.8, 121.0, 123.3, 124.9, 126.1, 129.4, 136.7, 145.6 ppm; MS: m/z = 395.16 (M^+).

2.7.19. 3, 3''-((4-nitrophenyl) methylene) bis (2-methyl-1H-indole) (3s, $C_{25}H_{21}N_3O_2$)

Light orange solid; M.p.: 217-219°C; FT-IR (KBr): ν= 3368 (NH), 1628 (C=C), 2940 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.58 (s, 1H, Ar-CH), 2.5 (s, 6H, $2CH_3$), 6.72-7.92 (m, 12H, Ar-H), 10.90 (bs, 2H,

NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 41.3 (Ar-CH), 12.4 (indolyl-CH_3), 111.5, 112.8, 118.6, 119.3, 120.8, 121.0, 123.3, 124.9, 126.1, 129.4, 136.7, 145.6 ppm; MS: m/z = 395.16 (M^+).

2.7.20. 3,3'-(thiophen-2-ylmethylene)bis(2-Phenyl-1H-indole) (3t, $C_{33}H_{24}N_2S$)

Yellow solid; M.p.: 196-197°C; FT-IR (KBr): ν = 3310 (NH), 1590 (C=C), 2923 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.38 (s, 1H, Ar-CH), 6.48-7.79 (m, 21H, Ar-H), 10.62 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 40.1 (Ar-CH), 109.4, 111.4, 119.3, 119.7, 120.5, 123.6, 124.7, 126.2, 126.9, 127.4, 129.6, 130.2, 131.7, 133.2, 136.7, 142.5 ppm; MS: m/z = 481.10 $(M+1)^+$.

2.7.21. 3,3'-(thiophen-2-ylmethylene)bis(2-methyl-1H-indole) (3u, $C_{23}H_{20}N_2S$)

Chocolate brown solid; M.p.: 130-131°C; FT-IR (KBr): ν = 3330 (NH), 1625 (C=C), 2920 (C-H) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.7 (s, 1H, Ar-CH), 2.5 (s, 6H, 2CH_3), 6.97-7.89 (m, 11H, Ar-H), 10.60 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 39.8 (Ar-CH), 12.4 (indolyl-CH_3), 111.4, 112.4, 116.4, 119.3, 121.7, 123.6, 124.7, 125.4, 126.2, 130.4, 135.6, 138.2 ppm; MS: m/z = 356.13 (M^+).

2.7.22. 3,3'-((5-nitrofuran-2yl)methylene)bis(2-methyl-1H-indole) (3v, $C_{23}H_{19}N_3O_3$)

Light yellow solid; M.p.: 130-131°C; FT-IR (KBr): ν = 3372 (NH), 1635 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ = 5.7 (s, 1H, Ar-CH), 2.5 (s, 6H, 2CH_3), 6.99-8.16 (m, 10H, Ar-H), 10.59 (bs, 2H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 39.7 (Ar-CH), 11.7 (indolyl-CH_3), 110.4, 111.2, 116.4, 119.3, 121.7, 123.6, 124.7, 125.4, 126.2, 130.4, 139.6, 140.2 ppm; MS: m/z = 385.14 (M^+).

3. RESULTS AND DISCUSSION

The efficient utilization *p*-toluenesulfonic acid as a catalyst for different organic reactions is due to its cost-effectiveness and easy availability. Initially, the reaction between indole (1) (2 mmol) with benzaldehyde (2) (1.1 mmol) utilizing water as solvent was carried out in presence of *p*-toluenesulfonic acid as a catalyst for 6 h, at room temperature, and the yield of 72% was obtained. In accordance to the result obtained, different Lewis acids have been utilized using acetonitrile as a solvent for the former reaction between compound 1 & 2, in order to know their catalytic efficiency. Among the reactions observed with different catalyst, *p*-toluenesulfonic acid (Table 1, entry 1), indicated its highest catalytic activity with the completion of reaction within 2 h. In order to optimize the reaction conditions, the effect of different solvents was also observed (Table 2), and the acetonitrile was found to be an effective solvent. It was also observed from the results (Table 2, entry 6) that optimum concentration of 5 mol % of *p*-Toluenesulfonic was required to achieve high yield in shorter reaction times. Thus, the reaction of indole (1) with carbonyl compounds (2) led to the formation of new bis(indolyl) methane derivatives 3(a-v) (Scheme 1) under the optimized reaction conditions in both conventional method and ultrasound irradiation method (Table 3).

Table 1: The reaction of indole (1) with benzaldehyde (2) in the presence of various catalysts using acetonitrile as solvent

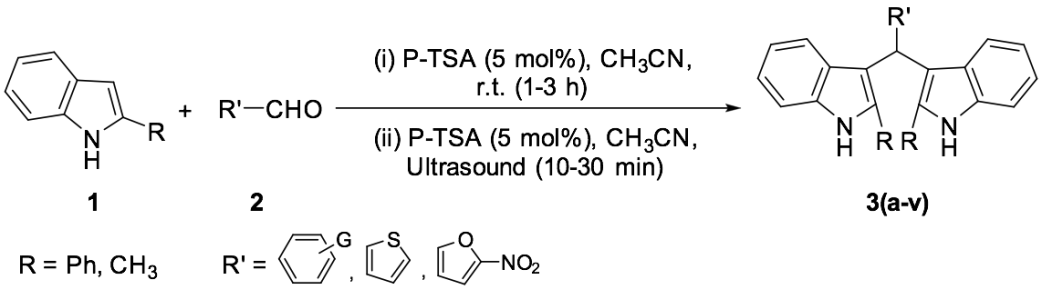
Entry	Catalyst[a]	Time (h)	Yield (%)[b]
1	$CH_3C_6H_4SO_3H$	1.5	86
2	$ZrCl_4$	2.5	79
3	Fe_3O_4	3	74
4	$ZrOCl_2$	3.5	78
5	H_3BO_3	12	65
6	Sulfamic acid	10	70
7	HF	8	72
8	$FeCl_3$	9	68
9	$HClO_4$	3	78
10	$LiClO_4$	10	45

[a] *5 mol% of the catalyst was used,* [b] *Yields referred to the isolated yield*

Table 2: The reaction of indole (1) with benzaldehyde (2) in the presence of various catalysts using acetonitrile as solvent

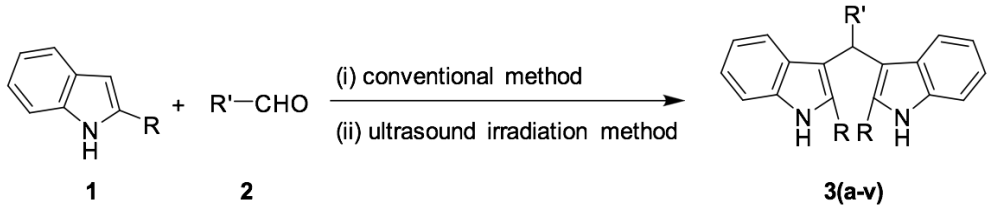
Entry	Solvent	Time (h)	Yield (%)[a]
1	Water	6	72
2	Methanol	10	60
3	Ethanol	9	65
4	1-Propanol	12	62
5	Tetrahydrofuran	8	80
6	CH_3CN	2.5	90
7	Dichloromethane	7.5	68
8	Toluene	18	40
9	Acetone	8.5	62
10	Benzene	10	55

[a] *Yields referred to the isolated yield*



Scheme

Table 3: Preparation of bis(indolyl)methanes (BIMs) 3(a-v) catalyzed by *P*-TSA in acetonitrile under conventional and ultrasound irradiation methods



Entry	R	R'CHO	R'	Time (min)		Isolated Yield (%)	
				(i)	(ii)	(i)	(ii)
1	Ph	PhCHO	C_6H_5 (**3a**)	90	18	86	92
2	Ph	4-FC_6H_4CHO	4-FC_6H_4 (**3b**)	90	22	79	86
3	Ph	2-ClC_6H_4CHO	2-ClC_6H_4 (**3c**)	82	25	81	89
4	Ph	4-ClC_6H_4CHO	4-ClC_6H_4 (**3d**	90	26	79	85
5	Ph	4-BrC_6H_4CHO	4-BrC_6H_4 (**3e**)	92	25	74	81
6	Ph	4-$CH_3C_6H_4CHO$	4-$CH_3C_6H_4$ (**3f**)	95	30	74	85
7	Ph	4-$OCH_3C_6H_4CHO$	4-$OCH_3C_6H_4$ (**3g**)	90	28	78	88
8	Ph	4-$NO_2C_6H_4CHO$	4-$NO_2C_6H_4$ (**3h**)	85	16	85	90
9	Ph	3-$NO_2C_6H_4CHO$	3-$NO_2C_6H_4$ (**3i**)	80	25	82	89
10	Ph	2-OH,4-$OCH_3C_6H_3CHO$	2-OH,4-$OCH_3C_6H_3$ (**3j**)	120	30	80	90
11	CH_3	PhCHO	C_6H_5 (**3k**	80	16	89	92
12	CH_3	4-FC_6H_4CHO	4-FC_6H_4 (**3l**)	90	25	74	85
13	CH_3	2-ClC_6H_4CHO	2-ClC_6H_4 (**3m**)	90	20	76	86
14	CH_3	4-ClC_6H_4CHO	4-ClC_6H_4 (**3n**)	90	22	79	87
15	CH_3	4-BrC_6H_4CHO	4-BrC_6H_4 (**3o**)	120	23	76	82
16	CH_3	4-$OCH_3C_6H_4CHO$	4-$OCH_3C_6H_4$ (**3p**)	96	20	80	86
17	CH_3	3-$OCH_3C_6H_4CHO$	3-$OCH_3C_6H_4$ (**3q**)	120	25	79	84
18	CH_3	4-$NO_2C_6H_4CHO$	4-$NO_2C_6H_4$ (**3r**)	90	14	82	92
19	CH_3	3-$NO_2C_6H_4CHO$	3-$NO_2C_6H_4$ (**3s**)	90	18	80	90
20	Ph	C_4H_3SCHO	C_4H_3S (**3t**)	85	22	82	92
21	CH_3	C_4H_3SCHO	C_4H_3S (**3u**)	80	16	89	94
22	CH_3	5-$NO_2C_4H_2OCHO$	5-$NO_2C_4H_2O$ (**3v**)	90	20	79	85

3.1. *In vitro* antioxidant activity of compounds by DPPH assay method

The compounds 3(a-v) were evaluated for antioxidant activities by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method. Ascorbic acid was used as standard. The percent radical scavenging activity (RSA%) of free radical production from DPPH was calculated by the following equation.

$$\%RSA = [(A_C - A_S)/A_C] \times 100 \quad (1)$$

Where A_C is the absorbance of the control and A_S is the absorbance of the tested samples. The antioxidant activity was expressed in terms of IC_{50} value which is

defined as the concentration (μg/mL) of compound at which 50% of DPPH reduction was observed, this may be to the presence of electron donating Substituents such as methoxy/hydroxyl at *para* position and the *meta* position in phenyl ring of bis(indolyl)methane derivatives as shown in Table 4 and Fig. 1. The compounds 3g, 3t, 3j, 3p and 3u, showed potent antioxidant activity. 2-(Bis(2-phenyl-1*H*-indol-3-yl) methyl)-5-methoxyphenol (3j) exhibited highest DPPH radical scavenging activity and minimum IC_{50} value when compared with other compounds. The compounds 3a, 3f, 3g, 3j, 3k, 3p, 3q and 3u showed good to moderate antioxidant activity, whereas the compounds 3b, 3c, 3d, 3e, 3l, 3m, 3n, 3o and 3v displayed least activity. However, the compounds 3h, 3i, 3r and 3s exhibited no activity.

Table 4: Antioxidant Activity of Synthesized Compounds

Compound	IC_{50} (μg/mL)	pIC_{50}(μg/mL)
3a	65.18	4.1859
3b	78.15	4.1071
3c	79.2	4.1013
3d	76.52	4.1162
3e	74.14	4.1299
3f	63.12	4.1998
3g	54.22	4.2658
3h	NA	NA
3i	NA	NA
3j	46.51	4.3325
3k	62.72	4.2026
3l	77.14	4.1127
3m	80.22	4.0957
3n	79.46	4.0999
3o	72.54	4.1394
3p	52.12	4.283
3q	61.17	4.2135
3r	NA	NA
3s	NA	NA
3t	57.18	4.2428
3u	52.7	4.2782
3v	92.15	4.0355
Ascorbic acid	40.35	-

3.2. 3D-QSAR Studies

The 18 indole derivatives of our research work are subjected to QSAR studies using the SYBYL-X 2.1.1 package (Tripos, Inc., USA). The biological activity of synthesized compounds was reported in IC_{50}, which were converted to the corresponding negative logarithm of IC_{50} (pIC_{50}= -logIC_{50}) (Table 4). Initially, the energy of the molecules was optimized by Tripos force field and Gasteiger-Huckel charge [39]. The energy minimization was accomplished with 10000 iterations and 0.01 Kcal mol^{-1} energy gradient convergence criterions. In the next step, the molecular alignment was done by pharma-cophore-based module (Fig. 2). Bis-indole scaffold is used as pharmacophore for molecular alignment (Fig. 3). The statistical parameters, the cross-validated correlation (q^2), the non-cross-validated correlation coefficient (r^2), and the standard error of estimate (SEE) are used for evaluation of the model. The partial least square (PLS) was used for cross-validation analysis, which regarded the descriptors of force fields as independent variables and the pIC_{50} values as the dependent variables for 3D-QSAR analysis. To develop a good model, the selection of training and test set was carried out in such a way that both the sets contain structurally diverse compounds and activities of all ranges. In the CoMFA method steric and electrostatic fields were calculated using Lennard-Jones and Coulombic potentials respectively. A 3D cubic lattice having a grid spacing of 2.0 Å was generated automatically to surround the aligned molecules in all directions. These grid points were generated using the Tripos force field, a sp^3 carbon atom probe with a Van der Waals radius of 1.52 Å, and a charge of +1.00 (default probe atom in SYBYL) to calculate various steric and electrostatic fields. The default energy cut off for steric and electrostatic fields was 30 kcal/mol [40].

The statistical result for the best CoMFA model was summarized in Table 5. In the best CoMFA model, the PLS regression analysis obtained the q2 of 0.192 and the ONC of 2. Then, the non-cross validation gave the r2 of 0.864 with the SEE of 0.031. The residual values are shown in the table which is the difference between the predicted p IC_{50} and the actual p IC_{50} were obtained near to zero shown in Table 6. The contributions of steric (Green region represents desirable steric bulk whereas yellow region represents undesirable steric bulk) and electrostatic (blue region represents requirement of positively charged substituents and the red region represents negatively charged substituents) fields to CoMFA model shown in Table 5.

The 3D contour map revealed that steric bulk near the green colour area is required to improve the activity shown in Fig. 4(a). While Fig. 4(b) revealed that addition of positive charged substituent near the blue region which indicated over phenyl ring, can improve the activity. While red region over one of the indole

rings indicated that the negative charged substituent can reduce the activity. Therefore, in future design considering above all points may lead to development of a better molecule.

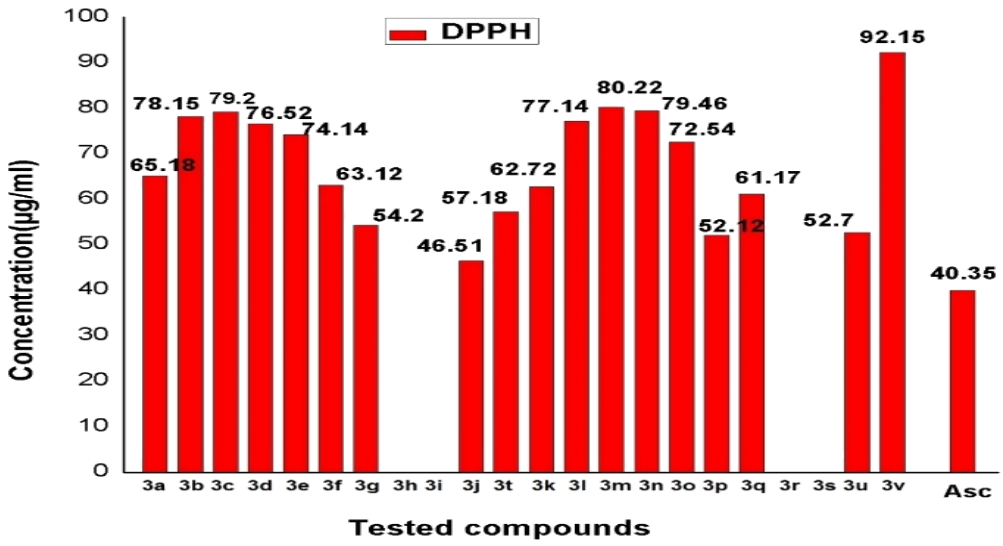


Fig. 1: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and IC_{50} values

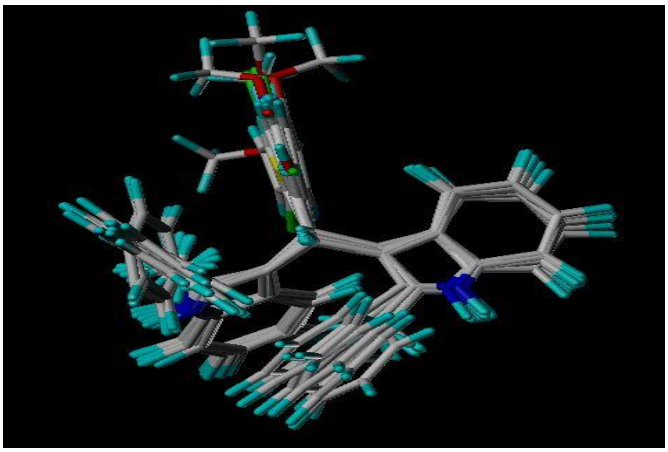


Fig. 2: Molecular alignment of 18 compound

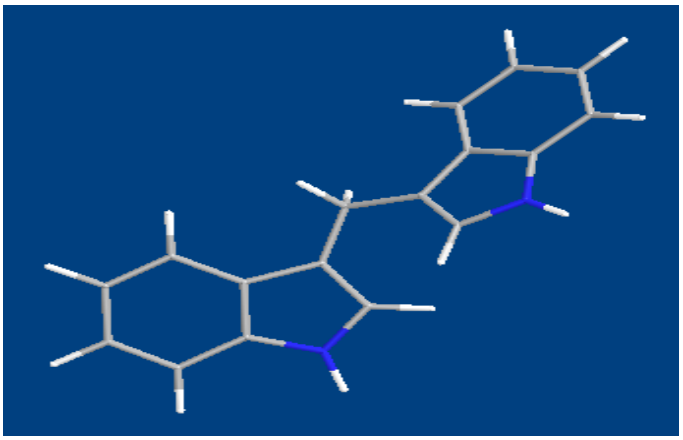


Fig. 3: Bis-indole template for alignment

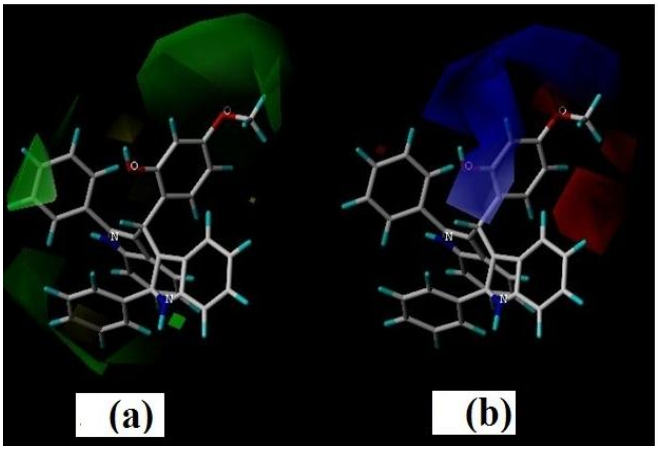


Fig. 4: 3D contour map of CoMFA model. Green region represents desirable steric bulk whereas yellow region represents undesirable steric bulk (a). Blue region represents requirement of positively charged Substituents and red region represents negatively charged Substituents (b).

Table 5: PLS Statistical Results of CoMFA

Entry	Statistical parameter	CoMFA values
1	q2	0.192
2	r2	0.864
3	SEE	0.0305
4	ONC	2
	Field contribution	
5	Steric	0.47
6	Electrostatic	0.53

Table 6: Experimented and predicted activity of synthesized molecules

Compound	Experimented value	Predicted value	Residual value
3a	4.1859	4.1515	0.0344
3b	4.1071	4.1068	0.0003
3c	4.1013	4.159	-0.0577
3d	4.1162	4.1549	-0.0387
3e	4.1299	4.135	-0.0051
3f	4.1998	4.2018	-0.002
3g	4.2658	4.1746	0.0912
3h	NA	NA	NA
3i	NA	NA	NA
3j	4.3325	4.1545	0.178
3k	4.2026	4.1562	0.0464
3l	4.1127	4.1084	0.0043
3m	4.0957	4.1326	-0.0369
3n	4.0999	4.1267	-0.0268
3o	4.1394	4.1324	0.007
3p	4.283	4.2578	0.0252
3q	4.2135	4.2091	0.0044
3r	NA	NA	NA
3s	NA	NA	NA
3t	4.2428	4.2594	-0.0166
3u	4.2782	4.2518	0.0264
3v	4.0355	4.0044	0.0311

4. CONCLUSION

The reaction of indole (1) with carbonyl compounds (2) in the presence of P-TSA (5 mol %) as a catalyst in acetonitrile was carried out to obtain bis(indolyl) methane derivatives 3(a-v) adopting conventional and ultrasonication methodologies. It was observed that the reaction completed in shorter reaction times, higher yields, in ultrasonication methodology, as compared to conventional methods. The synthesized compounds were tested for antioxidant activity, and it was observed that, 3j was the compound which showed the maximum antioxidant activity. Furthermore, to enhance the antioxidant property, computational investigation based on CoMFA models was performed. The best model suggested that by changing the steric and electrostatic property may lead to development of a potent molecule. Our suggested requirements of the molecular structures identified through 3D-QSAR are consistent with the experimental results, which can help in designing more active antioxidant activity.

Conflicts of interest

The authors declare no conflict of interest.

5. ACKNOWLEDGEMENTS

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