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Synthesis, Spectral Analysis, Molecular Docking and Biological Evaluation of Cyclohepta[*b*]indole Derivatives

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Abstract: A new series of specifically substituted cyclohepta[*b*]indole derivatives from the precursor thiophen-2-ylmethylene has been synthesized. The structures of synthesized derivatives were established by spectral and elemental analyses. The docking studies with protein kinase CK2 was performed, derivative **6c** exhibited the most excellent glide and E model score of –7.61 and –58.27, respectively. In-vitro anticancer activity against cervical cancer cell line (HeLa) was studied. The IC_{50} values were compared with the standard drug Ellipticine. Compounds **5c**, **6c** and **6d** showed better IC_{50} value when compared to the other molecules. The derivatives were evaluated for their antibacterial activity against the reference drugs Sparfloxacin and Norfloxacin using agar dilution method. The derivatives **4a–d** exhibited better MIC values against Gram-positive bacteria and Gram-negative bacteria when compared with remaining derivatives. Structure activity relationship (SAR) analyses established that the derivatives are potential lead compounds for future drug development studies.

Keywords: cyclohepta[*b*]indoles, antibacterial activity, molecular docking, HeLa, anticancer activity.

INTRODUCTION

NON communicable diseases (NCDs) such as cancer, heart disease and diabetes account for about 65% of the world's deaths. The global cancer burden is growing at an alarming rate. As per the latest information available, it is estimated that if cancer cases continue to increase at its present rate, in 2030 alone, about 21.6 million new cancer cases and 13.0 million cancer deaths would happen worldwide (Cancer Facts & Figures 2017). According to the statistics, cervical and breast cancers are the most commonly diagnosed cancers among women in most areas of the world.[1]

Current cancer treatments include surgery, radiotherapy and chemotherapy. Some of the major chemotherapeutic agents currently being used to treat cancer are Bleomycin, Avastin, Cisplatin and Docetaxel. The search for potent, harmless and selective anticancer compounds is a crucial aspect of modern cancer research. On the other hand, treatment of bacterial infections still remains a major and challenging therapeutic problem, due to the emergence of bacterial resistance to current therapeutic agents.[2] Therefore, there is a call for a new set of drugs in treating the pathogenic microorganisms and cancer causing cells.

Among the heterocyclic substructure the indole ring may be the most ubiquitous one. Owing to its great diversity in both structure and biological activity, it is not surprising that the indole ring is an important structural component in many pharmaceuticals.[3–5] Particularly fused-polycyclic indole derivatives bearing a cyclohepta[*b*]indole framework are potential candidates for drug discovery because this structural motif is present in a wide variety of biologically active alkaloids.[6–9]

The development of an efficient synthetic method of cyclohepta[*b*]indole derivatives has attracted broad attention in medicinal chemistry and synthetic organic chemistry. Extensive efforts are therefore focused on this topic.[10–13] Based on the promising therapeutic activities of cyclohepta[*b*]indole, this research aimed to explore and

synthesize novel substituted cyclohepta[*b*]indole derivatives. Furthermore, the newly synthesized compounds were evaluated for their active sites with protein kinase CK2 (casein kinase 2) by molecular docking study. The in vitro antibacterial and anticancer activity against HeLa human cervical cancer cell line were also carried out.

EXPERIMENTAL

Materials

All the chemicals and solvents were purchased from Sigma-Aldrich and Merck, India. The melting points were checked in open capillaries and are uncorrected. Purity of the products was monitored on silica gel 60 F254 coated TLC plates. The FT-IR spectra of the samples were recorded on *Shimadzu* spectrophotometer in 4000-400 cm^{-1}. ^{1}H-NMR spectra: These were recorded on Bruker Avance III (400 MHz) and Bruker Avance-300 (300 MHz) spectrometers. ^{13}C-NMR spectra: These were recorded on Bruker Avance III (100.6 MHz) and Bruker Avance-300 (75.4 MHz) instruments. Elemental analysis was performed on an *Elementar Vario EL III* C-H-N analyser.

General Procedure for the Synthesis of Compounds (3a–3d)

A mixture of respective 7,8,9,10-tetrahydro-5*H*-cyclohepta*[b]*indol-6-one (**1a**, 4 mmol) and thiophene-2-carbaldehyde (**2**, 4 mmol) was treated with 4 % alcoholic KOH (15 mL) and the mixture stirred for 6 h at room temperature. The precipitated yellow crystalline product **3a** was filtered off and washed with rectified spirit. Compounds **3b**–**3d** was prepared using a similar procedure.

7-THIOPHEN-2-YLMETHYLENE-7,8,9,10-TETRAHYDRO-5H-CYCLOHEPTA[*b*]INDOL-6-ONE (3a)

1.002 g (88 %); Yellow solid; m.p 162–165 °C; IR (KBr) ν_{max} / cm^{-1}: 3312 (N-H); 1623 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ / ppm: 2.14 (q, 2H, C_9-H, J = 5.3 Hz), 3.02 (t, 2H, C_8-H_2, J = 5.6 Hz), 3.13 (t, 2H, C_{10}-H_2, J = 4.7 Hz), 7.04–7.80 (m, 8H, $C_{3'}$ $C_{4'}$,$C_{5'}$-thiophene-H, C_2-methylene- H, C_1,C_2,C_3,C_4-aromatic-H), 9.02 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 25.4, 26.4, 28.0, 112.4, 112.6, 121.7, 122.4, 126.3, 128.3, 128.4, 129.8, 130.2, 133.4, 134.3, 135.1, 136.2, 138.3, 185.7; *Anal*. Calcd mass fractions of element, w / %, for $C_{18}H_{15}NOS$ (M_r = 293) are: C, 73.72; H, 5.11; N, 4.78; S, 10.94. Found: C, 73.78; H, 5.16; N, 4.77; S, 10.92.

2-METHYL-7-THIOPHEN-2-YLMETHYLENE-7,8,9,10-TETRAHYDRO-5H-CYCLOHEPTA[*b*]INDOL-6-ONE (3b)

0.9957 g (85 %); Yellow solid; m.p 178–180 °C; IR (KBr) ν_{max} / cm^{-1}: 3319 (N-H), 1622 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ / ppm: 2.17 (q, 2H, C_9-H, J = 5.7 Hz), 2.45 (s, C_2-3H), 3.05–3.09 (t, 2H, C_8-H_2, J = 5.7 Hz), 3.16-3.20 (t, 2H, C_{10}-H_2 , J = 6.3 Hz), 7.11–7.86 (m, 7H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_2-methylene- H, C_1, C_3,C_4-aromatic-H), 9.03 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm : 21.5, 25.4, 26.4, 28.0, 111.4, 111.5, 120.5, 123.0, 127.4, 128.6, 128.9, 129.4, 129.5, 132.8, 133.1, 136.8, 138.7, 185.4; *Anal*. Calcd mass fractions of element, w / %, for $C_{19}H_{17}NOS$ (M_r = 307) are: C, 74.26; H, 5.53; N, 4.56; S, 10.42. Found: C, 74.33; H, 5.50; N, 4.57; S, 10.44.

2-CHLORO-7-THIOPHEN-2-YLMETHYLENE-7,8,9,10-TETRAHYDRO-5H-CYCLOHEPTA[*b*]INDOL-6-ONE (3c)

0.9832 g (81 %); Yellow solid; m.p 210–212 °C; IR (KBr) ν_{max} / cm^{-1}: 3294 (N-H), 1628 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ / ppm: 2.20-2.27 (q, 2H, C_4-H, J = 5.5 Hz), 3.06-3.10 (t, 2H, C_3-H_2, J = 4.7 Hz), 3.13-3.19 (t, 2H, C_5-H_2, J = 6.3 Hz), 7.12–7.89 (m, 7H, $C_{3'}$, $C_{4'}$, $C_{5'}$-thiophene-H; C_2-methylene-H, C_1, C_3 ,C_4-aromatic-H); 9.16 (s,N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 25.3, 26.3, 27.9, 113.1, 113.3, 122.4, 123.7, 127.3, 129.3, 129.4, 129.8, 130.2, 133.2, 133.7, 135.1, 136.3, 138.7, 185.4; *Anal*. Calcd mass fractions of element, w / %, for $C_{18}H_{14}ClNOS$ (M_r = 327) are: C, 66.05; H, 4.28; N, 4.28; S, 9.78. Found: C, 66.11; H, 4.29; N, 4.22; S, 9.80.

2-BROMO-7-THIOPHEN-2-YLMETHYLENE-7,8,9,10-TETRAHYDRO-5H-CYCLOHEPTA[*b*]INDOL-6-ONE (3d)

0.9651 g (79 %); Yellow solid; m.p 182–184 °C; IR (KBr) ν_{max} / cm^{-1}: 3296 (N-H), 1628 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$) δ / ppm: 2.17 (q, 2H, C_4-H, J = 5.3 Hz), 3.06 (t, 2H, C_3-H_2, J = 5.4 Hz), 3.13 (t, 2H, C_5-H_2, J 6.3 Hz), 7.04-7.80 (m,7H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_2-methylene-H, C_1,C_3,C_4-aromatic-H), 9.02 (s,N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 25.3, 26.3, 27.9, 112.5, 112.9, 120.5, 122.5, 125.7, 126.9, 127.5, 129.3, 130.2, 133.2, 133.9, 134.9, 136.3, 138.7, 185.4; *Anal*. Calcd mass fractions of element, w / %, for $C_{18}H_{14}BrNOS$: (M_r = 371) are: C, 58.22; H, 3.77; N, 3.77; S, 8.62. Found: C, 58.20; H, 3.72; N, 3.80; S, 8.62.

General Procedure for the Synthesis of Compounds (4a–4d)

Respective 7-thiophen-2-ylmethylene-7,8,9,10-tetrahydro-5*H*-cyclohepta*[b]*indol-6-one (**3a**, 1 mmol) was dissolved in absolute ethanol (20 mL) and hydrazine hydrate (0.5 mL, 10 mmol) was added. This mixture was refluxed in oil bath for 30 minutes. Then the solvent was removed under reduced pressure. The crude reaction mixture was poured into ice cold water and the solid obtained was filtered off, washed with water, dried and purified over column chromatography with petroleum ether: ethyl acetate mixture (1 : 2), to get **4a** as a yellow prism. Compounds **4b–4d** was prepared using a similar procedure.

3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-4H-PYRAZOLO[4',3':6,7]CYCLOHEPTA[1,2-*b*]INDOLE (4a)

0.1781 g (79 %); Yellow prism; m.p 150–152 °C; IR (KBr) ν_{max} / cm^{-1}: 3427 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.63 (q, 2H, C_4-H, J = 8.8 Hz), 2.13 (t, 2H, C_3-H_2, J = 8.4 Hz), 2.89 (t, 2H, C_5-H_2 J = 7.6 Hz), 6.93-7.74 (m, 7H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_7,C_8,C_9,C_{10}-aromatic-H), 7.95 (s, 1H, 2NH); 10.95 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 26.1, 27.0, 27.8, 109.1, 109.4, 112.3, 116.6, 120.3, 121.6, 122.3, 123.8, 125.8, 126.8, 128.5, 136.7, 149.5, 152.7, 157.6; *Anal*. Calcd mass fractions of element, *w* / %, for $C_{18}H_{15}N_3S$ (M_r = 305) are: C, 70.82; H, 4.92; N, 13.77; S, 10.49. Found: C, 70.80; H, 4.91; N, 13.75; S, 10.47.

8-METHYL-3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-4H-PYRAZOLO[4',3':6,7] CYCLOHEPTA[1,2-*b*]INDOLE (4b)

0.1732 g (75 %);Yellow prism; m.p 164–166 °C; IR (KBr) ν_{max} / cm^{-1}: 3366 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.70 (q, 2H, C_4-H, J = 10.4 Hz), 2.14 (t, 2H, C_3-H_2, J = 10.8Hz), 2.43 (s, 1H, C_7-CH_3), 3.17 (t, 2H, C_5-H_2 J = 6.4Hz), 6.51–7.71 (m, 6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_7,C_9,C_{10}-aromatic-H); 8.06 (s, 1H, 2NH); 11.32 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 21.5, 25.8, 26.7, 27.5, 111.4, 112.0, 116.1, 120.5, 123.1, 123.3, 128.4, 128.5, 129.3, 133.1, 135.2, 136.5, 144.1, 152.4; Anal. Calcd mass fractions of element, *w* / %, for $C_{19}H_{17}N_3S$ (M_r = 319) are: C, 71.47; H, 5.33; N, 13.16; S, 10.03. Found: C, 71.40; H, 5.32; N, 13.17; S, 10.04.

8-CHLORO-3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-4H-PYRAZOLO[4',3':6,7] CYCLOHEPTA[1,2-*b*]INDOLE (4c)

0.1794 g (72 %); Yellow prism; m.p 180-182 °C; IR (KBr) ν_{max} / cm^{-1}: 3375 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.61 (q, 2H, C_4-H, J = 10.0 Hz), 2.12 (t, 2H, C_3-H_2, J = 8.8 Hz), 2.90 (t, 2H, C_5-H_2 J = 8.0 Hz), 6.94–7.85 (m, 6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_7,C_9,C_{10}-aromatic-H), 8.09 (s, 1H, 2NH); 11.20 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 25.6, 26.6, 27.3, 112.2, 113.0, 113.3, 116.7, 122.6, 123.8, 123.9, 129.3, 129.8, 133.8, 135.2, 136.0, 144.4, 152.2, 158.6; *Anal*. Calcd mass fractions of element, *w* / %, for $C_{18}H_{14}ClN_3S$ (M_r = 339) are: C, 63.71; H, 4.13; N, 12.38; S, 9.43. Found: C, 63.68; H, 4.09; N, 12.34; S, 9.41.

8-BROMO-3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-4H-PYRAZOLO[4',3':6,7] CYCLOHEPTA[1,2-*b*]INDOLE (4d)

0.1693 g (64 %); Yellow prism; m.p 172–174 °C; IR (KBr) ν_{max} / cm^{-1}: 3297 (N-H); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.70 (q, J = 10.0 Hz, 2H), 2.09 (t, J = 10.0 Hz, 2H), 2.70 (t, J = 12.4 Hz, 2H), 6.94–7.83 (m, 6H), 8.05 (s, 1H), 10.99 (s, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ: 25.9, 26.6, 27.5, 112.4, 113.3, 113.6, 116.6, 122.4, 123.8, 123.9, 129.3, 129.8, 133.4, 135.1, 136.2, 144.3, 152.3, 158.7; *Anal*. Calcd mass fractions of element, *w* / %, for $C_{18}H_{14}BrN_3S$: (M_r = 384) are: C, 56.25; H, 3.64; N, 10.93; S, 8.33. Found: C, 56.23; H, 3.62; N, 10.89; S, 8.31.

General Procedure for the Synthesis of Compounds (5a–5d)

7-Thiophen-2-ylmethylene-7,8,9,10-tetrahydro-5*H*-cyclohepta*[b]*indol-6-one (**3a**, 1 mmol) was mixed with hydroxylamine hydrochloride (14 mmol) and pyridine (5 mL). The reaction mixture was refluxed in oil bath for 30 minutes. After the reaction was completed, the mixture was poured into crushed ice. The resulting solid separated was filtered off, washed with dilute HCl and water. The substance was dried and purified over column chromatography with petroleum-ether: ethyl acetate solvent mixture (1 : 5), to get **5a** as a yellow prism. Compounds **5b–5d** was prepared using a similar procedure.

3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-ISOXAZOLO[4',3':6,7]CYCLOHEPTA[1,2-*b*] INDOLE (5a)

0.1552 g (57 %); Yellow prism; m.p 152–154 °C; IR (KBr) ν_{max} / cm^{-1}: 3292 (N-H), 1739 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.63 (q, 2H, C_4-H, J = 8.8 Hz), 2.13 (t, 2H, C_3-H_2, J = 8.4 Hz), 2.89 (t, 2H, C_5-H_2 J = 7.6 Hz), 6.93-7.74 (m,7H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_7,C_8,C_9,C_{10}-aromatic-H), 10.95 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 22.6, 25.6, 26.5, 109.4, 109.7, 112.0, 116.3, 120.6, 121.8, 122.1, 137.3, 141.5, 143.7, 145.6, 147.0, 151.8, 156.6, 157.8; *Anal*. Calcd mass fractions of element, *w* / %, for $C_{18}H_{14}N_2OS$ (M_r = 306) are: C, 70.58; H, 4.57; N, 9.15; S, 10.45. Found: C, 70.55; H, 4.52; N, 9.17; S, 10.42.

8-METHYL-3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-ISOXAZOLO[4',3':6,7]CYCLOHEPTA [1,2-*b*]INDOLE (5b)

0.1530 g (58 %); Yellow prism; m.p 167–169 °C; IR (KBr) ν_{max} / cm^{-1}: 3293 (N-H), 1741 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.70 (q, 2H, C_4-H, J = 10.4 Hz), 2.14 (t, 2H, C_3-H_2, J = 10.8 Hz), 2.43 (s, 1H, C_7-CH_3), 3.17 (t, 2H, C_5-H_2 J = 6.4 Hz), 6.51–7.71 (m, 6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_7,C_9,C_{10}-aromatic-H), 11.32 (s, N-H). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 21.6, 25.8, 26.6, 27.5, 111.9, 119.1, 121.4, 122.0, 126.4, 127.2, 127.8, 128.4, 130.5, 136.3, 136.5, 137.0, 139.8, 150.1, 157.6; *Anal*. Calcd mass fractions of element, *w* / %, for $C_{19}H_{16}N_2OS$ (M_r = 320) are: C, 71.25; H, 5.00; N, 8.75; S, 10.00. Found: C, 71.29; H, 4.98; N, 8.71; S, 10.02.

8-CHLORO-3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-ISOXAZOLO[4',3':6,7]CYCLOHEPTA [1,2-*b*]INDOLE (5c)

0.1603 g (55 %); Yellow prism; m.p 172–174 °C; IR (KBr) ν_{max} / cm^{-1}: 3285 (N-H), 1741 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.61 (q, 2H, C_4-H, J = 10.0 Hz), 2.12 (t, 2H, C_3-H_2, J = 8.8 Hz), 2.90 (t, 2H, C_5-H_2 J = 8.0 Hz), 6.94–7.85 (m,6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_7,C_9,C_{10}-aromatic-H), 11.20 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 25.9, 26.6, 27.5, 113.5, 115.8, 117.4, 120.2, 121.5, 122.5, 126.7, 136.4, 137.8, 139.8, 141.6, 141.7, 141.8, 152.3, 158.7; *Anal*. Calcd mass fractions of element, *w* / %, for $C_{18}H_{13}ClN_2OS$ (M_r =

340) are: C, 63.52; H, 3.82; N, 8.23; S, 9.41. Found: C, 63.50; H, 3.78; N, 8.25; S, 9.39.

8-BROMO-3-THIOPHEN-2-YL-2,5,6,11-TETRAHYDRO-ISOXAZOLO[4',3':6,7]CYCLOHEPTA [1,2-*b*]INDOLE (5d)

0.1594 g (51 %); Yellow prism; m.p 168–170 °C; IR (KBr) ν_{max} / cm^{-1}: 3286 (N-H), 1740 (C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.70 (q, 2H, C_4-H, J = 10.0 Hz), 2.09 (t, 2H, C_3-H_2, J = 10.0 Hz), 2.70 (t, 2H, C_5-H_2 J = 12.4 Hz), 6.94-7.83 (m,6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_7,C_9,C_{10}-aromatic-H), 10.99 (s, N-H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ / ppm: 25.6, 26.6, 27.3, 113.6, 120.3, 121.4, 122.5, 124.5, 126.8, 127.1, 128.8, 130.5, 133.8, 135.2, 136.0, 144.4, 152.2, 158.6; *Anal*. Calcd mass fractions of element, w / %, for $C_{18}H_{13}BrN_2OS$ (M_r = 385) are: C, 56.10; H, 3.37; N, 7.32; S, 8.31. Found: C, 56.10; H, 3.37; N, 7.32; S, 8.31.

General Procedure for the Synthesis of Compounds (6a–6d)

7 – Thiophen – 2 – ylmethylene - 7, 8, 9, 10 – tetrahydro - 5*H* - cyclo-hepta*[b]*indol-6-one (**3a**, 1 mmol) was mixed with guanidium nitrate (10 mmol) in glacial acetic acid (5 mL). The reaction mixture was refluxed in oil bath for 5 hours. The reaction was monitored by TLC. After the reaction was completed, the mixture was poured into crushed ice. The resulting solid separated was filtered off, washed with water. The substance was dried and purified over column chromatography with petroleum-ether: ethyl acetate solvent mixture (5 : 1), to get **6a** as a yellow solid. Compounds **6b**–**6d** was prepared using a similar procedure.

2-AMINO- 4-THIOPHEN-2-YL-5,6,7,12-TETRAHYDRO-PYRIMIDO[5',6':6,7]CYCLOHEPTA [1,2 *b*]INDOLE (6a)

0.1971 g (70 %); Yellow solid; m.p 138-140 °C; IR (KBr) ν_{max} / cm^{-1}: 3342 (N-H), 3275, 3196 (NH_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.87–1.88 (m, 2H, C_4-H), 1.94–1.99 (m, 2H, C_3-H_2), 2.75–2.73 (m, 2H, C_5-H_2), 5.00 (s, 2H, NH_2), 6.92–7.57 (m,7H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_8,C_9,C_{10},C_{11}-aromatic-H), 11.22 (s, N-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ / ppm: 23.6, 26.8, 29.7, 113.3, 121.0, 122.7, 123.2, 126.8, 128.6, 129.0, 129.1, 136.9, 140.5, 140.6, 142.1, 143.8, 150.0, 154.2, 155.2; *Anal*. Calcd mass fractions of element, w / %, for $C_{19}H_{16}N_4S$ (M_r = 332) are: C, 68.67; H, 4.81; N, 16.86; S, 9.63. Found: C, 68.63; H, 4.78; N, 16.82; S, 9.60.

2-AMINO- 9-METHYL- 4-THIOPHEN-2-YL-5,6,7,12-TETRAHYDROPYRIMIDO[5',6':6,7]CYCLOHEPTA-[1,2-*b*]INDOLE (6b)

0.1938 g (68 %); Yellow solid; m.p 189–191 °C; IR (KBr) ν_{max} / cm^{-1}: 3351 (N-H), 3289, 3190 (NH_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.87–1.88 (m, 2H, C_4-H), 1.94–1.97 (m, 2H, C_3-H_2), 2.37–2.75 (m, 2H, C_5-H_2), 5.00 (s, 2H, NH_2), 6.92–7.46 (m,6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_8,C_{10},C_{11}-aromatic-H), 11.17(s, N-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ / ppm: 21.16, 23.6, 26.8, 29.7, 113.3, 121.0, 122.7, 123.2, 126.8, 128.6, 129.0, 129.1, 136.9, 140.5, 140.6, 142.1, 143.8, 150.0, 154.2, 155.2 ppm; *Anal*. Calcd mass fractions of element, w / %, for $C_{20}H_{18}N_4S$ (M_r = 346) are: C, 69.36; H, 5.20; N, 16.18; S, 9.24. Found: C, 69.32; H, 5.24; N, 16.17; S, 9.22.

2-AMINO- 9-CHLORO- 4-THIOPHEN-2-YL-5,6,7,12-TETRAHYDROPYRIMIDO[5',6':6,7] CYCLOHEPTA-[1,2-*b*]INDOLE (6c)

0.1860 g (71 %); Yellow solid; m.p 218–220 °C; IR (KBr) ν_{max} / cm^{-1}: 3322 (N-H), 3267, 3176 (NH_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.87–1.88 (m, 2H, C_4-H), 1.94–1.99 (m, 2H, C_3-H_2), 2.73–2.75 (m, 2H, C_5-H_2), 5.00 (s, 2H, NH_2), 6.92–7.57 (m,6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_8,C_{10},C_{11}-aromatic-H), 11.22(s, N-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ / ppm: 25.4, 25.7, 26.4, 113.5, 121.1, 122.5, 123.9, 126.3, 128.9, 129.0, 129.5, 136.8, 140.6, 140.6, 142.1, 143.8, 150.0, 154.2, 155.3; *Anal*. Calcd mass fractions of element, w / %, for $C_{19}H_{15}ClN_4S$ (M_r = 366) are: C, 62.29; H, 4.09; N, 15.30; S, 8.74. Found: C, 62.32; H, 4.11; N, 15.28; S, 8.73.

2-AMINO-9-BROMO-4-THIOPHEN-2-YL-5,6,7,12-TETRAHYDROPYRIMIDO[5',6':6,7] CYCLOHEPTA-[1,2-*b*]INDOLE (6d)

0.1702 g (65 %); Yellow solid; m.p 212–214 °C; IR (KBr) ν_{max} / cm^{-1}: 3345 (N-H), 3269, 3144 (NH_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ / ppm: 1.87–1.88 (m, 2H, C_4-H), 1.94–1.99 (m, 2H, C_3-H_2), 2.73–2.75 (m, 2H, C_5-H_2), 5.00 (s, 2H, NH_2), 6.92–7.57 (m,6H, $C_{3'}$,$C_{4'}$,$C_{5'}$-thiophene-H; C_8,C_{10},C_{11}-aromatic-H), 11.21(s, N-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ / ppm: 25.3, 25.6, 26.4, 113.5, 121.1, 122.5, 123.9, 126.3, 128.9, 129.0, 129.5, 136.8, 140.6, 140.6, 142.1, 143.8, 150.0, 154.2, 155.3; Anal. Calcd mass fractions of element, w / %, for $C_{19}H_{15}BrN_4S$ (M_r = 411) are: C, 55.47; H, 3.64; N, 13.62; S, 7.78. Found: C, 55.45; H, 3.68; N, 13.60; S, 7.72.

In vitro Antibacterial Studies

All the cyclohepta[*b*]indole derivatives were studied for their antibacterial activity against clinically isolated two Gram-positive bacteria (*Bacillus subtilis (B.S)* and *Staphylococcus aureus (S.A)*) and five Gram negative bacteria (*Escherichia coli (E.C)*, *Proteus vulgaris (P.V)*, *Salmonella typhi (S.T)*, *Pseudomonas aureus (P.A)* and *Klebsiella pneumonia (K.P)*) using conventional agar dilution method.[14] The minimum inhibitory concentrations (MICs) values were determined by comparing the values with reference bacterial drugs as Sparfloxacin and Norfloxacin. All the cultures were prepared by Muller Hinton agar and the turbidity of all bacterial cultures was adjusted to 0.5 McFarland standards by preparing a bacterial suspension of three to five well-isolated colonies of the similar morphological type chosen

from an agar plate culture. The cultures were further diluted 1,000-fold to get an inoculums size of 1.5×10^5 CFU / mL. The synthesized compounds and standard bacterial drugs (50 mg) were dissolved in dimethyl formamide (DMF) (0.5 mL) and the solution was diluted with water (4.5 mL) to get a stock solution of 10,000 mg L^{-1} of each compound. Further progressive double dilution with Muller–Hinton broth was performed to obtain the required concentrations of 2,500–2.1 μg mL^{-1}. To make sure that the solvent had no effect on the bacterial growth, a control test was carried out with a test medium supplemented with DMF at the same dilutions as used in the experiment. In each microwell inoculated with 75 μL of the serial dilutions, 75 μL of the bacterial suspension was added in a series of 12 microwells. Incubation of the cultures overnight at 37 °C was done and the growth measured.

Molecular Docking Studies

Molecular docking studies were done by the *Schrödinger* Glide program[15] (version 8.5, *Schrödinger*, LLC, New York 2010). The three-dimensional structures of CK2 protein were predicted and downloaded through Robetta server. In order to apprehend the docking glide score results, a Maestro user interface (version 8.5, *Schrödinger,* LLC, New York 2010) was executed. To confirm the best docking and to validate the docking score, the protocol was evaluated by re-docking. Structures of all the compounds were sketched using ACD/chemsketch[16] (Freeware version). With the help of the Glide Grid generation wizard we can describe the docking space which predicts the biologically active site. Glide docking was accomplished with the help of SP (Single precision) and XP (Extra precision) docking procedure.

In vitro Anticancer Studies

The *in vitro* anticancer property of the synthesized cyclohepta[*b*]indole derivatives was carried out by MTT assay.[17] The human cervical cancer cell line (*HeLa*) was acquired from National Centre for Cell Science (NCCS), Pune, and cultured in complete EMEM growth medium (EMEM + 10 % FBS). For screening test, the cells were seeded into 96-well plate at plating density of 10,000 cells / wells. And the plates were incubated to allow cell attachment at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h. Then the cells were treated with the synthesized cyclohepta[*b*]indole derivatives at different concentrations. The samples were dissolved in DMSO and additionally diluted in serum free medium to make five concentrations. 100 μL per well of each concentration was added to plates to get final concentrations of 100, 50, 25, 12.5 and 6.25 μM. The total volume in each well was 200 μL and the plates were incubated at 37 °C, 5 % CO_2, 95 % air and 100 % relative humidity for 48 h. The medium without samples were considered as control. Three replicates were maintained for each concentration. After incubation, 15 μL of MTT solution (5 mg mL^{-1}) in phosphate buffered saline (PBS) was added to each well and incubated for 4 h at 37 °C. The medium with MTT was then flicked off and the formed Formosan products were dissolved in 100 μL of DMSO and then the absorbance was monitored at 570 nm using micro plate reader. The % cell inhibition was calculated using the following formula. % cell inhibition = 100 - absorbance (sample) / absorbance (control) × 100. Nonlinear regression graph was plotted against % cell inhibition and Log_{10} concentration and using Graph Pad Prism software IC_{50} were determined.

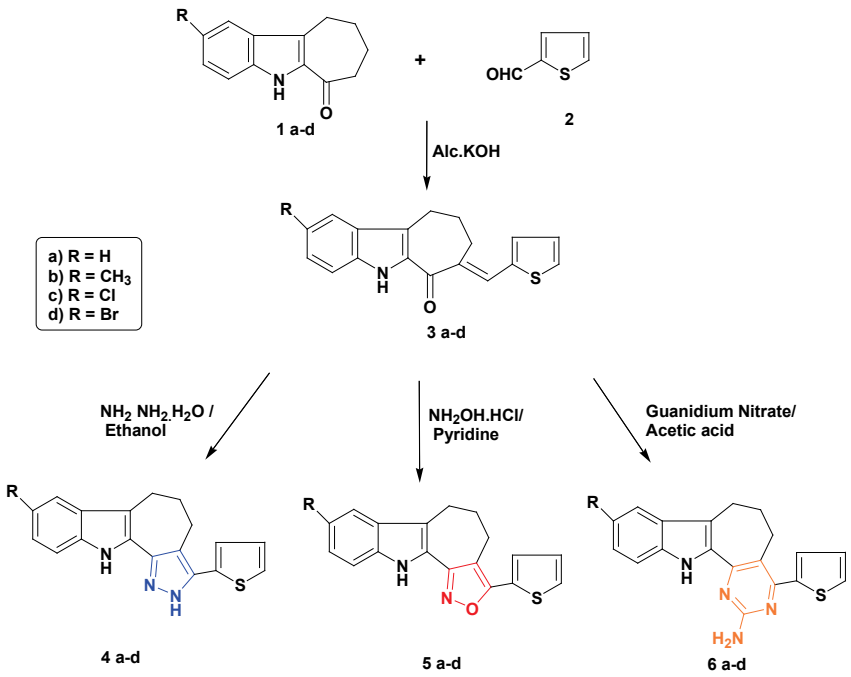
RESULTS AND DISCUSSION

Chemistry

The precursor thiophen-2-ylmethylene **3a–d** was synthesized by mixed aldol condensation of 1,4-dihydro-2H-cyclopenta[*b*]indol-3-one **1a–d** with thiophene-2-carbaldehyde **2**. Further the reaction of thiophen-2-ylmethylene derivatives **3a–d** with hydrazine hydrate, hydroxylamine hydrochloride and guanidium nitrate leads to highly substituted cyclohetpa[*b*]indoles **4a–d**, **5a–d** and **6a–d** in one-pot addition reactions. The synthetic routes are shown in the Scheme 1. The structures of the products were established on the basis of IR, ^{1}H NMR, ^{13}C NMR data and elemental analysis.

The formation of **3a** was confirmed from its ^{1}H NMR spectrum, which shows that the C_2-methylene proton and thiophene-CH signals along with the aromatic proton signals and the disappearance of C_7-methylene proton signal. The IR spectrum of **3a** showed sharp and strong bands at 3312 and 1623 cm^{-1} assigned to NH and carbonyl group respectively. The spectral and analytical data suggested the structure of **3a** to be a thiophen-2-ylmethylene compound. The proton NMR spectrum of **4a** showed two broad singlet at 10.95 ppm and 7.77 ppm, attributed to indole-NH and pyrazole-NH protons, respectively. The other aromatic protons appeared as multiplet in the region 6.93–7.54 ppm which confirmed the formation of **4a**. The IR spectrum of **5a** registered absorption band at 1626 cm^{-1} assigned to C=N functional group. Its proton NMR spectrum showed one broad singlet at 11.55 ppm assigned for indole-NH. The rest of the aromatic protons appeared as a multiplet between the regions δ 7.05–7.90 ppm. Analytical data are in accordance with the proposed structure for compound **5a**.

The ^{1}H NMR spectrum of **6a** showed a broad singlet at 11.22 ppm accounted for indole-NH. The methylene protons disappeared from the aliphatic region and further peaks appeared in the aromatic region undoubtedly indicating that the system was completely aromatized. The presence of an amino group in the substrate was inferred



Scheme1. Synthesis of substituted cyclohetpa[*b*]indoles.

Table 1. In-vitro antibacterial activity of synthesized compounds against (MICs in μg mL^{-1}).

Compounds	Minimum Inhibitory Concentrations / μg mL^{-1} (MIC)						
	B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*	*S. typhi*	*P. aureus*	*K. pneumoniae*
3a	286.3	–[a]	483.1	246.4	237.2	106.8	724.6
3b	396.5	638.2	273.7	485.6	547.1	162.5	366.5
3c	174.0	724.6	–[a]	196.5	321.3	141.1	994.2
3d	–[a]	173.7	326.8	661.5	799.7	131.7	–[a]
4a	263.5	564.0	291.6	81.5	166.5	116.5	1012.4
4b	82.0	–[a]	163.5	361.5	–[a]	156.5	876.5
4c	39.5	162.5	–[a]	157.5	443.5	83.5	572.5
4d	157.2	234.5	89.5	132.5	101.7	112.5	890.5
5a	218.3	146.5	–[a]	247.5	543.5	56.2	513.5
5b	84.25	113.5	427.25	143.8	282.6	97.5	970.5
5c	85.0	–[a]	126.3	291.4	–[a]	156.3	1280.6
5d	61.9	264.6	105.2	349.5	784.4	39.7	1957.1
6a	246.4	185.6	116.5	161.5	197.7	107.5	836.8
6b	237.2	247.1	121.3	99.7	246.3	161.5	179.5
6c	106.8	212.5	–[a]	231.7	593.5	180.0	714.5
6d	124.6	166.5	94.2	128.3	438.5	31.2	472.5
Sparfloxacin[b]	9.76	4.97	166.3	7.8	1500	163.8	1320.0
Norfloxacin[b]	–[a]	49.06	515	392.25	825	57.4	973.4

Lower MIC values indicate that higher antimicrobial activity.

[a] No inhibition observed.

[b] Standard antibacterial drugs.

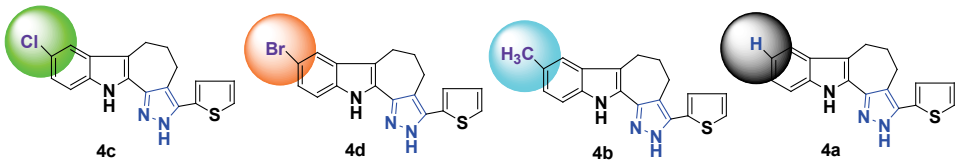


Figure 1. Role of electron-withdrawing group in increasing the efficiency of antibacterial activities.

from a broad singlet at 5.00 ppm. The IR spectrum showed absorptions at 3443, 3189 and 3157 cm^{-1} which were assigned to N–H and NH_2 functional groups. Analytical data are in accordance with the proposed structure for compound **6a**. The ^{13}C NMR spectra of the compounds are characteristic and helped in assigning the number of different carbon atoms. The structures of the other compounds were assigned in similar way with all spectral and analytical data.

In vitro Evaluation of Antibacterial Activities

The newly synthesized cyclohepta[*b*]indole derivatives were tested for their *in vitro* antibacterial activities against two Gram-positive and five Gram-negative bacteria strains. The results revealed that the derivatives exhibited good antibacterial profile against three Gram-negative bacteria *S. typhi*, *P. aureus* and *K. Pneumonia*. Compounds **4d** (89.5 μg mL^{-1}) and **6d** (94.2 μg mL^{-1}) exhibit better activity against *E. coli* than the standard drug Sparfloxacin (166.3 μg mL^{-1}) and Norfloxacin (515 μg mL^{-1}). Compound **4a** (81.5 μg mL^{-1}) exhibits better antibacterial activity against *P. vulgaris* than Norfloxacin (627 μg mL^{-1}). Compound **4d** (101.7 μg mL^{-1}) shows good activity against *S. typhi*. Compound **6b** (179.5 μg mL^{-1}) exhibits the best activity among all the molecules synthesized against *K. pneumoniae* that was better than those of Sparfloxacin (1320 μg mL^{-1}) and Norfloxacin (973 μg mL^{-1}). Standard drug Norfloxacin did not exhibit any activity against *B. subtilis* microorganism. Compounds **4b** and **5c** did not exhibit any inhibition against *S. typhi* and *P. aureus*. All the MIC values are presented in Table 1.

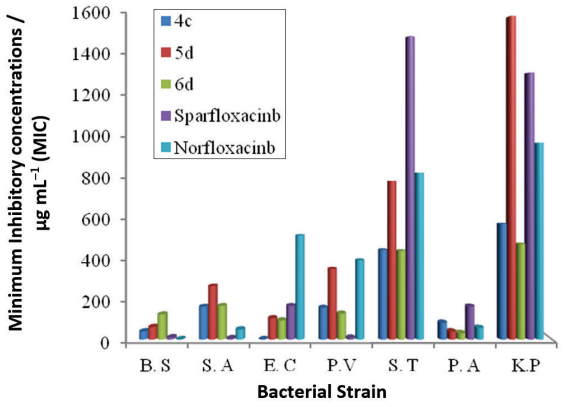


Figure 2. Representative antibacterial activities of most potent compounds.

Regarding the structure activity relationship (SAR), a variety of 8-substituted cyclohepta[*b*]indole analogues containing CH_3, Cl, Br groups have been exploited for their antibacterial activity. The investigation reveals that introduction of halogen (chloro and bromo) groups positively influence the antibacterial effectiveness. This property is truly aligned with the literature.[18] Further compound with an unsubstituted cyclohepta[*b*]indole ring exhibited only least activity. Figure 1 clearly shows this pattern among pyrazolo derivatives **4a–4d**.

The pyrazole moiety[19] enhances antibacterial activity in **4a–d**, inducing better antibacterial activity against four bacterial pathogens when compared to all other compounds. Only a moderate improvement of antibacterial activity was noted in the presence of a isoxazole **5a–d** or pyrimidine moiety **6a–d**. Compounds **3a–d** showed the least activity among the series. Representative antibacterial activities of most potent compounds are displayed in Figure 2.

Molecular Docking Studies

In an attempt to get deeper understanding about the mechanism of anticancer activity and structure activity relationships (SAR) of the newly synthesized hetero-annulated cyclohepta[*b*]indoles, we performed docking studies using the *Schrödinger* Glide program with Human Protein Kinase CK2 protein. It is a ubiquitous serine/threonine protein kinase, product of a tetramer containing two catalytic and regulatory subunits (α and / or α') linked with two molecules of the β subunits. CK2 is a multifunctional protein kinase that plays a major role in cell growth, cell proliferation and cell apoptosis. Numerous reports have demonstrated the over-expression, dysregulation and hyperactivation of CK2 in many types of cancers.[20,21]

Table 2. Molecular docking data of compounds (**4c**, **5c** and **6c**) with protein kinase CK2.

Compound	Glide score / kcal mol^{-1}	E model score	Glide energy
4c	–7.081	–53.589	–37.755
5c	–7.298	–53.162	–36.784
6c	–7.615	–58.270	–40.200

The glide and E model scores of the selected compounds (**4c, 5c and 6c**) are presented in Table 2. The docking results show that the binding mode in compound **4c** was attractively bound to CK2 via hydrophobic interaction, and Pi-Pi stacked interaction. The compound **4c** enclosed by VAL27, VAL35, ALA50, LEU151, ALA165, ILE79, LEU70, MET167, PHE169, TYR32. The amino acid residue PHE97 interacts in pi-pi stacked interaction with thiophene ring. VAL27, VAL35, LEU151, ALA50, ILE79, PHE97, MET167, LEU70, PHE169, ALA165, TYR32 residues are enclosed by hydrophobic interaction in compound **5c**. The compound **6c** is surrounded by hydrophobic interaction of amino acids residues PHE169, LEU70, ALA165, VAL27, MET167, ILE79, PHE97, ALA50, LEU151, and VAL35. The Tyrosinal interacts in pi-pi stacked interaction with pyrimidine ring (Figure 3).

The scoring functions of the docking program ranked that the binding interactions of the intermediate were less than those of the cyclised products. The compounds **6a**, **6b**, **6c** and **6d** showed better binding interaction compared to the intermediate; this might be due to the presence of the pyrimido group. The next best interactions were found among the compounds **5a**, **5b**, **5c** and **5d** which hold the isoxazolo moiety. Among the cyclised products, compound **6c** showed the best lowest binding energy and ligand efficiency, this might be due to the presence of the pyrimido group which was further reinforced by favourable electrostatic interaction of the chloro group at the 9 position of the indole moiety. In general it was found that the pyrimido moiety favours better binding interactions compared to isoxazolo, pyrazolo moieties and intermediates.

Anticancer Properties of Cyclohepta[*b*]indole Derivatives

The in vitro anticancer activities of the synthesized compounds (**3a–6d**) were evaluated by a cell viability assay method against a human cervical cancer cell line (HeLa). A clinically used antitumor agent Ellipticine was used as a positive control. The anticancer properties of these compounds were shown by IC_{50} (the concentration that causes a 50 % reduction of the cell growth). The results are given in Figure 4. The in vitro anticancer assay proves that the compounds **5c, 6c** and **6d** have potent anticancer effect on human cervical cancer cells. The estimated IC_{50} values were 17.14 µM for **5c**, 12.92 µM for **6c**, 13.06 µM for **6d**. Therefore, it is evident that the compound **6c** has a cell growth inhibition value closer to the standard drug Ellipticine. From the Figure 5, it is evident that all the compounds display a dose-dependent anticancer activity. The maximum anticancer property was obtained at 100 µM. The percentage of cell growth inhibition is found to be 58.26 % for compound **5c,** 88.13% for compound **6d** and 100 % for compound **6c**.

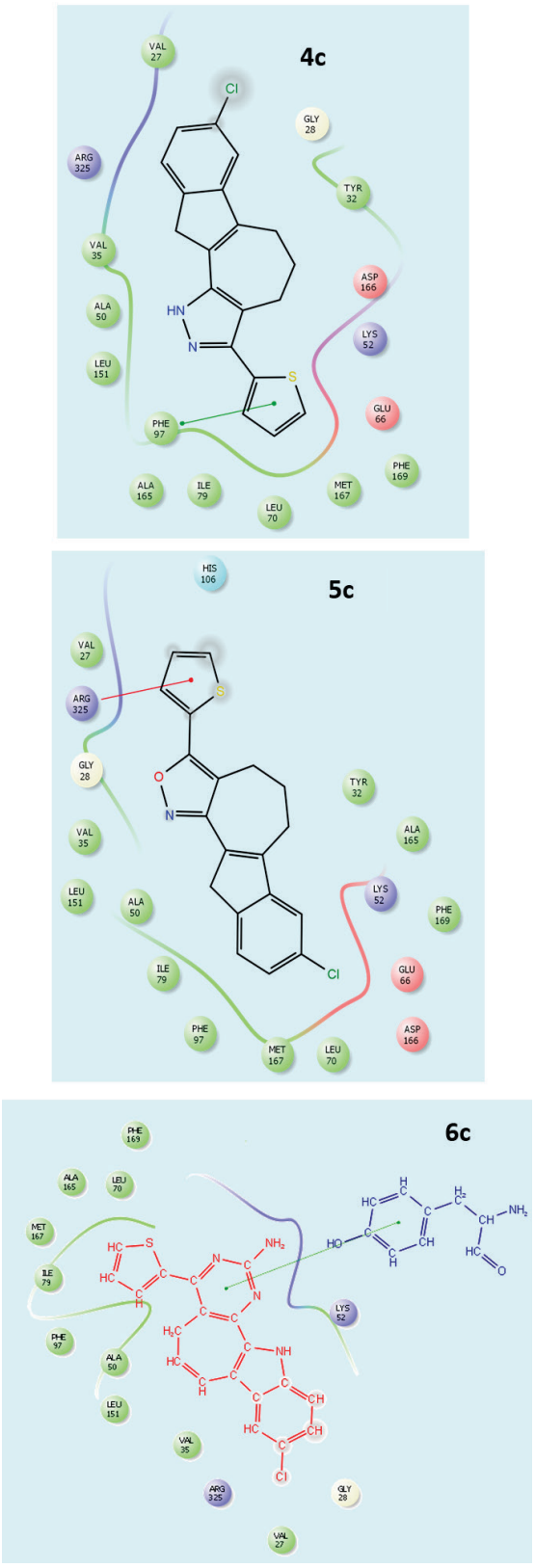


Figure 3. Docking model structure of compounds **4c, 5c** and **6c** into the Protein kinase.

Structure Activity Relationship (SAR) Studies

The present study evaluates the effect of several substituents and from the results of anticancer activity of the synthesized cyclohepta*[b]*indoles the following structure activity relationships can be derived:

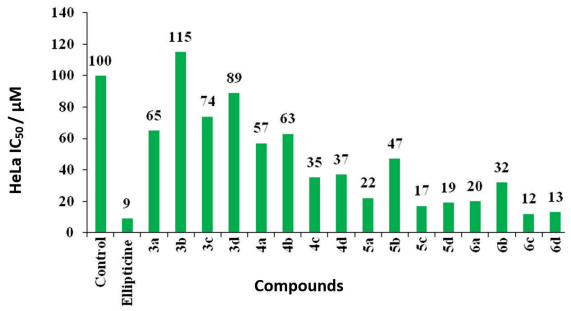


Figure 4. Anticancer activity (IC_{50})of synthesised compounds (**3a–6d**) and Ellipticine used as standard.

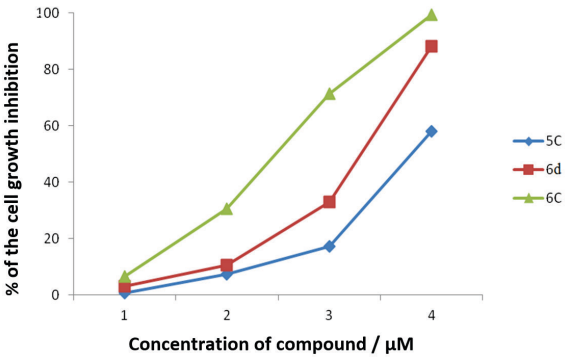


Figure 5. Effect of % cell growth inhibition of the compounds **5c**, **6c** and **6d** in different concentration (µM).

(i) Among the synthesized compounds, the compound **6c** displayed the strongest anticancer activity against HeLa. This might be due to the presence of the pyrimido moiety[22] which enhanced the anticancer activity. The biological properties like anticancer, antiviral, antibacterial, urinary tract infection treatment and vasodilation were improved when the 5 and 6 position of pyrimidine fused with heterocyclic ring. Literature survey revealed that the pyrimidine substituted heterocycles induced cell apoptosis through tubulin polymerization. The structure activity relationship analysis also confirms that pyrimidine group is essential for the induction of apoptosis and extreme anticancer activity.[23]

(ii) The next most active compounds were **5a, 5b, 5c** and **5d** which displayed good anticancer activity. This might be due to the presence of an isoxazolo group[24] which boosts the anticancer activity.

(iii) Subsequently, compounds **4a**, **4b**, **4c** and **4d** also showed good anticancer activity with IC_{50} values < 65 µM against HeLa which was due to the presence of pyrazolo moiety which enhanced the anticancer activity.

(iv) It was noted that the intermediates showed comparatively less cytotoxic activity than the cyclised derivatives (Figure 6).

(v) In general, it was noted that among the cyclised derivatives, the derivatives bearing an electron-withdrawing[25] chlorine and bromine in the indole ring enhanced the anticancer activity more than the electron-donating methyl group and the unsubstituted derivative.

CONCLUSION

Molecular docking study results revealed clearly that these molecules show significant molecular binding interaction with Protein kinase CK2. The structure activity relationship

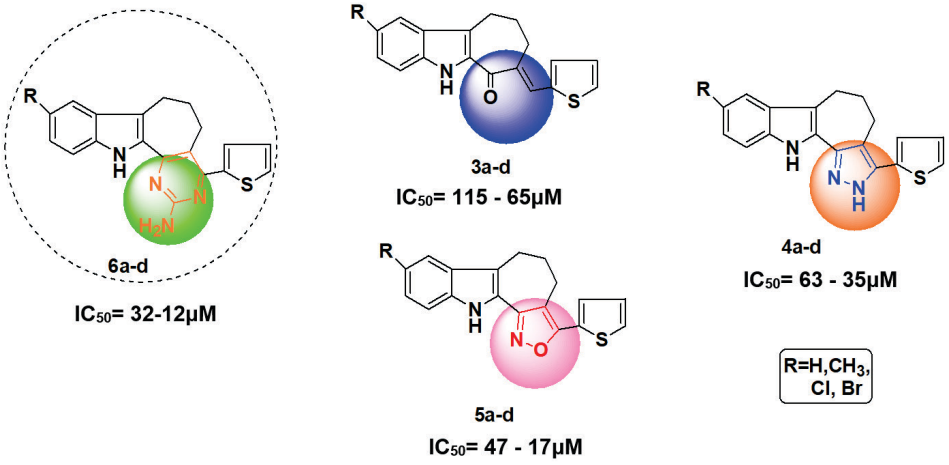


Figure 6. Structure activity relationship of compounds **6a-d** with **3a-d, 4a-d** and **5a-d**.

of antimicrobial studies discovered that the halogen substituted cyclohepta[*b*]indoles display higher activity than their counterparts. Similarly, compounds **5c, 6c** and **6d** displayed better anticancer activity against other derivatives. Further investigations of cyclohepta[*b*]indole derivatives could lead to more potent compounds as promising candidates for the development of new antimicrobial and anticancer agents.

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Supplementary Information. Supporting information to the paper is attached to the electronic version of the article at: https://doi.org/10.5562/cca3375.

PDF files with attached documents are best viewed with Adobe Acrobat Reader which is free and can be downloaded from Adobe's web site.

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