

Synthesis, Biological Evaluation and Structure Activity Relationship of Substituted pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocycloocta[b]indoles

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Abstract

A new class of heterocycles, (3-7) specifically substituted pyrazolo-, isoxazolo- and pyrimidocycloocta[b]indoles, has been synthesized by condensation of substituted 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones (2) with hydrazine hydrate, hydroxylamine hydrochloride, phenylhydrazine, urea and thiourea, respectively. The structures of the compounds were established by IR, ¹H NMR, ¹³C NMR and the compounds have been screened for *in vitro* antibacterial and antitumor activities against two human cancer cell lines (A549 and MCF7). Among the compounds screened, 6b and 7b emerged as the most active anti bacterial and the most potent anti proliferative compounds against both the tested cell lines. Structure activity relationship (SAR) analyses confirmed that these two compounds are potential lead for future drug discovery studies.

Keywords: 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones; Claisen condensation; IC₅₀ the cycloocta[b]indole core; Anti tumour activity

Introduction

The design of molecules that spontaneously organize into helical architecture is of desirable interest [1] because of their fascinating structural features as well as their potential applications. Alkaloids that contain indole units such as prenylated indoles, carbazoles, indolo quinolone, cycloocta[b]indole have often showed high levels of biological activities such as antiameobic, antiplasmodic, antiprotozoal and antihypertensive activities [2-5]. Among them, cycloocta[b]indole is an emerging class of biologically relevant indole analogue that has been found in many naturally occurring alkaloids such as macroline, ajmaline, macrocarpamine, villalstonine, O-acetyl macralstonine and macralstonine. The cycloocta[b]indole framework has recently been a subject of biology-oriented synthesis (BIOS) [6] and their analogues are promising targets for developing a novel class of potent and selective Mycobacterium protein tyrosine phosphatase B (MptpB) inhibitors against Mycobacterium tuberculosis [7]. The diverse biological activities of these alkaloids (Figure 1) validate the cycloocta[b]indole core as a promising scaffold for the generation of bioactive compounds.

Although several reports are available on the synthesis of functionalized cyclohepta[b]indoles [8,9] and carbazoles, the reports regarding the synthesis and utilization of cycloocta[b]indoles are scant. The reports available in the literature describing the synthetic methods towards Macroline and Sarpagine are problematic which involves multi step preparations of appropriate precursors, harsh reaction or poor region selectivity of the cyclization step. The structural, biological and pharmacological importance of cycloocta[b]indoles, and the lack of reports on their synthesis have prompted us to devise a reliable synthetic route to prepare these classes of compounds using different methodologies. Hence this manuscript presents the results of detailed investigations on the synthesis and *in vitro* evaluation of the antibacterial and antitumor properties of novel annulated cycloocta[b]indoles. To the best of our knowledge and in the open literature, this is the first report on the synthesis and biological evaluation of pyrazolo-, isoxazolo-, and pyrimidocycloocta[b]indoles.

Experimental

Chemistry

General procedure for the synthesis of 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydro cycloocta[b]indol-6-ones (2): An appropriate 5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (1, 0.001 mol), was added in portion over a period of 5 min to a well cooled vigorously stirred mixture of sodium methoxide (2.5 g of sodium in 25 mL of methanol) and ethyl formate (15 mL). The mixture was stirred in ice bath for another half an hour and then allowed to stand at room temperature for 24 h. At the end of the period, ice water was added to the yellow solid mass and acidified with concentrated hydrochloric acid. The precipitate obtained was filtered and dried. The brown solid mass thus separated out was then purified by column chromatography over silica gel using petroleum ether:ethyl acetate (99:1) as eluent to yield the corresponding 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2).

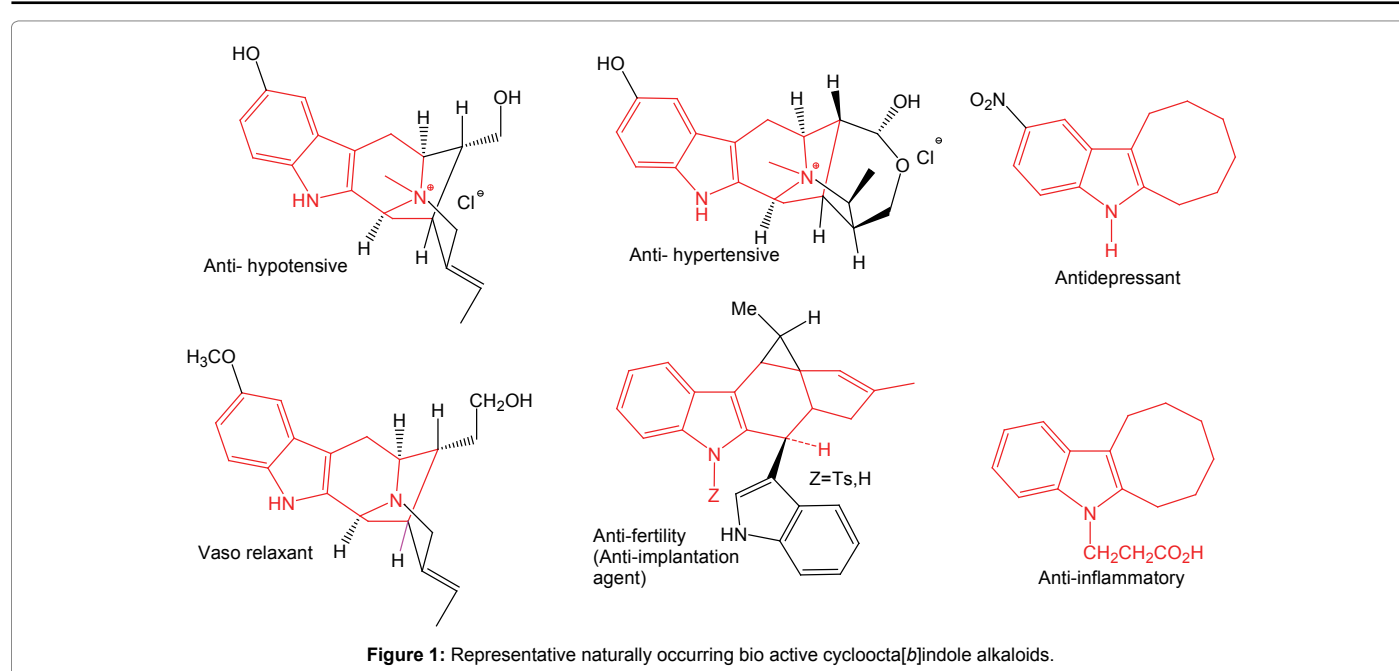
7-(Hydroxymethylene)-2-methyl-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2a): Yellow solid; yield: 90%; m.p. 185-187°C; IR (KBr, cm⁻¹) ν_{max}: 3313 (N-H), 1625 (C=O); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 15.62 (d, 1H, C₇-CHOH, J=8.05 Hz), 8.89 (b s, 1H, N₅-H), 7.90 (d, 1H, C₇-CHOH, J=8.05 Hz), 7.49-7.19 (m, 3H, C₁, C₃ and C₄-H), 3.03-2.72 (m, 4H, C₈ and C₁₁-H), 2.48 (s, 3H, C₂-CH₃), 1.68-1.49 (m, 4H, C₉ and C₁₀-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_C: 182.0 (C=O), 163.7 (C₇-CHO), 142.8 (C_{4a}), 137.8 (C_{5a}), 135.9 (C_{11b}), 131.4 (C₃), 126.9 (C_{11a}), 122.6 (C₁), 120.5 (C₃), 117.0 (C₇), 112.0 (C₄), 29.7 (C₁₀), 27.8 (C₈), 27.2 (C₉), 24.8 (C₁₁), 21.2 (C₂-CH₃); MS: m/z (M⁺, 255); Anal. calcd. for: C₁₆H₁₇NO₂: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.30; H, 6.76; N, 5.54%.

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2-Chloro-7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2b): Yellow solid; yield: 82%; m.p.170-172°C; IR (KBr, cm^{-1}) ν_{max} : 3353 (N-H), 1619 (C=O); ^1H NMR (500 MHz, CDCl_3) (ppm) δ_{H} : 15.47 (d, 1H, $\text{C}_7\text{-CHOH}$, $J=8.50$ Hz), 8.78 (b s, 1H, $\text{N}_5\text{-H}$), 7.60 (d, 1H, $\text{C}_7\text{-CHOH}$, $J=8.50$ Hz), 7.36-7.20 (m, 3H, C_1 , C_3 and $\text{C}_4\text{-H}$), 2.98-1.40 (m, 8H, C_8 , C_9 , C_{10} and $\text{C}_{11}\text{-H}$); ^{13}C NMR (125 MHz, CDCl_3) (ppm) δ_{C} : 180.0 (C=O), 166.7 ($\text{C}_7\text{-CHO}$), 141.8 (C_{4a}), 138.6 (C_{3a}), 137.8 (C_{11b}), 129.5 (C_2), 127.7 (C_{11a}), 123.3 (C_1), 122.2 (C_3), 118.1 (C_7), 113.5 (C_4), 30.0 (C_{10}), 28.1 (C_8), 26.8 (C_9), 25.2 (C_{11}); MS: m/z (M^+ , 275); Anal. calcd. for: $\text{C}_{15}\text{H}_{14}\text{ClNO}_2$: C, 65.34; H, 5.12; N, 5.08. Found: C, 65.39; H, 5.16; N, 5.04%.

7-(Hydroxymethylene)-4-methyl-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2c): Yellow solid; yield: 80%; m.p.150-152°C; IR (KBr, cm^{-1}) ν_{max} : 3345 (N-H), 1614 (C=O); ^1H NMR (500 MHz, CDCl_3) (ppm) δ_{H} : 15.59 (d, 1H, $\text{C}_7\text{-CHOH}$, $J=8.00$ Hz), 8.79 (b s, 1H, $\text{N}_5\text{-H}$), 7.75 (d, 1H, $\text{C}_7\text{-CHOH}$, $J=8.00$ Hz), 7.48-7.19 (m, 3H, C_1 , C_2 and $\text{C}_3\text{-H}$), 3.10-2.87 (m, 4H, C_8 and $\text{C}_{11}\text{-H}$), 2.50 (s, 3H, $\text{C}_4\text{-CH}_3$), 1.78-1.69 (m, 4H, C_9 and $\text{C}_{10}\text{-H}$); ^{13}C NMR (125 MHz, CDCl_3) (ppm) δ_{C} : 179.7 (C=O), 164.6 ($\text{C}_7\text{-CHO}$), 143.7 (C_{4a}), 136.9 (C_{5a}), 134.7 (C_{11b}), 128.7 (C_{11a}), 123.4 (C_2), 122.5 (C_3), 121.0 (C_4), 118.6 (C_1), 116.1 (C_7), 28.6 (C_{10}), 27.2 (C_8), 26.7 (C_9), 25.3 (C_{11}), 17.0 ($\text{C}_4\text{-CH}_3$); MS: m/z (M^+ , 255); Anal. calcd. for: $\text{C}_{16}\text{H}_{17}\text{NO}_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.30; H, 6.76; N, 5.54%.

7-(Hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2d): Yellow solid; yield: 79%; m.p.161-163°C; IR (KBr, cm^{-1}) ν_{max} : 3372 (N-H), 1610 (C=O); ^1H NMR (500 MHz, CDCl_3) (ppm) δ_{H} : 15.58 (d, 1H, $\text{C}_7\text{-CHOH}$, $J=8.00$ Hz), 8.94 (b s, 1H, $\text{N}_5\text{-H}$), 7.58 (d, 1H, $\text{C}_7\text{-CHOH}$, $J=8.00$ Hz), 7.37-7.20 (m, 4H, C_1 , C_2 , C_3 and $\text{C}_4\text{-H}$), 3.05-1.40 (m, 8H, C_8 , C_9 , C_{10} and $\text{C}_{11}\text{-H}$); ^{13}C NMR (125 MHz, CDCl_3) (ppm) δ_{C} : 176.8 (C=O), 165.4 ($\text{C}_7\text{-CHO}$), 144.2 (C_{4a}), 139.0 (C_{5a}), 135.1 (C_{11b}), 128.0 (C_{11a}), 122.5 (C_2), 121.7 (C_1), 120.2 (C_3), 117.7 (C_7), 112.6 (C_4), 29.3 (C_{10}), 28.7 (C_8), 27.0 (C_9), 26.2 (C_{11}); MS: m/z (M^+ , 241); Anal. calcd. for: $\text{C}_{15}\text{H}_{15}\text{NO}_2$: C, 74.67; H, 6.27; N, 5.81. Found: C, 74.74; H, 6.21; N, 5.84%.

General procedure for the synthesis of 4,5,6,7-tetrahydropyrazolo [3',4':8,7]-12H-cycloocta[b]indoles (3): The reaction mixture

consisting of the appropriate 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2, 0.001 mol), hydrazine hydrate (0.5 mL) was refluxed in absolute ethanol (15 mL) for 5 h. After the completion of the reaction, the excess solvent was removed by distillation and the mixture was poured into ice-water, filtered, dried and purified by column chromatography (eluent: petroleum ether:ethyl acetate, 85:15) and recrystallized from ethanol to give the respective 4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (3) as yellow prism.

9-Methyl-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (3a): Yellow prism; yield: 68%; m.p.210-212°C; IR (KBr, cm^{-1}) ν_{max} : 3394 (N-H), 1600 (C=N); ^1H NMR (500 MHz, CDCl_3) (ppm) δ_{H} : 9.01 (b s, 1H, $\text{N}_{12}\text{-H}$), 7.57 (s, 1H, $\text{N}_2\text{-H}$), 7.48-7.20 (m, 4H, C_3 , C_8 , C_{10} and $\text{C}_{11}\text{-H}$), 3.07-2.69 (m, 4H, C_4 and $\text{C}_7\text{-H}$), 2.52 (s, 3H, $\text{C}_9\text{-CH}_3$), 1.94-1.78 (m, 4H, C_5 and $\text{C}_6\text{-H}$); ^{13}C NMR (125 MHz, CDCl_3) (ppm) δ_{C} : 134.5 (C_{11a}), 131.8 (C_2), 129.0 (C_{12b}), 128.2 (C_9), 126.8 (C_{12a}), 125.9 (C_{7b}), 121.0 (C_8), 120.2 (C_{10}), 114.5 (C_{3a}), 113.1 (C_{7a}), 110.2 (C_{11}), 29.0 (C_6), 27.2 (C_5), 25.1 (C_7), 23.1 (C_4), 21.1 ($\text{C}_9\text{-CH}_3$); MS: m/z (M^+ , 251); Anal. calcd. For: $\text{C}_{16}\text{H}_{17}\text{N}_3$: C, 76.46; H, 6.82; N, 16.72. Found: C, 76.50; H, 6.77; N, 16.80%.

9-Chloro-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (3b): Yellow prism; yield: 62%; m.p.220-222°C; IR (KBr, cm^{-1}) ν_{max} : 3332 (N-H), 1614 (C=N); ^1H NMR (500 MHz, CDCl_3) (ppm) δ_{H} : 9.31 (b s, 1H, $\text{N}_{12}\text{-H}$), 7.45 (s, 1H, $\text{N}_2\text{-H}$), 7.33-7.00 (m, 4H, C_3 , C_8 , C_{10} and $\text{C}_{11}\text{-H}$), 2.92-1.50 (m, 8H, C_4 , C_5 , C_6 and $\text{C}_7\text{-H}$); ^{13}C NMR (125 MHz, CDCl_3) (ppm) δ_{C} : 135.7 (C_{11a}), 131.8 (C_2), 130.0 (C_{12b}), 127.9 (C_9), 125.0 (C_{12a}), 124.1 (C_{7b}), 122.3 (C_8), 121.7 (C_{10}), 115.3 (C_{3a}), 114.0 (C_{7a}), 111.8 (C_{11}), 29.1 (C_6), 27.9 (C_5), 26.8 (C_7), 25.5 (C_4); MS: m/z (M^+ , 271); Anal. calcd. for: $\text{C}_{15}\text{H}_{14}\text{N}_3$: C, 66.30; H, 5.19; N, 15.46. Found: C, 66.35; H, 5.17; N, 15.50%.

11-Methyl-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (3c): Yellow prism; yield: 66%; m.p.191-193°C; IR (KBr, cm^{-1}) ν_{max} : 3367 (N-H), 1602 (C=N); ^1H NMR (500 MHz, CDCl_3) (ppm) δ_{H} : 9.21 (b s, 1H, $\text{N}_{12}\text{-H}$), 7.59 (s, 1H, $\text{N}_2\text{-H}$), 7.50-7.08 (m, 4H, C_3 , C_8 , C_9 and $\text{C}_{10}\text{-H}$), 3.17-2.88 (m, 4H, C_4 and $\text{C}_7\text{-H}$), 2.61 (s, 3H, $\text{C}_{11}\text{-CH}_3$), 2.12-1.98 (m, 4H, C_5 and $\text{C}_6\text{-H}$); ^{13}C NMR (125 MHz, CDCl_3)

(ppm) δ_c : 136.7 (C_{11a}), 132.5 (C₃), 131.6 (C_{12b}), 127.5 (C_{12a}), 126.3 (C_{7b}), 124.0 (C₉), 121.0 (C₁₀), 120.5 (C₁₁), 119.2 (C₉), 116.7 (C_{3a}), 114.1 (C_{7a}), 28.4 (C₆), 26.8 (C₅), 25.7 (C₇), 24.0 (C₄), 18.9 (C₁₁-CH₃); MS: *m/z* (M⁺, 251); Anal. calcd. for: C₁₆H₁₇N₃: C, 76.46; H, 6.82; N, 16.72. Found: C, 76.50; H, 6.77; N, 16.80%.

4,5,6,7-Tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (3d): Yellow prism; yield: 64%; m.p.197-199°C; IR (KBr, cm⁻¹) ν_{max} : 3329 (N-H), 1607 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 8.98 (b s, 1H, N₁₂-H), 7.69 (s, 1H, N₂-H), 7.41-7.06 (m, 5H, C₃, C₈, C₉, C₁₀ and C₁₁-H), 3.01-2.10 (m, 8H, C₄, C₅, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 137.5 (C_{11a}), 132.9 (C₃), 131.4 (C_{12b}), 126.1 (C_{7b}), 125.6 (C_{12a}), 123.9 (C₉), 122.6 (C₉), 121.0 (C₁₀), 114.9 (C_{3a}), 113.4 (C_{7a}), 112.6 (C₁₁), 28.8 (C₆), 27.2 (C₅), 25.7 (C₇), 24.6 (C₄); MS: *m/z* (M⁺, 237); Anal. calcd. for: C₁₅H₁₅N₃: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.97; H, 6.31; N, 17.66%.

General procedure for the synthesis of 4,5,6,7-tetrahydroisoxazolo[3',4':8,7]-12H-cycloocta[b]indoles (4): The reaction mixture consisting of the appropriate 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2, 0.001 mol), hydroxylamine hydrochloride (0.34 g) and glacial acetic acid (5 mL) was refluxed at 120°C for 4 h. After the completion of the reaction, it was cooled and poured into crushed ice, the solid thus separated out was filtered, washed with water, dried and purified by column chromatography (eluent: petroleum ether:ethyl acetate, 85:15) and recrystallized from ethanol to give the respective 4,5,6,7-tetrahydroisoxazolo[3',4':8,7]-12H-cycloocta[b]indole (4).

9-Methyl-4,5,6,7-tetrahydroisoxazolo[3',4':8,7]-12H-cycloocta[b]indole (4a): Yellow prism; yield: 63%; m.p.210-212°C; IR (KBr, cm⁻¹) ν_{max} : 3426 (N-H), 1611 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 8.93 (b s, 1H, N₁₂-H), 8.19-7.09 (m, 4H, C₃, C₈, C₉ and C₁₁-H), 3.08-2.67 (m, 4H, C₄ and C₇-H), 2.41 (s, 3H, C₉-CH₃), 1.70-1.49 (m, 4H, C₅ and C₆-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 159.7 (C_{12b}), 151.8 (C₃), 132.2 (C_{11a}), 128.7 (C₉), 127.4 (C_{7b}), 126.1 (C_{12a}), 123.4 (C₈), 120.7 (C₁₀), 115.5 (C_{7a}), 113.7 (C_{3a}), 111.5 (C₁₁), 27.8 (C₆), 26.7 (C₅), 24.7 (C₇), 24.0 (C₄), 21.2 (C₉-CH₃); MS: *m/z* (M⁺, 252); Anal. calcd. for: C₁₆H₁₆N₂O: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.20; H, 6.34; N, 11.04%.

9-Chloro-4,5,6,7-tetrahydroisoxazolo[3',4':8,7]-12H-cycloocta[b]indole (4b): Yellow prism; yield: 58%; m.p.204-206°C; IR (KBr, cm⁻¹) ν_{max} : 3414 (N-H), 1617 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 8.78 (b s, 1H, N₁₂-H), 8.09-7.08 (m, 4H, C₃, C₈, C₉ and C₁₁-H), 3.01-1.60 (m, 8H, C₄, C₅, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 157.4 (C_{12b}), 150.6 (C₃), 134.1 (C_{11a}), 129.2 (C_{7b}), 128.0 (C₉), 125.2 (C_{12a}), 122.3 (C₈), 121.5 (C₁₀), 114.1 (C_{7a}), 112.6 (C_{3a}), 111.0 (C₁₁), 28.9 (C₆), 27.2 (C₅), 25.6 (C₇), 24.5 (C₄); MS: *m/z* (M⁺, 272); Anal. calcd. for: C₁₅H₁₃ClN₂O: C, 66.06; H, 4.80; N, 10.07. Found: C, 66.10; H, 4.75; N, 10.12%.

11-Methyl-4,5,6,7-tetrahydroisoxazolo[3',4':8,7]-12H-cycloocta[b]indole (4c): Yellow prism; yield: 56%; m.p.219-221°C; IR (KBr, cm⁻¹) ν_{max} : 3435 (N-H), 1619 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 8.96 (b s, 1H, N₁₂-H), 8.03-7.10 (m, 4H, C₃, C₈, C₉ and C₁₀-H), 3.10-2.92 (m, 4H, C₄ and C₇-H), 2.43 (s, 3H, C₁₁-CH₃), 1.81-1.74 (m, 4H, C₅ and C₆-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 160.0 (C_{12b}), 152.2 (C₃), 134.1 (C_{11a}), 129.9 (C_{7b}), 127.5 (C_{12a}), 124.2 (C₉), 122.0 (C₁₀), 121.2 (C₁₁), 118.8 (C₈), 114.2 (C_{7a}), 112.6 (C_{3a}), 27.9 (C₆), 26.8 (C₅), 24.9 (C₇), 23.8 (C₄), 19.0 (C₁₁-CH₃); MS: *m/z* (M⁺, 252); Anal. calcd. for: C₁₆H₁₆N₂O: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.20; H, 6.34; N, 11.04%.

4,5,6,7-Tetrahydroisoxazolo[3',4':8,7]-12H-cycloocta[b]indole (4d): Yellow prism; yield: 55%; m.p.197-199°C; IR (KBr, cm⁻¹) ν_{max} : 3429 (N-H), 1613 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 9.01 (b s, 1H, N₁₂-H), 8.22-7.18 (m, 5H, C₃, C₈, C₉, C₁₀ and C₁₁-H), 3.04-1.67 (m, 8H, C₄, C₅, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 158.1 (C_{12b}), 151.3 (C₃), 135.4 (C_{11a}), 130.3 (C_{7b}), 124.0 (C_{12a}), 123.1 (C₉), 122.0 (C₈), 121.3 (C₁₀), 113.1 (C_{7a}), 111.9 (C_{3a}), 110.5 (C₁₁), 29.3 (C₆), 27.8 (C₅), 26.5 (C₇), 25.2 (C₄); MS: *m/z* (M⁺, 238); Anal. calcd. for: C₁₅H₁₄N₂O: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.66; H, 5.88; N, 11.80%.

General procedure for the synthesis of 1-phenyl-4,5,6,7-tetrahydropyrazolo[4',5':8,7]-12H-cycloocta[b]indoles (5): The reaction mixture consisting of the appropriate 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2, 0.001 mol), phenyl hydrazine (0.54 g) and glacial acetic acid (5 mL) was refluxed at 120°C for 10 h. After the completion of the reaction, it was cooled and poured into crushed ice, the solid thus separated out was filtered, washed with water, dried and purified by column chromatography (eluent: petroleum ether:ethyl acetate, 97:3) and recrystallized from ethanol to give the respective 1-phenyl-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (5).

9-Methyl-1-phenyl-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (5a): Yellow solid; yield: 66%; m.p.203-205°C; IR (KBr, cm⁻¹) ν_{max} : 3321 (N-H), 1623 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 7.53 (s, 1H, C₃-H), 7.07 (b s, 1H, N₁₂-H), 7.72-6.90 (m, 8H, C₈, C₉, C₁₀, C₁₁, C₂', C₃', C₄', C₅' and C₆'-H), 3.18-2.77 (m, 4H, C₄ and C₇-H), 2.40 (s, 3H, C₉-CH₃), 1.80-1.59 (m, 4H, C₅ and C₆-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 140.7 (C₁'), 138.0 (C₃'), 133.1 (C_{11a}'), 130.8 (C₉'), 129.2 (C₃' and C₅'), 127.5 (C₂' and C₆'), 126.5 (C_{7b}'), 126.1 (C_{12b}'), 124.8 (C₄'), 123.2 (C_{12a}'), 122.4 (C₈'), 121.6 (C₁₀'), 119.3 (C_{3a}'), 113.8 (C_{7a}'), 111.4 (C₁₁'), 29.5 (C₆'), 28.3 (C₅'), 26.7 (C₇'), 25.3 (C₄'), 21.3 (C₉-CH₃); MS: *m/z* (%) 327 (M⁺, 327); Anal. calcd. for: C₂₂H₂₁N₃: C, 80.70; H, 6.46; N, 12.83. Found: C, 80.75; H, 6.41; N, 12.79%.

9-Chloro-1-phenyl-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (5b): Yellow solid; yield: 60%; m.p.236-238°C; IR (KBr, cm⁻¹) ν_{max} : 3327 (N-H), 1598 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 7.57 (s, 1H, C₃-H), 7.16 (b s, 1H, N₁₂-H), 7.45-6.95 (m, 8H, C₈, C₉, C₁₀, C₁₁, C₂', C₃', C₄', C₅' and C₆'-H), 3.32-1.62 (m, 8H, C₄, C₅, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 141.5 (C₁'), 139.4 (C₃'), 134.3 (C_{11a}'), 129.2 (C₃' and C₅'), 128.2 (C_{7b}'), 127.8 (C₉'), 127.5 (C₂' and C₆'), 126.8 (C_{12b}'), 125.5 (C₄'), 124.0 (C_{12a}'), 122.8 (C₈'), 121.2 (C₁₀'), 118.4 (C_{3a}'), 112.6 (C_{7a}'), 110.8 (C₁₁'), 28.9 (C₆'), 27.8 (C₅'), 26.5 (C₇'), 24.9 (C₄); MS: *m/z* (M⁺, 347); Anal. calcd. for: C₂₁H₁₈ClN₃: C, 72.51; H, 5.22; N, 12.08. Found: C, 72.55; H, 5.17; N, 12.13%.

11-Methyl-1-phenyl-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (5c): Yellow solid; yield: 63%; m.p.220-222°C; IR (KBr, cm⁻¹) ν_{max} : 3341 (N-H), 1605 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 7.63 (s, 1H, C₃-H), 7.27 (b s, 1H, N₁₂-H), 7.50-7.04 (m, 8H, C₈, C₉, C₁₀, C₂', C₃', C₄', C₅' and C₆'-H), 2.93-2.67 (m, 4H, C₄ and C₇-H), 2.21 (s, 3H, C₁₁-CH₃), 2.12-1.90 (m, 4H, C₅ and C₆-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c : 142.0 (C₁'), 140.1 (C₃'), 135.1 (C_{11a}'), 129.8 (C₃' and C₅'), 128.0 (C₂' and C₆'), 127.3 (C_{7b}'), 126.0 (C_{12b}'), 125.3 (C₄'), 124.7 (C_{12a}'), 123.8 (C₉'), 122.5 (C₁₀'), 121.9 (C₁₁'), 119.4 (C₈'), 117.6 (C_{3a}'), 112.8 (C_{7a}'), 29.1 (C₆'), 28.6 (C₅'), 26.8 (C₇'), 25.2 (C₄'), 18.8 (C₁₁-CH₃); MS: *m/z* (M⁺, 327); Anal. calcd. for: C₂₂H₂₁N₃: C, 80.70; H, 6.46; N, 12.83. Found: C, 80.75; H, 6.41; N, 12.79%.

1-Phenyl-4,5,6,7-tetrahydropyrazolo[3',4':8,7]-12H-cycloocta[b]indole (5d): Yellow solid; yield: 61%; m.p.244-246°C; IR (KBr, cm⁻¹) ν_{max} : 3334 (N-H), 1595 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H : 7.60 (s, 1H, C₃-H), 7.23 (b s, 1H, N₁₂-H), 7.49-6.92 (m, 9H, C₈, C₉, C₁₀, C₁₁, C₂', C₃', C₄', C₅' and C₆'-H), 3.00-1.91 (m, 8H, C₄, C₅, C₆

and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 140.1 (C₁'), 138.5 (C₃), 135.0 (C_{11a}), 129.6 (C₃' and C₅'), 127.8 (C_{7b}), 126.9 (C₂' and C₆'), 125.7 (C_{12b}), 124.5 (C₄'), 124.0 (C_{12a}), 123.8 (C₉), 122.1 (C₈), 121.5 (C₁₀), 116.4 (C_{3a}), 113.9 (C_{7a}), 112.5 (C₁₁), 29.0 (C₆), 28.3 (C₅), 27.8 (C₇), 25.4 (C₄); MS: *m/z* (M⁺, 313); Anal. calcd. for: C₂₁H₁₉N₃: C, 80.48; H, 6.11; N, 13.41. Found: C, 80.53; H, 6.16; N, 13.45%.

General procedure for the synthesis of 2-hydroxy-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indoles (6): A mixture of an appropriate 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2, 0.001 mol), urea (0.12 g) and glacial acetic acid (6 mL) was refluxed at 120°C for 5 h. After the completion of the reaction, it was cooled and poured into crushed ice with stirring. The solid thus separated out was filtered, dried and purified by column chromatography (eluent: petroleum ether:ethyl acetate, 75:25) and recrystallized from ethanol to give the respective 2-hydroxy-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (6).

2-Hydroxy-10-methyl-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (6a): Yellow solid; yield: 71%; m.p.222-224°C; IR (KBr, cm⁻¹) ν_{max}: 3418 (O-H), 3251 (N-H), 1621 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 11.68 (s, 1H, C₂-OH), 9.38 (b s, 1H, N₁₃-H), 7.54-7.11 (m, 4H, C₄, C₉, C₁₁ and C₁₂-H), 3.03-2.82 (m, 4H, C₅ and C₈-H), 2.42 (s, 3H, C₁₀-CH₃), 1.78-1.57 (m, 4H, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 162.4 (C₂), 150.2 (C₄), 146.9 (C_{13b}), 134.6 (C_{12a}), 131.9 (C₁₀), 129.0 (C_{8b}), 124.0 (C_{13a}), 121.0 (C₉), 120.2 (C₁₁), 118.6 (C_{4a}), 113.2 (C_{8a}), 110.4 (C₁₂), 27.9 (C₇), 25.1 (C₆), 23.6 (C₈), 22.6 (C₅), 21.6 (C₁₀-CH₃); MS: *m/z* (M⁺, 279); Anal. calcd. for: C₁₇H₁₇N₃O: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.15; H, 6.18; N, 15.10%.

2-Hydroxy-10-chloro-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (6b): Yellow solid; yield: 70%; m.p.198-200°C; IR (KBr, cm⁻¹) ν_{max}: 3424 (O-H), 3275 (N-H), 1608 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 11.38 (s, 1H, C₂-OH), 9.21 (b s, 1H, N₁₃-H), 7.39-7.03 (m, 4H, C₄, C₉, C₁₁ and C₁₂-H), 3.00-1.63 (m, 8H, C₅, C₆, C₇ and C₈-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 165.1 (C₂), 153.2 (C₄), 148.3 (C_{13b}), 133.4 (C_{12a}), 130.4 (C_{8b}), 129.2 (C₁₀), 123.4 (C_{13a}), 122.0 (C₉), 121.1 (C₁₁), 119.1 (C_{4a}), 112.5 (C_{8a}), 111.6 (C₁₂), 28.5 (C₆), 27.6 (C₇), 25.2 (C₈), 24.3 (C₅); MS: *m/z* (M⁺, 299); Anal. calcd. for: C₁₆H₁₄ClN₃O: C, 64.11; H, 4.71; N, 14.02. Found: C, 64.16; H, 4.67; N, 14.08%.

2-Hydroxy-12-methyl-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (6c): Yellow solid; yield: 74%; m.p.214-216°C; IR (KBr, cm⁻¹) ν_{max}: 3476 (O-H), 3308 (N-H), 1602 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 11.10 (s, 1H, C₂-OH), 8.99 (b s, 1H, N₁₃-H), 7.50-7.08 (m, 4H, C₄, C₉, C₁₀ and C₁₁-H), 3.10-2.75 (m, 4H, C₅ and C₈-H), 2.50 (s, 3H, C₁₂-CH₃), 1.98-1.66 (m, 4H, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 160.7 (C₂), 151.5 (C₄), 145.5 (C_{13b}), 135.7 (C_{12a}), 131.0 (C_{8b}), 124.6 (C_{13a}), 123.6 (C₁₀), 121.0 (C₁₁), 120.8 (C₁₂), 119.8 (C₉), 118.6 (C_{4a}), 113.2 (C_{8a}), 27.9 (C₆), 25.1 (C₇), 23.6 (C₈), 22.6 (C₅), 19.2 (C₁₂-CH₃); MS: *m/z* (M⁺, 279); Anal. calcd. for: C₁₇H₁₇N₃O: C, 73.10; H, 6.13; N, 15.04. Found: C, 73.15; H, 6.18; N, 15.10%.

General procedure for the synthesis of 2-mercapto-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indoles (7): A mixture of an appropriate 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (2, 0.001 mol), thiourea (0.152 g) and glacial acetic acid (6 mL) was refluxed at 120°C for 5 h. After the completion of the reaction, it was cooled and poured into crushed ice with stirring. The solid thus separated out was filtered, dried and purified by column chromatography (eluent: petroleum ether:ethyl acetate, 75:25) and recrystallized from ethanol to give the respective 2-mercapto-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (7).

2-Mercapto-10-methyl-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (7a): Yellow solid; yield: 74%; m.p.248-250°C; IR (KBr, cm⁻¹) ν_{max}: 3423 (N-H), 1155 (C=S); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 8.89 (b s, 1H, N₁₃-H), 7.76-7.04 (m, 4H, C₄, C₉, C₁₁ and C₁₂-H), 3.19-2.92 (m, 5H, C₂-SH, C₅ and C₈-H), 2.36 (s, 3H, C₁₀-CH₃), 2.05-1.89 (m, 4H, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 172.2 (C₂), 153.9 (C₄), 152.0 (C_{13b}), 133.4 (C_{12a}), 130.8 (C₁₀), 127.8 (C_{8b}), 126.1 (C_{13a}), 122.1 (C₉), 121.3 (C₁₁), 120.4 (C_{4a}), 115.8 (C_{8a}), 113.7 (C₁₂), 29.4 (C₇), 27.9 (C₆), 24.8 (C₈), 23.7 (C₅), 21.4 (C₁₀-CH₃); MS: *m/z* (M⁺, 295); Anal. calcd. for: C₁₇H₁₇N₃S: C, 69.12; H, 5.80; N, 14.22; S, 10.85. Found: C, 69.18; H, 5.75; N, 14.17; S, 10.90%.

2-Mercapto-10-chloro-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (7b): Yellow solid; yield: 69%; m.p.265-267°C; IR (KBr, cm⁻¹) ν_{max}: 3395 (N-H), 1158 (C=S); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 8.85 (b s, 1H, N₁₃-H), 7.68-7.10 (m, 4H, C₄, C₉, C₁₁ and C₁₂-H), 3.44-2.10 (m, 9H, C₂-SH, C₅, C₆, C₇ and C₈-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 173.6 (C₂), 155.3 (C₄), 151.4 (C_{13b}), 134.5 (C_{12a}), 132.1 (C₁₀), 128.1 (C_{8b}), 125.5 (C_{13a}), 121.1 (C₉), 120.8 (C₁₁), 120.6 (C_{4a}), 114.8 (C_{8a}), 112.6 (C₁₂), 28.6 (C₇), 27.2 (C₆), 26.7 (C₈), 24.6 (C₅); MS: *m/z* (M⁺, 315); Anal. calcd. for: C₁₆H₁₄ClN₃S: C, 60.85; H, 4.47; N, 13.31; S, 10.15. Found: C, 60.81; H, 4.52; N, 13.27; S, 10.20%.

2-Mercapto-12-methyl-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (7c): Yellow solid; yield: 72%; m.p.244-246°C; IR (KBr, cm⁻¹) ν_{max}: 3378 (N-H), 1152 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 9.08 (b s, 1H, N₁₃-H), 7.62-7.18 (m, 4H, C₄, C₉, C₁₀ and C₁₂-H), 3.39-2.71 (m, 5H, C₂-SH, C₅ and C₈-H), 2.48 (s, 3H, C₁₂-CH₃), 2.09-1.96 (m, 4H, C₆ and C₇-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 170.6 (C₂), 152.4 (C₄), 151.5 (C_{13b}), 136.2 (C_{12a}), 128.5 (C_{8b}), 127.0 (C_{13a}), 124.1 (C₁₀), 122.1 (C₁₁), 121.3 (C₁₂), 120.4 (C_{4a}), 119.9 (C₉), 114.2 (C_{8a}), 28.1 (C₇), 27.2 (C₆), 26.5 (C₈), 25.3 (C₅), 18.8 (C₁₂-CH₃); MS: *m/z* (M⁺, 295); Anal. calcd. for: C₁₇H₁₇N₃S: C, 69.12; H, 5.80; N, 14.22; S, 10.85. Found: C, 69.18; H, 5.75; N, 14.17; S, 10.90%.

2-Mercapto-5,6,7,8-tetrahydropyrimido[5',6':8,7]-13H-cycloocta[b]indole (7d): Yellow solid; yield: 70%; m.p.211-213°C; IR (KBr, cm⁻¹) ν_{max}: 3360 (N-H), 1157 (C=N); ¹H NMR (500 MHz, CDCl₃) (ppm) δ_H: 9.20 (b s, 1H, N₁₃-H), 7.70-7.10 (m, 5H, C₄, C₉, C₁₀, C₁₁ and C₁₂-H), 3.49-1.56 (m, 9H, C₂-SH, C₅, C₆, C₇ and C₈-H); ¹³C NMR (125 MHz, CDCl₃) (ppm) δ_c: 171.3 (C₂), 153.5 (C₄), 152.5 (C_{13b}), 133.8 (C_{12a}), 129.3 (C_{8b}), 124.6 (C_{13a}), 122.9 (C₁₀), 121.8 (C₉), 120.5 (C₁₁), 119.5 (C_{4a}), 112.9 (C_{8a}), 111.7 (C₁₂), 28.9 (C₇), 28.0 (C₆), 27.2 (C₈), 25.7 (C₅); MS: *m/z* (M⁺, 281); Anal. calcd. for: C₁₆H₁₅N₃S: C, 68.30; H, 5.37; N, 14.93; S, 11.40. Found: C, 68.35; H, 5.31; N, 15.00; S, 11.35%.

Biology

Anti-bacterial activity: The anti-microbial activity was done by well diffusion method (Cappuccino and Sherman) against the test organism such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus sp*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp*, *Salmonella sp*, *Proteus sp*, *Shigella sp*, *Bacillus sp*, and *Candida albicans*. By using a sterile cork borer, wells were punctured in Mueller Hinton agar plates previously seeded with one of the test organism. One hundred microliter of supernatant of each isolate was added in each well. The diameters of inhibition were determined after 24 h of incubation at 37°C.

Anti tumor activity: Cytotoxicity studies of the compounds along with Doxorubicin were carried out on MCF-7 breast cancer and in A549 non-small cell lung cancer (NSCLC) celllines and which were obtained from National Centre for Cell Science, Pune, India. Cell viability was carried out using the MTT assay method. The HeLa and AGS cells were grown in Eagles minimum essential medium containing 10% fetal

bovine serum (FBS). For the screening experiment, the cells were seeded into 96-well plates in 100 μ L of the respective medium containing 10% FBS, at a plating density of 10,000 cells/well, and incubated at 37°C, under conditions of 5% CO₂, 95% air, and 100% relative humidity for 24 h prior to the addition of compounds. The compounds were dissolved in DMSO and diluted in the respective medium containing 1% FBS. After 24 h, the medium was replaced with the respective medium with 1% FBS containing the compounds at various concentrations and incubated at 37 °C under conditions of 5% CO₂, 95% air, and 100% relative humidity for 48 h. After 48 h, 10 μ L of MTT (5 μ g/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4 h. The medium with MTT was then flicked off, and the formed formazan crystals were dissolved in 100 μ L of DMSO. The absorbance was then measured at 570 nm using a microplate reader. The percentage of cell inhibition was determined using the following formula, and a graph was plotted with the percentage of cell inhibition versus concentration. From this, the IC₅₀ value was calculated: % inhibition = [mean OD of untreated cells (control)/mean OD of treated cells (control)] \times 100. The results were expressed as the concentration at which there was 50% inhibition (IC₅₀).

Results and Discussion

Chemistry

Synthesis of isoxazolo-, pyrido- and pyrazolo- cycloocta[b]indoles: As part of our ongoing studies on the development of facile methods for the synthesis of organic compounds from readily available starting materials, specifically 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones (**2**), a putative synthon, which can be obtained by Claisen condensation of 5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones (**1**) with ethylformate in the presence of sodium methoxide as shown in Scheme 1. We would like to describe here the reaction of these readily available compounds (**2**) with hydrazine hydrate, hydroxylamine hydrochloride, phenylhydrazine, urea and thiourea leading to highly-substituted pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocycloocta[b]indoles in one-pot addition reactions to form a library of new pyrazolo-, isoxazolo-, pyrimido- and mercaptopyrimidocycloocta[b]indoles (Scheme 2). IR, ¹H NMR, ¹³C NMR data and elemental analyses were used to ascertain the structures of all compounds.

As a model reaction we initially investigate the reaction of 5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones (**1**) with ethyl formate (Scheme 1). A major reason for our interest in these compounds was the possibility of their transformation into a number of annulated cycloocta[b]indoles possessing different heterocyclic systems using simple reactions with various agents. The structures of

the products were substantiated on the basis of IR, ¹H NMR, ¹³C NMR data and elemental analysis. The IR spectrum of **2a** showed sharp and strong bands at 3313 and 1625 cm⁻¹ assigned to hydroxyl (overlapped with NH) and carbonyl group respectively. Further the disappearance of C₇-methylene protons signals and the appearance of OH and olefinic-CH signals as two doublets at δ 15.62 ($J=8.05$ Hz) and δ 7.90 ($J=8.05$ Hz) and also the appearance of carbazole NH proton at δ 8.89 in its ¹H-NMR spectrum suggested the structure of **2a** to be a hydroxyl methylene compound. The rest of the aromatic protons appeared in the corresponding region.

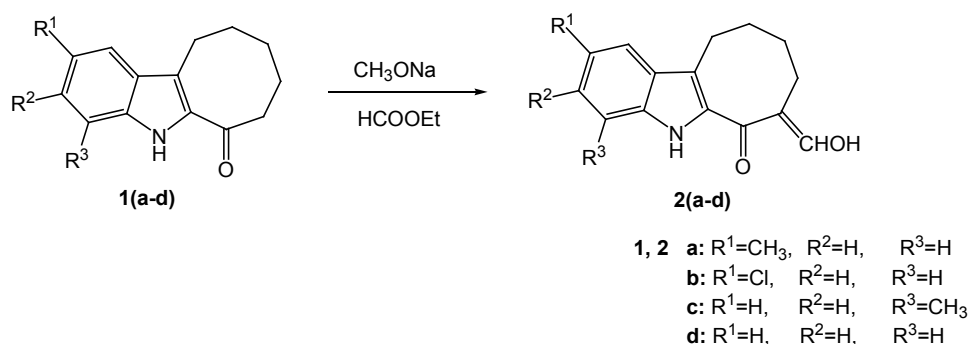
In the next step we expanded the range of nucleophiles and investigated the construction of pyrazolo-, isoxazolo- and pyrimidocycloocta[b]indoles from the precursor, 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones (**2**) upon reaction with hydrazine hydrate, hydroxylamine hydrochloride, phenylhydrazine, urea and thiourea as shown in Scheme 2.

We first focused our attention towards the synthesis of pyrazolocycloocta[b]indoles **3** from 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones (**2**) upon reaction with hydrazine hydrate. The proton NMR spectrum of **3a** revealed the appearance of two broad singlets at δ 9.01 and δ 7.57 accounted for indole-NH and pyrazolo-NH protons respectively. The other aromatic protons appeared as multiplet in the region δ 7.48-7.20. The remaining protons appear in the corresponding region. Mass spectrum and elemental analysis data support the molecular formula as C₁₆H₁₇N₃. The identities of the other compounds **3b** to **3d** were established in the same way with all spectroscopic data readily assignable.

In an anticipation to derive isoxazolocycloocta[b]indoles (**4**), the corresponding 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (**2**) was treated with hydroxylamine hydrochloride in acetic acid. The IR spectrum of **4a** registered absorption band at 1611 cm⁻¹ assigned to C=N functional group. The proton NMR spectrum of **4a** showed one broad singlet at δ 8.93 accounted for indole-NH. The rest of the aromatic protons appeared as a multiplet between the region δ 8.19-7.09. Analytical data are in accordance with the proposed structure for compound **4**.

In order to extend the above synthesis, we carried out the reaction of 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (**2**) with phenyl hydrazine in glacial acetic acid for 6 h to afford a single product (**5**) (Scheme 2). The ¹H-NMR spectrum of **5a** exhibited two singlets at δ 7.53 and δ 7.07 for C₃- and N-H proton. The structure of **5** is fully supported by spectral and analytical data.

In conjunction with this work, we believed that the targeted



Scheme 1: Synthesis of 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-ones.

pyrimidocycloocta[b]indole (**6**) was accomplished by the reaction of 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (**2**) with urea in glacial acetic acid for 5 hours at 120°C (Scheme 2). The IR spectrum of **6a** revealed the presence of hydroxy and N-H functional groups at 3418 cm^{-1} and 3251 cm^{-1} . The proton NMR spectrum of **6a** showed two broad singlets at δ 11.68 and δ 9.38 due to the presence of C_2 -OH and N_{13} -H. The rest of the aromatic protons appeared between the region δ 7.54-7.11. The elemental analysis and mass spectral data were compatible with the molecular formula $\text{C}_{17}\text{H}_{17}\text{N}_3\text{O}$.

Further the reaction of 7-(hydroxymethylene)-5,7,8,9,10,11-hexahydrocycloocta[b]indol-6-one (**2**) with thiourea in glacial acetic acid was also investigated with a view to synthesize mercaptopyrimidocycloocta[b]indole (**7**) as shown in Scheme 2. In the IR spectrum of **7a** the absorption band at 1155 cm^{-1} corresponds to C=S group which strongly support the structure of the obtained product as **7**. Furthermore, the ^1H NMR gave strong evidence for the formation of compound **7**. Moreover, structure **7** was supported by mass spectrum and elemental analysis, which was compatible with assigned structure.

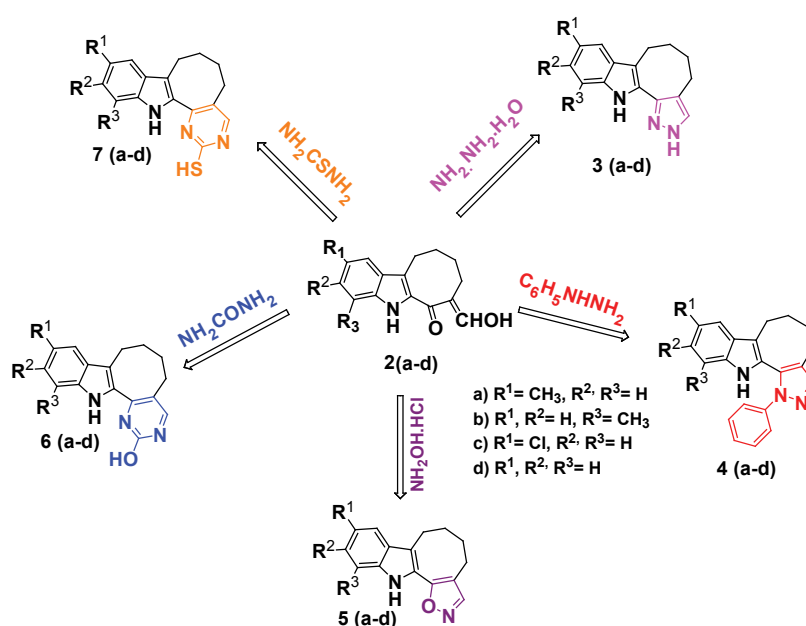
Biology

Antibacterial activity: The newly synthesized cycloocta[b]indoles were screened for their *in vitro* anti bacterial activities against one Gram-positive bacterium *Staphylococcus aureus* and Gram-negative bacteriae *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella Typhi*, *Vibrio cholera* and *Shigella dysenteriae* by the well diffusion method using Streptomycin and Ampicillin as control drugs. The pathogens were cultured on the nutrient agar medium and DMSO was used as solvent and as negative control. The susceptibility was assessed on the basis of diameter of zone of inhibition against gram-positive and gram-negative bacteria. Inhibition zones were measured and compared with the current antibacterial drugs Streptomycin and Ampicillin. The inhibitory activity of **2a-d** against bacteria is summarized in Table 1, together with the results obtained for streptomycin and Ampicillin which were used as reference drugs.

The antibacterial activity results revealed that the majority of the synthesized compounds showed varying degrees of inhibition against the tested micro organisms. Of particular relevance is that compounds **3b**, **6b** and **7b** had effective antibacterial properties against multidrug-resistant strains of *S. aureus* and *E. coli*, *K. pneumoniae*, *S. typhi* and *V. cholerae* with zone of inhibition values ranging between 27-09 mm respectively.

The results of the antimicrobial screening lead to the following assumptions about the structural activity relationship (SAR):

- Analysis of the structure-activity relationships shows that the substituent at the C_2 -position ($\text{R}=\text{CH}_3$, Cl, H) in the intermediate and C_9 -/ C_{10} - position in the cyclized product plays a key role in determining the efficacy of antimicrobial activity.
- The pyrazole moiety [10] in pyrazolocycloocta[b]indoles positively influences in the antibacterial effectiveness, inducing good antimicrobial activity against all the four bacterial pathogens when compared to all other compounds.
- The introduction of electron withdrawing group at the C_2 position in the intermediate and C_9 -/ C_{10} - position in the cyclized product contributed to an activity enhancement, with respect to the electron donating CH_3 group. The role of electron withdrawing group in improving antimicrobial activities had been reported in the literature [11].
- The introduction of a hydroxyl substituted pyrimidine moiety into the cycloocta[b]indole core causes decrease in the inhibitory activity against both the gram positive and gram negative bacterial strain when compared with the introduction of mercapto pyrimidine group due to the decrease in lipophilicity [12].
- Compounds with an unsubstituted cycloocta[b]indole ring exhibited only least activity at greater than 160 mg/ml (data not shown).



Scheme 2: Synthesis of isoxazolo-, pyrido- and pyrazolo-cycloocta[b]indoles.

Compound	Bacteria (zone of inhibition in mm)					
	^a <i>S. aureus</i>	^b <i>E. coli</i>	^c <i>K. pneumoniae</i>	^d <i>S. typhi</i>	^e <i>V. cholerae</i>	^f <i>Shigella sp.</i>
2a	07	08	14	12	9	17
2b	08	07	05	08	09	03
2c	06	03	05	04	11	07
2d	06	05	08	11	9	7
3a	08	07	06	03	11	10
3b	12	11	13	09	27	11
3c	02	03	05	02	12	06
3d	04	11	09	10	07	05
4a	07	09	05	02	10	03
4b	06	08	01	10	09	04
4c	07	11	03	04	11	07
4d	01	-	02	-	04	03
5a	08	06	05	10	08	07
5b	10	11	10	08	15	04
5c	02	04	07	08	06	03
5d	-	06	-	11	-	-
6a	05	08	06	09	04	07
6b	10	09	12	11	15	14
6c	18	14	13	16	19	20
6d	-	-	08	06	09	-Nil-
7a	04	06	08	03	09	05
7b	16	16	13	11	26	15
7c	07	03	08	05	09	02
7d	-	05	-	-	08	06
Streptomycin (150 µg/mL)	09	-	12	10	08	12
Ampicillin (150 µg/mL)	12	13	11	-	17	09

^a*Staphylococcus aureus*, ^b*Escherichia coli*, ^c*Klebsiella pneumoniae*, ^d*Salmonella typhi*, ^e*Vibrio cholerae*, ^f*Shigella species*

Table 1: Anti-bacterial activity of the products (2a-7d).

Antitumor activity: The antitumor activity of compounds 2a-7d were assayed with respect to Doxorubicin by evaluating cell proliferation in MCF-7 breast cancer and in A549 non-small cell lung cancer (NSCLC) cell lines. After 72 h, all the tested compounds showed significant inhibition of cell proliferation (Table 2). In general, all of the synthesized compounds showed a certain degree of anticancer activity against A549 and MCF7 cell lines. As shown in Table 2, some of the tested compounds showed moderate to potent anticancer activities against both the tested cells. The most interesting compounds (2b-7b) had comparable IC₅₀ to the reference substance, ranging between 7 ± 1.224-24.26 ± 2.70 against A549 cell line.

Among the intermediates 2(a-d), the promising compound was found to be 2b which exhibits excellent activity against both the cell lines (A549 IC₅₀ = 20.06 ± 3.79 µg/mL and MCF7 IC₅₀ = 21.96 ± 3.29 µg/mL). This may be due to the presence of electron withdrawing chloro group at 2nd position. It could be seen from Table 2 that, among the cyclized product, the compound 7b with electron-withdrawing chloro group at 10th position and SH groups at 2nd position exhibited the highest cytotoxicity against both A549 (IC₅₀ = 5.02 ± 2.132 µg/mL) and MCF7 (IC₅₀ = 12.35 ± 2.16 µg/mL) cell line. The next highest activity was shown by 6b which is having hydroxy group at 2nd position and chloro group at 10th position with IC₅₀ values A549 (IC₅₀ = 7.00 ± 1.224 µg/mL) and MCF7 (IC₅₀ = 11.00 ± 1.256 µg/mL). Compound 3b showed IC₅₀ values A549 (IC₅₀ = 13.31 ± 2.48 µg/mL) and MCF7 (IC₅₀ = 15.98 ± 1.75 µg/mL) was found to be the next active compound that is having pyrazolo and chloro group. The next highest active compound was 4b having isoxazolo moiety and chloro group with IC₅₀ = 20.06 ± 3.79 µg/mL for A549 and IC₅₀ = 21.96 ± 3.29 µg/mL for MCF cell line. Compound 5b

Compounds	IC ₅₀ (µg/mL)	
	A549 ^a	MCF7 ^b
2a	28.62 ± 2.43	27.34 ± 2.44
2b	20.06 ± 3.79	21.96 ± 3.29
2c	38.84 ± 4.33	37.79 ± 4.18
2d	44.27 ± 3.64	49.45 ± 2.34
3a	26.24 ± 1.33	30.41 ± 3.35
3b	13.31 ± 2.48	15.98 ± 1.75
3c	47.26 ± 2.76	35.77 ± 1.59
3d	43.16 ± 2.65	47.56 ± 3.81
4a	28.63 ± 1.40	29.96 ± 3.71
4b	20.06 ± 3.79	21.96 ± 3.29
4c	42.32 ± 2.56	37.14 ± 3.83
4d	49.18 ± 1.80	48.26 ± 1.17
5a	48.21 ± 1.85	50.41 ± 2.43
5b	24.26 ± 2.70	27.23 ± 3.47
5c	32.00 ± 3.51	25.22 ± 2.04
5d	45.88 ± 2.10	54.61 ± 3.84
6a	22.79 ± 2.67	30.13 ± 3.74
6b	7.00 ± 1.224	11 ± 1.256
6c	26.21 ± 1.262	32 ± 1.220
6d	30.16 ± 3.435	45 ± 2.478
7a	24 ± 3.131	31 ± 1.334
7b	5.02 ± 2.132	12.35 ± 2.16
7c	29.12 ± 1.456	34 ± 2.126
7d	35.12 ± 3.546	46 ± 2.417
Doxorubicin	6.00 ± 1.237	12.355 ± 2.38

^aHuman Lung adenocarcinoma, ^bHuman Breast adenocarcinoma

Table 2: In vitro cytotoxic activity of the products (2a-7d).

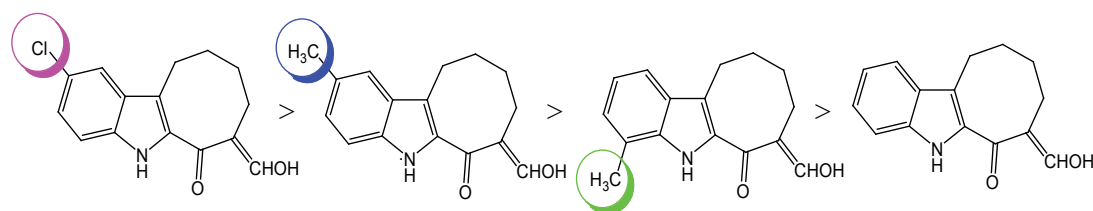


Figure 2: The decreasing order of activity of intermediates.

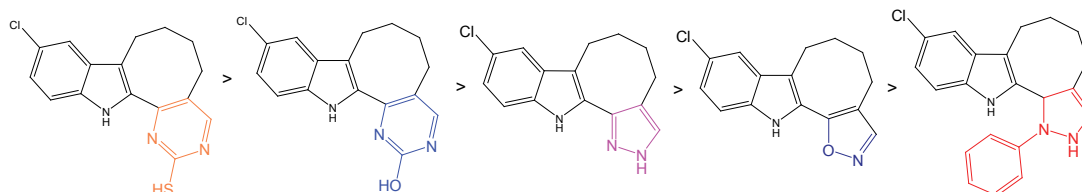


Figure 3: The decreasing order of activity of cyclized products.

having phenyl group at 1st position and chloro group at 9th position was found to exhibit slightly decreased anticancer potency against (A549 IC_{50} = 24.26 ± 2.70 μ g/mL and MCF7 27.23 ± 3.47) cell line.

Compounds **7a**, **6a**, **3a**, **4a**, **5a**, **7c**, **6c**, **3c**, **4c** and **5c** showed moderate activity ranging between 22.79 ± 2.67 - 49.45 ± 2.34 against both the cell lines. Compounds **7d**, **6d**, **3d**, **4d** and **5d** showed least activity ranging between 35.12 ± 3.54 - 54.61 ± 3.84 .

Structure activity relationship: The structure-activity relationship analysis of the data reported in Table 2 shows that,

- Among the intermediates as well as cyclized products, compounds having chloro group was found to be the most active compound compared to the non-halogenated compounds. Out of the intermediates formed, compound **2b** exhibited excellent cytotoxicity. The reason for the efficacy was attributed to the stronger electron withdrawing nature of chlorine [13] that is presented in compound **2b**. For the intermediates the order of activity was shown in Figure 2.
- Among the cyclized products, compound **7b**, was found to be the promising one having chloro group at the 10th position and SH group at 2nd position. This may be due to the presence of mercapto pyrimido [14,15] moiety which enhances the cytotoxic activity. The next potent compound was **6b** that contains hydroxyl group at 2nd position and chloro group at the 10th position. Compound **3b** showed next highest activity which is having pyrazolo moiety and chloro group. The next active compound was **4b** that is having isoxazolo group. The least activity was shown by compound **5b** that contains substituted phenyl pyrazolo group. For the cyclized products the order of activity was shown in Figure 3.

Conclusion

Novel hetero annulated cycloocta[b]indoles were synthesized by efficient synthetic routes and their anti bacterial and anti proliferative activities were assessed. The chloro substituted cycloocta[b]indoles exhibited high anti bacterial activity than their methyl substituted and unsubstituted counter parts. Furthermore, most of them had high anti proliferative activity against breast MCF-7 and lung A549 tumor cell lines. **7b** emerged as the most active antibacterial compound against multidrug-resistant *S. aureus* and the most potent antiproliferative compound against A549 and MCF-7 cells.

While the investigation at the molecular level identified only in part the targets and the binding modes of these new molecules need to be further elucidated, the combination of potent activity against Gram positive bacteria and cancer cell lines make these agents of interest in the search for potential cancer chemotherapeutics. These compounds, possessing both anticancer and antibacterial activity, have a promising therapeutic potential due to their selective cytotoxicity coupled with the ability to reduce the danger of bacterial infections in the frequently immune compromised cancer patient. Furthermore, the screening for anti proliferative properties showed that all products may have a good future as new potent antitumor agent.

To the best of our knowledge, this is the first report on the bio active potential of cycloocta[b]indole counter parts against the A549 and MCF-7 cancer cell lines with IC_{50} value 5 ± 2.132 . This result could be used as a basis for the development of a structure oriented drug discovery program and further study is underway.

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