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Synthesis, Characterization and Evaluation of Anti-inflammatory Activity of Novel Indoline Derivatives

Dhineshkumar Manoharan [a*], Kannan Kulanthai[a] and Gnanavel Sadhasivam[a]
[a*] Department of chemistry, Government College of Engineering, Salem-636011, Tamil Nadu, India.
[a] Department of chemistry, Government College of Engineering, Salem-636011, Tamil Nadu, India.
[*]E-mail: dhineshkavi2020@gmail.com, Phone: + 91 9787809030

Abstract

Series of indoline derivatives were synthesized using N-(4-aminophenyl) indoline-1-carbothiamide as a precursor. The structures of synthesized compounds were confirmed by FT-IR, ^{1}H-NMR, ^{13}C-NMR and LC-MS. The *in vitro* anti-inflammatory activity of synthesized indoline derivatives were examined by standard anti-denaturation assay. The compounds **4a** (IC_{50} = 62.2 µg/ml) and **4b** (IC_{50} = 60.7 µg/ml) showed potent inhibition on protein denaturation. The compounds **5a** (IC_{50} = 97.8 µg/ml) exhibits moderate inhibition on protein denaturation.

Keywords: indoline, anti-inflammatory , anti-denaturation, ^{1}H-NMR .

1. Introduction

Heterocyclic compounds containing five or six membered ring are essential for their various biological activities [1]. Indoline the potent basic pharmacodynamic nucleus, has been reported to possess a wide variety of biological properties such as antimicrobial [2-5], anticancer [6-10], anti-diabetic [11], anti-inflammatory [12-13], antitubulin[14], anti-hyperlipidemic [15], anti-atherosclerotic [15], anticoagulant [16], tyrosinekinase inhibitor [17], antiperoxidative [18], antioxidant [19], anti-hepatocellularcarcinoma [20], Antimitoic [21] and Antagonists[22-23].

In addition, the indoline nucleus is incorporated in various natural products such as alkaloids [24]. Encouraged by the above observations and considering the interesting pharmacological profile of indoline, we synthesized N-(4-aminophenyl)indoline-1-carbothioamide scaffold based compounds as anti-inflammatory agents. The biological activity and structure–activity relationship (SARs) of the newly synthesized indoline derivatives discussed.

2. EXPERIMENTAL

2.1. Chemistry

The synthetic starting materials such as reagents and solvents were used in analytical grade or the highest quality commercially available and were purchased from Sigma-Aldrich Chemical Co., Merck Chemical Co. Melting points were recorded by labtronics digital melting points apparatus. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 solvent on Bruker 300 MHz spectrophotometer using tetramethylsilane as an internal reference. The apparent resonance multiplicity is described as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), td (triplet of doublet), q (quartet) and m (multiplet). Infrared measurements were recorded in the range 400-4000 cm^{-1} by Perkin Elmer. Mass spectra were recorded on a Thermo LCQ Deca XP MAX at 70eV. Thin layer chromatography (TLC) analysis was carried out on 5 x 20 cm plate coated with silica gel GF_{254}.

2.1.1. Synthesis of N-(4-nitrophenyl) indoline-1-carbothioamide (1)

To a solution of indoline (10 g, 0.0835 mol) in 200 mL of THF, 4-nitrophenyl isothiocyanate (16.53 g, 0.0918 mol) was added at 0^{o}C. The resulting reaction mass was allowed to RT and stirred for 4 hr. The reaction mass was concentrated under reduced pressure to give the compound **1** as a yellow solid; Yield 80%; m.p 173-174 ^{O}C; IR (KBr) ν_{max} in cm^{-1} : 3434, 3300 (NH stretch), 3074 (CH stretch, aromatic), 2919, 2850 (CH stretch, aliphatic), 1532, 1319, (NO_2 stretch), 1477 (CH_2 bend), 1270 (C=S), 909 (C-N stretch) ; ^{1}H-NMR (DMSO-d_6) δ ppm: 3.13 (t, 2H, *J* = 8.1 Hz), 4.32 (t, 2H, *J* = 8.2 Hz), 7.05 (td, 1H, *J* = 0.9, 7.9 Hz), 7.18 (td, 1H, *J* = 1.2, 8.1 Hz), 7.33 (d, 1H, *J* = 7.9 Hz), 7.71 (d, 2H, *J* = 9.9 Hz), 7.80 (d, 1H, *J* = 8.0 Hz), 8.19 (d, 2H, *J* = 9.9 Hz), 10.39 (s,1H); ^{13}C-NMR (DMSO-d_6) δ ppm: 26.6, 54.2, 116.0, 121.9, 123.9, 124.1, 125.6, 126.8, 134.3, 142.1, 142.2, 146.8, 177.5; LC-MS calculated for ($C_{15}H_{13}N_3O_2S$) m/z $[M + H]^+$ 299.07, found m/z 300.0665.

2.1.2. Synthesis of N-(4-aminophenyl) indoline-1-carbothioamide (2)

To a solution of compound 1 (10 g, 0.0338 mol) in 100 mL 12N HCl, $SnCl_2$ (63.88 g, 0.338 mol) was added at RT. The resulting reaction mass was stirred for 3 hr. The completion of the reaction was monitored by TLC. The reaction mass was diluted with 350 mL of cold water. The resulting reaction mass was basified to pH 8 with 40% NaOH. The aqueous layer was extracted with ethyl acetate (3 x 150 mL) and water (2 x 30 mL), brine (1 x 300 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to afford compound **2**. Brown solid; Yield 72% ; mp 209-210 ^{o}C; IR (KBr) ν_{max} in cm^{-1} : 3398 (NH stretch), 3055 (CH stretch, aromatic), 2969 (CH stretch, aliphatic), 1610 (NH bend), 1447 (CH_2 bend), 1209 (C=S), 932 (C-N stretch) ; ^{1}H-NMR (DMSO-d_6) δ ppm: 3.08 (t, 2H, *J* = 8.1 Hz), 4.21 (t, 2H, *J* = 8.2 Hz), 5.05 (s, 2H), 6.52 (d, 2H, *J* = 8.7 Hz), 6.95 (t, 3H, *J* = 7.2 Hz), 7.12 (t, 1H, *J* = 7.8 Hz), 7.24 (d,1H, *J* = 7.9 Hz), 8.29 (d, 1H, *J* = 8.1 Hz), 9.41 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 52.8, 113.3, 116.1, 122.5, 124.9, 126.1, 126.8, 128.5, 133.3, 143.4, 146.3, 178.5; LC-MS calculated for ($C_{15}H_{15}N_3S$) m/z $[M + H]^+$ 269.100, found m/z 270.0979.

2.1.3. Synthesis of 1-(4-fluorophenyl)-3-(4-(indoline-1-carbothioamido)phenyl)thiourea (3a)

To a solution of compound 2 (0.4 g, 0.0015 mol) in 12 mL THF, 4-fluoro phenyl isothiocyanate (0.300 g, 0.0019 mol) was added at 0^{o}C. The reaction mixture was allowed to RT and stirred for 2 hr. The progress of the reaction was monitored by TLC. The reaction mass was concentrated under reduced pressure and separated between ethyl acetate (1 x 50 mL) and 2N HCl (1 x 25 mL). The resulting ethyl acetate layer was washed with brine (1 x 40 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound **3a.** Pal yellow solid; Yield 32%; mp 259-261 ^{o}C; IR (KBr) v_{max} in cm^{-1}: 3424 (NH stretch), 2926 (CH stretch, aromatic), 2581 (CH stretch, aliphatic), 1590 (NH bend), 1449 (CH_2 bend), 1291 (C-N stretch), 1211 (C=S), 761 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.10 (t, 2H, *J* = 8.3 Hz), 4.26 (t, 2H, *J* = 8.1 Hz), 6.99 (t, 1H, *J* = 7.8 Hz), 7.13-7.15 (m, 3H), 7.29 (d, 1H, *J* = 8.3 Hz), 7.27-7.50 (m, 6H), 8.09 (d, 1H, *J* = 8.4 Hz), 9.76 (s, 3H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 53.3, 114.8, 115.1, 123.0, 123.6, 125.2, 126.0, 126.4, 133.7, 135.7, 143.0, 157.8, 160.2,178.2, 179.9; LC-MS calculated for ($C_{22}H_{19}FN_4S_2$) m/z [M + H]$^{+}$ 422.1, found m/z 423.0681.

2.1.4. Synthesis of 1-(4-(indoline-1-carbothioamido) phenyl)-3-(3-methoxy phenyl)urea (3b)

To a solution of compound 2 (0.5 g, 0.0018 mol) in 15 mL THF, 3-methoxy phenyl isocynate (0.322 g, 0.0024 mol) was added at 0^{o}C. The reaction was carried out at RT and stirred for 1 hr. The completion of reaction was monitored by TLC. To the resulting residue, diethyl ether was added and the solid was filtered off. The resulting white solid was dried under vacuum to afford compound **3b**. Yield 76% ; mp 246-248 ^{o}C ; IR (KBr) v_{max} in cm^{-1}: 3287 (NH stretch), 2950 (CH stretch, aromatic), 2838 (CH stretch, aliphatic), 1649 (C=O), 1558 (NH bend), 1491 (CH_2 bend), 1291 (C-N amide), 1230 (C=S), 1160 (C-O), 763 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.10 (t, 2H, *J* = 8.1 Hz), 3.70 (s, 3H), 4.27 (t, 2H, *J* = 8.1 Hz), 6.52 (dd, 1H, *J* = 2.1, 8.1 Hz), 6.92-7.00 (m, 2H), 7.11-7.27 (m, 6H), 7.43 (d, 2H, *J* = 8.7 Hz), 8.23 (d, 1H, *J* = 8.2 Hz), 9.41 (d, 2H, *J* = 9.1 Hz), 9.67 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 53.1, 54.8, 103.5, 106.8, 110.1, 116.1, 117.6, 122.8, 125.0, 125.8, 126.2, 129.4, 133.6, 136.9, 141.2, 141.2, 143.2, 152.7, 159.6, 178.4 ; LC-MS calculated for ($C_{23}H_{22}N_4O_2S$) m/z [M + H]$^{+}$418.15, found m/z 419.1155.

2.1.5. Synthesis of N-(4-(tosylamino) phenyl) indoline-1-carbothio amide (4a)

To a solution of compound 2 (0.4 g, 0.00150 mol) in 10 mL THF, pyridine (0.474 mL, 0.006 mol) was added at 0^{o}C followed by 4-toluene sulfonyl chloride (0.285 g, 0.00150 mol) in 2 mL 1,2-dichloroethane was added . The reaction mixture was allowed to RT and stirred for 3 hr. The progress of the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (1 x 30 mL), 10 % $NaHCO_3$ (2 x 25 mL), brine (2 x 50 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound **4a**. White solid; Yield 48 % mp 220-222 ^{o}C; IR (KBr) v_{max} in cm^{-1} : 3423 (NH stretch), 3282, 3059 (CH stretch, aromatic), 2921 (CH stretch, aliphatic), 1595 (NH bend), 1516 (CH_2 bend), 1371, 1154 (S=O), 749 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.34 (s, 3H), 3.08 (t, 2H, *J* = 8.4 Hz), 4.22 (t, 2H, *J* = 8.4 Hz), 6.95-7.09 (m, 3H), 7.11 (t, 1H, *J* = 7.8 Hz), 7.20-7.27 (m, 3H), 7.35 (d, 2H, *J* = 8.1 Hz), 7.64 (d, 2H, *J* = 8.1 Hz), 8.9 (d, 1H, *J* = 8.1 Hz), 9.63 (s, 1H), 10.18 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ ppm: 20.9, 26.6, 53.1, 116.0, 120.1, 123.0, 125.1, 125.66, 126.3, 126.6, 129.6, 133.6, 134.2, 136.2, 136.7, 142.8, 143.1, 178.1; LC-MS calculated for ($C_{22}H_{21}N_3O_2S_2$) m/z [M + H]$^{+}$ 423.11, found m/z 424.0771.

2.1.6. Synthesis of N-(4-{[(2,6-dichlorophenyl)sulfonyl]amino}phenyl) indoline-1-carbothio amide (4b)

Prepared as reported above for 4a, starting from compound 2 and 2,6-dichlorobenzene-1-sulfonyl chloride. This reaction was carried out at room temperature for 3 hr. Red solid; Yield 37 % ; mp 215-217 ^{o}C ; IR (KBr) v_{max} in cm^{-1}: 3432 (NH stretch), 3360, 3099 (CH stretch, aromatic), 2922, 2850 (CH stretch, aliphatic), 1600 (NH bend), 1519 (CH_2 bend), 1387, 1165 (S=O), 747 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.09 (t, 2H, *J* = 8.1 Hz), 4.21 (t, 2H, *J* = 8.2 Hz), 7.04-7.07 (m, 2H), 7.14 (d, 1H, *J* = 8.5 Hz), 7.23-7.26 (m, 3H), 7.52-7.71 (m, 3H), 8.10 (d, 1H, *J* = 8.4 Hz), 9.63 (s,1H), 10.78 (s,1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 27.1, 53.7, 116.5, 119.6, 123.5, 125.6, 126.3, 126.8, 127.6, 130.7, 132.4, 133.7, 134.2, 134.92, 136.8, 143.4,178.7 ; LC-MS calculated for ($C_{21}H_{17}Cl_2N_3O_2S_2$) m/z [M + H]$^{+}$ 477.01, found m/z 477.9745

2.1.7. Synthesis of 2-(1H-indol-3-yl)-N-(4-(indoline-1-carbothio amido) phenyl) acetamide (5a)

To a solution of compound 2 (0.4 g, 0.00150 mol) in 12 mL , 3-indole acetic acid (0.316 g, 0.00181 mol), EDC.HCl (0.370 g, 0.00195 mol) and HOBt (0.202 g, 0.00150 mol) was added. The mixture was cooled to 0^{o}C and triethylamine (0.818 mL, 0.006 mol) was added. The resulting solution was allowed to RT and stirred for 8 hr. The reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure and resulting crude product was separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (1 x 30 mL), 10% $NaHCO_3$ (2 x 20 mL), brine (2 x 50 mL) and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to afford compound **5a**. Pal yellow solid; Yield 40%; mp 243-245 ^{O}C; IR (KBr) v_{max} in cm^{-1}: 3406 (NH stretch), 3310, 3184 (CH stretch, aromatic), 2904 (CH stretch, aliphatic), 1657 (C=O), 1527 (NH bend), 1482 (CH_2 bend), 1291 (C-N), 1254 (C=S), 788 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.09 (t, 2H, *J* = 8.1 Hz), 3.72 (s, 2H), 4.24 (t, 2H, *J* = 8.1 Hz), 6.97 (td, 2H, *J* = 1.2, 6.9 Hz), 7.0-7.15 (m, 2H), 7.28 (m, 4H), 7.35 (d, 1H, *J* = 8.1 Hz), 7.54 - 7.62 (m,3H), 8.16 (d, 1H, *J* = 8.3 Hz), 9.67 (s, 1H), 10.12 (s, 1H), 10.92 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 33.7, 53.1, 108.6, 111.35, 116.0, 118.3, 118.6, 118.9, 120.9, 122.9, 123.8, 125.1, 126.3, 127.2, 133.6, 135.0, 136.1, 136.2, 143.1, 169.5, 178.4 ; LC-MS calculated for ($C_{25}H_{22}N_4OS$) m/z [M + H]$^{+}$426.15, found m/z 427.1205.

2.1.8. Synthesis of 3-fluoro-N-(4-(indoline-1-carbothio amido)phenyl)benzamide (5b)

Prepared as reported above for 5a, starting from compound 2 and 3-fluorobenzoic acid. This reaction was carried out at RT for 7 hr. White solid; Yield 24 % ; mp 220-222 ^{o}C ; IR (KBr) ν_{max} in cm^{-1} : 3422 (NH), 3336, 3271 (CH stretch aromatic), 2930 (CH stretch aliphatic), 1657 (C=O), 1593 (NH bend), 1516 (CH_2 bend), 1388 (C-F), 1317 (C-N), 746 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.17 (t, 2H, *J* = 8.1 Hz), 4.27 (t, 2H, *J* = 8.4 Hz), 6.99 (t, 1H, *J* = 7.2 Hz), 7.15 (t, 1H, *J* = 8.1 Hz), 7.32 (d, 1H, *J* = 7.2 Hz), 7.36 (d, 2H, *J* = 8.7 Hz), 7.47 (t, 1H, *J* = 7.8 Hz), 7.56-7.63 (m, 1H), 7.71 (d, 2H, *J* = 8.5 Hz), 7.76-7.83 (m, 2H), 8.15 (d, 1H, *J* = 8.4 Hz), 9.75 (s, 1H), 10.33 (s,1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 53.2, 114.5, 116.0, 118.3, 120.3, 123.8, 125.1, 126.3, 130.5, 130.6, 133.7, 135.5, 135.8, 137.2, 143.0, 160.7, 163.1, 163.9, 178.4 ; LC-MS calculated for ($C_{22}H_{18}FN_3OS$) m/z $[M + H]^+$ 391.12, found m/z 392.0887

2.1.9. Synthesis of 2-(2-fluorophenyl)-N-(4-(indoline-1-carbothio amido) phenyl)acetamide (5c)

Prepared as reported above for 5a, starting from compound 2 and 2-fluoro phenylacetic acid. This reaction was carried out at RT for 7 hr. Yellow solid; Yield 24 % ; mp 220-222 ^{o}C ; IR (KBr) ν_{max} in cm^{-1} : 3328 (NH stretch), 3289, 3066 (CH stretch, aromatic), 2632 (CH stretch, aliphatic), 1672 (C=O), 1600 (NH bend), 1510 (CH_2 bend), 1388 (C-F), 1317 (C-N), 744 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 3.10 (t, 2H, *J* = 8.4 Hz), 3.73 (s, 2H), 4.25 (t, 2H, *J* = 8.4 Hz), 6.97 (t, 1H, *J* = 6.6 Hz), 7.11-7.21 (m, 3H), 7.27 (d, 4H, *J* = 6.8 Hz), 7.31-7.42 (m, 1H), 7.54 (d, 2H, *J* = 8.6 Hz), 8.16 (d, 1H, *J* = 8.3 Hz), 9.69 (s, 1H), 10.23 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.7, 36.2, 53.1, 115.1, 116.0, 118.9, 122.9, 123.0, 124.1, 125.1, 126.3, 128.7, 128.7, 131.9, 133.6, 135.2, 143.0, 159.4, 161.8, 167.8, 178.4 ; LC-MS calculated for ($C_{23}H_{20}FN_3OS$) m/z $[M + H]^+$ 405.13, found m/z 406.0980.

2.1.10. Synthesis of N-(4-(indoline-1-carbothio amido) phenyl)cyclopentanecarboxamide (5d)

Prepared as reported above for 5a, starting from compound 2 and cyclopentane carboxylic acid. This reaction was carried out at RT for 8 hr. White solid; Yield 27 % ; mp 213-215 ^{o}C ; IR (KBr) ν_{max} in cm^{-1} : 3298 (NH stretch), 3040 (CH stretch, aromatic), 2928, 2860 (CH stretch, aliphatic), 1670 (C=O), 1602 (NH bend), 1518 (CH_2 bend), 1317 (C-N), 744 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 1.56-1.748 (m, 7H), 1.84 (d, 2H, *J* = 8.1 Hz), 3.10 (t, 2H, *J* = 8.1 Hz), 4.25 (t, 2H, *J* = 8.4 Hz), 6.98 (t, 1H, *J* = 7.5 Hz), 7.13 (t, 1H, *J* = 7.5 Hz), 7.27 (d, 3H, *J* = 8.4 Hz), 7.55 (d, 2H, *J* = 8.7 Hz), 8.16 (d, 1H, *J* = 8.1 Hz), 9.70 (s, 1H), 9.82 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 26.1, 27.2, 30.5, 45.7, 53.6, 116.5, 119.4, 123.43, 125.6, 125.8, 126.8, 134.1, 135.4, 136.8, 143.6, 174.6, 178.9 ; LC-MS calculated for ($C_{21}H_{23}N_3OS$) m/z $[M + H]^+$ 365, found m/z 366.1384.

2.1.11. Synthesis of N-(4-(indoline-1-carbothio amido) phenyl)acetamide (5e)

To a soluction of compound 2 (0.4 g, 0.00150 mol) in 10 mL of THF, triethylamine (0.757 mL, 0.0075 mol) was added at 0^{o}C followed by acetyl chloride was added. The reaction mixture was allowed to RT and stirred for 1 hr. The reaction was monitored by TLC. The reaction mass was concentrated under reduced pressure and separated between ethyl acetate (50 mL) and water (50 mL). The combined ethyl acetate layer was washed with 2N HCl (1 x 25 mL), 10 % $NaHCO_3$ (2 x 20 mL), brine (2 x 50 mL) and dried over anhydrous Na_2SO_4. The ethyl acetate layer was concentrated under reduced pressure to afford compound 5e, white solid; Yield 42 % mp 200-202 ^{O}C; IR (KBr) ν_{max} in cm^{-1} : 3428 (NH stretch), 3303, 3044 (CH stretch, aromatic), 2928 (CH stretch, aliphatic), 1661 (C=O), 1600 (NH bend), 1517 (CH_2 bend), 1315 (C-N), 743 (CH bend); ^{1}H-NMR (DMSO-d_6) δ ppm: 2.21 (s, 3H), 3.10 (t, 2H, J = 8.4 Hz), 4.25 (t, 2H, J = 8.4 Hz), 6.9 (t, 1H, J = 7.5 Hz), 7.14 (t, 1H, J = 7.5 Hz), 7.27 (d, 3H, J = 8.7 Hz), 7.52 (d, 2H, J = 8.7 Hz), 8.16 (d, 1H, J = 8.5 Hz), 9.67 (s, 1H), 9.94 (s, 1H) ; ^{13}C-NMR (DMSO-d_6) δ ppm: 23.9, 26.5, 116.0, 118.8, 122.9, 125.4, 126.3, 133.6, 134.9, 136.1, 143.0, 168.0, 178.4 ; LC-MS calculated for ($C_{17}H_{17}N_3OS$) m/z $[M + H]^+$ 311.11, found m/z 312.0977.

2.2. PHARMACOLOGY

2.2.1. In vitro anti-inflammatory activity (Anti-denaturation assay)

The experiment was carried out with small modification [25]. The standard drug and synthesized compounds were dissolved in minimum quantity of dimethyl Formamide (DMF) and diluted with phosphate buffer (0.2 M, PH 7.4). Final concentration of DMF in all solution was less than 2.5 %. Test solution (4 ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 37°C in incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in water bath for 15 min. After cooling, the turbidity was measured at 660 nm. Percentage of inhibition of denaturation was calculated from control where no drug was added. The diclofenac sodium was used standard drug. The percentage inhibition of denaturation was calculated by using the following formula.

$$\% \text{ of Inhibition} = (At - Ac) \times 100 / At$$

At = O.D. of test solution

Ac = O.D. of control

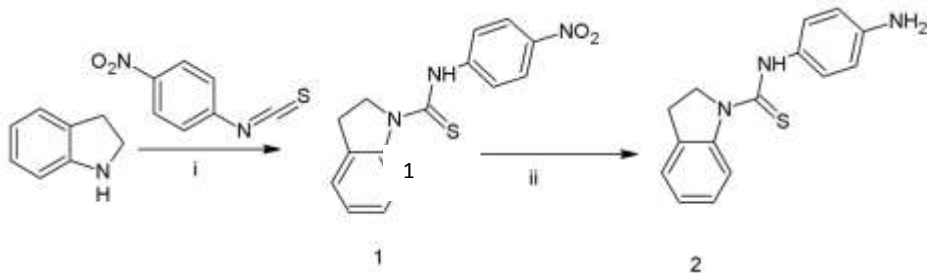
3. RESULTS AND DISCUSSION

3.1 Chemistry

The synthetic scheme for the preparation of intermediates and final compounds are shown in scheme **(1-3).** The intermediate-1 was prepared [26] by using the reagent 4-nitrophenyl isothiocyanate. The FT-IR spectrum of intermediate-1

had characteristic NH absorption peaks at 3434 cm^{-1} and 3300 cm^{-1}. The absorption peaks for C=S seen at 1270 cm^{-1}. The NO_2 stretching frequency observed at 1319 cm^{-1} and 1532 cm^{-1}. In the ^{1}H-NMR spectra of compound-I, the proton signal of thiourea NH was observed at δ 10.39 (1H, singlet). Characteristic peaks of thiocarbamide compound were observed at δ 10.39 ppm. In ^{13}C-NMR of intermediate-2, the characteristic peaks at thiocarbamide C=S resonate at 177.5 ppm. The intermediate-2 was prepared [27] by reduction of intermediate-1 by $SnCl_2$. The FT-IR spectra of intermediate-2 showed absorption band at 3398 cm^{-1} due to NH stretching vibration from resulting amine group formed. In the FT-IR spectrum of intermediate-2, the reduction of NO_2 group was confirmed by disappearance of NO_2 stretching vibration at 1319 cm^{-1} and 1532 cm^{-1}. In the ^{1}H-NMR spectra of intermediate-2, the characteristic amine proton peak seen as broad singlet at δ 5.05 ppm. The resulting compound was also confirmed by TLC, which was exhibited ninhydrin activity. The intermediate-2 was taken a common scaffold for synthesis of proposed indoline derivatives. The synthetic path way for intermediate-2 is outlined below.

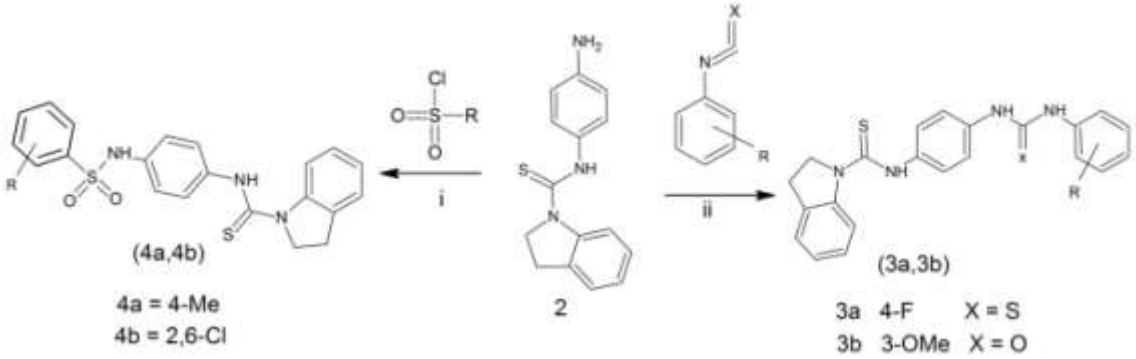
Scheme-1



Scheme.1. Reagents and conditions: (i). 4-nitrophenyl isothiocyanate, THF, 0 ^oC ; (ii) $SnCl_2$, 12N HCl, RT , 0 ^oC.

The sulfonamide derivates 4a and 4b were prepared [28] by reaction between corresponding sulfonyl chloride and key intermediate-2. The reacton was carried out in dichloroethane with pyridine base at 90 ^oC. The FT-IR spectrum resulted in sulfonamide derivatives exhibited absorption wave length at 1154 cm^{-1} and 1387 cm^{-1}. Which is the characteristic peak of (S=O) group. The ^{1}H-NMR spectra of synthesized sulfonamide derivatives showed new singlet peak at δ 9.63 ppm.

The desired title compounds **3a** and **3b** were obtained in resonable yield [29] by treating key intermediate-2 with corresponding isocyanate and isothiocyanate respectively. In the FT-IR spectrum of **3a** characteristic C=S seen at 1211 cm^{-1} while in **3b** characteristic C=O 1649 cm^{-1}. In the ^{1}H-NMR of 3a, NH proton of thiourea group resonated at δ 9.78 ppm. In the case of 3b, NH proton of new urea group resonated at δ 9.67 ppm. According to ^{13}C-NMR of 3a and 3b, the compounds has displayed characteristic thiourea carbonyl carbon C=S at δ 179.9 ppm and urea carbonyl carbon C=O at δ 159.6 ppm. The synthetic scheme for preparation of sulfonaminde, urea and thiourea derivatives are shown in **Scheme-2.**



Scheme-2 Reagents and conditions (i). Pyridine, Dichloroethane, 90 ^oC. (ii). THF, 0^oC.

From the above key intermediate-2, the carboxamide derivatives (**5a-d**) were prepared[30] by using reagent 1-Ethyl -3-(3-dimethylaminopropyl) carbodiimide (EDCI). The corresponding acid chloride was used for preparing [31] the amide derivatives 5e. The FT-IR spectrum of all the carboxamide derivatives has strong C=O absorption, which showed wave length between 1657-1672 cm^{-1}. The ^{1}H-NMR spectra of all carboxamide derivatives exhibited broad singals at δ 9.7-10.1ppm is assigned to NH. In addition, ^{13}C-NMR spectra of all carboxamide derivatives displayed signals at δ 163.9-174.6 ppm. All the FT-IR, ^{1}H-NMR and mass spectral data of compounds (**5a-e**) were in accordance with the proposed molecular structures. The purity of synthesized compounds was monitored by TLC. The synthetic scheme for the preparation of all carboxamide derivatives are depicated in **Scheme-3.**

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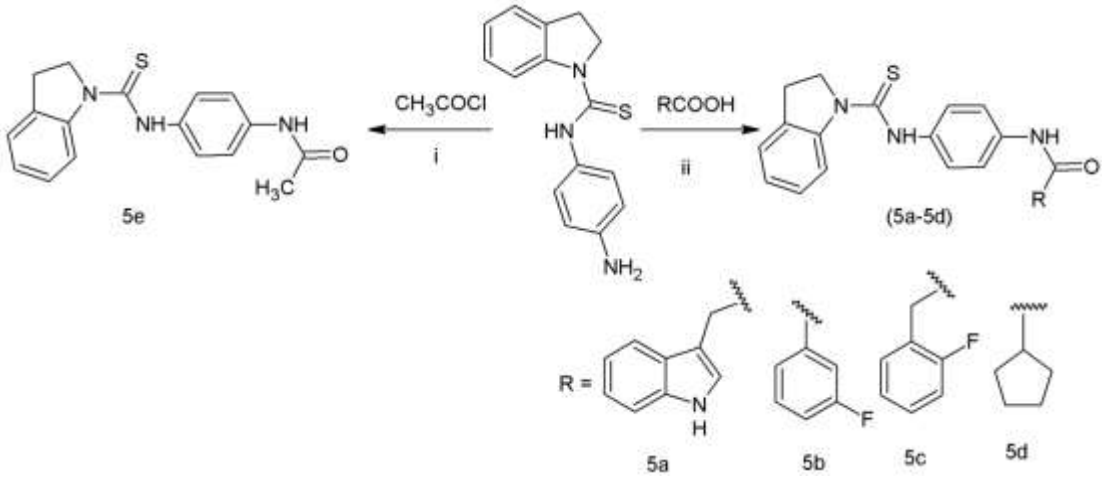

Scheme-3. Reagents and conditions (i). TEA, MDC, 0°C., (ii). EDCI, HOBt, TEA, THF, 0°C;

3.2 Anti-inflammatory activity

The anti-inflammatory activities of all the above indoline derivatives were examined by standard anti-denaturation assay. The inhibition efficiency of all synthesized indoline derivatives were tested at a concentration ranging from 50 µg/ml to 250 µg/ml. The percentage of inhibition and IC_{50} values of all indoline derivatives are listed in Table 1. The anti-denaturation activity study related that the indoline derivatives 4a (IC_{50} = 62.2 µg/ml) and 4b (IC_{50} = 60.7 µg/ml) showed excellent inhibition activity that is comparable with standard drug diclofenac sodium (IC_{50} = 54.2 µg/ml). Furthermore, in the carboxamide derivatives particularly compound 5a (IC_{50} = 97.8 µg/ml) showed moderate anti-denaturation activity. The derivatives 3a (IC_{50} = 115.4 µg/ml) and 3b (IC_{50} = 109.1 µg/ml) were found to be significant anti-denaturation activity. The compounds 5c, 5d and 5e did not show noticeable activity.

Table 1. Anti-inflammatory activity of indoline derivatives (anti-denaturation assay)

compound	% inhibition					IC_{50} (µg/ml)
	50 µg/ml	100 µg/ml	150 µg/ml	200 µg/ml	250 µg/ml	
3a	33.3	52.7	58.6	65.3	70.4	115.4
3b	35.4	53.1	60.1	64.9	69.7	109.1
4a	**45.3**	**60.1**	**68.3**	**80.7**	**86.9**	**62.2**
4b	**44.5**	**61.1**	**69.3**	**81.2**	**86.3**	**60.7**
5a	39.2	51.6	61.3	70.6	81.8	97.8
5b	17.3	27.5	45.3	59.4	64.7	180.1
5c	30.4	43.7	55.2	60.3	67.8	142.5
5d	19.2	30.4	45.6	58.4	64.3	177.4
5e	19.8	27.4	40.3	51.1	60.7	198.8
Diclofenac sodium	**47.3**	**61.8**	**70.6**	**81.5**	**90.4**	**54.2**

The *in vitro* anti-inflammatory study revealed that the synthesized final compound, containg sulfonamide group showed very good inhibition on protein denaturation. Furthermore, carboxamide based final compound bearing indole acetic acid group showed significant activity when comparing with other carboxamide based derivatives. Urea and thiourea based compounds showed noticeable inhibition on protein denaturation, which is less than sulphonamide derivatives.

The synthesized of compounds **4a** and **4b** could offer an excellent framework that may lead to the discovery of new potent anti-inflammatory agents.

4. CONCLUSION

It could be concluded that compounds **4a** (Synthesis of N-(4-(tosylamino) phenyl) indoline-1-carbothio amide) and **4b** (Synthesis of N-(4-{[(2,6-dichlorophenyl)sulfonyl]amino}phenyl) indoline-1-carbothioamide) showed potent inhibition on protein denaturation. Based on the above findings the novel indoline derivatives **4a, 4b** selected for future optimisation and development in animal models for detailed efficacy studies.

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