International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
Journal DOI: 10.7439/ijpc
CODEN: IJPCH3 (American Chemical Society)
Research Article

Docking, Synthesis and Anticancer Activity of Some Newer Carbazole Derivatives

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Abstract

Numerous carbazole derivatives were designed by the Chemsketch software followed by 3D optimization. Docking studies were performed using AUTODOCK 4.2.6 software to check their binding interactions with eukaryotic topoisomerase-I, based on the crystal structure of Human Topoisomerse-I-DNA complex (PDB ID: 1A35). Results of docking studies of designed carbazole derivatives were compared on the basis of their minimum binding energy with a well known topoisomerase-I inhibitor i.e. Rebeccamycin,. Above results were used to find out active compounds and two series of such active compounds i.e. 2-[(4, 5-dihydro-2-substitutedphenyl)imidazol-1-ylamino]-1-(9*H*-carbazol-9-yl)ethanone **(3a-3e)** and 2-(9*H*-carbazol-9-yl)-*N'*-[{(4-substitutedphenyl)(piperazin-1-yl)}methyl]acetohydrazide **(3a'-3e')** were synthesized. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, MASS spectrometry and elemental analysis and also screened for their *invitro* anticancer activity against human breast cancer cell line (MCF 7) by sulphorodamine B (SRB) assay method. GI_{50} was measured by using 10, 20, 40 and 80 µg/ml concentrations of tested compounds along with the standard i.e. Rebeccamycin. Results revealed that the tested compounds **3c**, **3e, and 3a'** were comparable to Adriamycin having GI_{50}<10µg/ml. Compound **3c** and **3a'** were found to be most active among all the tested compounds.

Keywords: Carbazole, Molecular Docking, Anticancer activity, Human Breast Cancer cell line (MCF7), Sulphorodamine B assay.

1. Introduction

Cancer cells are different from the normal cells in a number of biochemical processes, mainly during the control of cell growth and division. These cells have high proliferative index as compare to normal cells. Therefore targeting of proliferative pathway is considered as effective strategy for cell death via apoptosis or prevention of cell division via cell cycle arrest for combating the disease. A substantial number of new anti-neoplastic agents have been discovered. Even though major findings have been observed in the chemotherapeutic management of cancer patients but a laborious task is still considered necessary for the discovery of new clinically important anticancer agents. Due to the resistance and toxicity drawbacks of traditional cytotoxic treatments the combined anticancer therapies or multi acting drugs are clinically preferred., A more active and selective chemotherapeutic agent is therefore still needed for promising anticancer approach [1-3].

Topoisomerases are the enzymes involved in a number of cellular processes, such as transcription, replication and recombination. The importance of their role in cellular processes can be understood by the fact that they are the target of numerous anticancer agents. They are classified into two types: type I and type II .Topoisomerase which are capable of changing the topology of DNA via transient breaks on one or two of the DNA strands to allow passage of either a single or double DNA strand through the break followed by relegation. There are certain carbazole derivatives such as Strausporine, Rebeccamycine etc. which inhibit the topoisomerase I significantly and exhibit anticancer activity [4-8].

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The synthesis of various hetero annulated carbazole derivatives has attracted substantial attention in recent years because there are several naturally occurring compounds that have similar structural frameworks, displaying a wide range of biological activities such as antioxidant, antidiabetic, antimicrobial, anticancer, antitubercular, antipsychotic and anticonvulsant activity [9-17]. It has been noticed that introduction of additional heterocyclic rings to the carbazole core tends to exert profound influence in increasing the anticancer activity. We recently investigated and reported the discovery of various carbazole derivatives having potent anticancer properties [18-21]. With this in mind, we envisaged the design and synthesis of a combination of carbazole with heterocyclic moiety like piperazine and imidazole to obtain therapeutically active anticancer agents.

2. Material and Methods

2.1 Docking studies

Experimentally around 500 structures based on carbazole moiety were drawn and 3D optimized by ChemSketch software and docked with eukaryotic topoisomerase-I by using AUTODOCK 4.2.6 software in order to obtain the basic protein-ligand interactions. The ligands having conformational stability and structural diversity were docked with the crystal structure of Human Topoisomerse-I-DNA complex (PDB ID: 1A35). Carbazole derivative docks perpendicular to the DNA backbone, projects outward from the major groove and makes a network of potential hydrogen bonds with the active site of topoisomerase I residues. Active site was involved in hydrogen bonding with nitrogen atom of carbazole derivatives and gives rise to the inhibitory activity. Results of docking studies of heterocyclic compounds were compared with the standard drug i.e. Adriamycin, a wellknown topoisomerase-I inhibitor [6-8], on the basis of their minimum binding energy. Above results were used to separate active compounds from inactive ones. Among the docked molecules 20 compounds were found to be close to minimum binding energy of the standard drug (-7.25 Kcal/mol).

2.2 Chemistry

The purity of all the newly synthesized compounds were checked by TLC on pre-coated silica gel aluminum sheets (Type 60 GF_{254}, Merck) and the spots were detected by exposure to iodine vapors and UV-lamp at λ 254 nm. The melting points were determined in open capillary tubes and were uncorrected. The infrared (FT-IR) spectra were recorded on 470-Shimadzu infrared spectrophotometer using the KBr disc prepared by pressed pellet technique and υ_{max} are expressed in cm^{-1}. NMR spectra were measured in DMSO-d_6 as solvent at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) on a BRUKER AVANCE-300 spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts (δ) are given in parts per millions (ppm) and Coupling constants (J) are given in Hertz (Hz). Spin multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Mass spectra were obtained on Shimadzu 2010A LC-MS spectrometer. Elemental analysis was carried on Elemental Vario EL III Carlo Erba 1108 and the values were within ±0.04% of the theoretical values.

2.2.1 Synthesis of compound 1-2 and 1'-2'

Compounds **1-2** and **1'-2'** were synthesized by following the scheme as given in literature [18-20].

2.2.2 General procedure for the synthesis of 3a-3e

To a solution of compound **2** (0.0027 mol) in 30 ml of ethanol:dioxane (9:1 v/v) in an iodine flask, glyoxal (0.0027 mol) and ammonium acetate (0.0027 mol) were added and refluxed for 21 h. The completion of the reaction was monitored by TLC using 5% chloroform in benzene. The solvent was evaporated by using rotary evaporator and the crude product was recrystallized from ethanol to give compound **3a-3e**.

2-[4,5-dihydro-2-(4-nitrophenyl)imidazol-1-ylamino]-1-(9H-carbazol-9-yl)ethanone (3a) Yield 58%; Bright yellow crystal; IR (KBr, ν, cm^{-1}): 3482 (N-H), 3014 (C-H, aromatic), 2960 (C-H, aliphatic), 1680 (C=O), 1642 (C=N), 1549 (N-O, asymmetric), 1338 (N-O, symmetric) 842 (840 (C-H, def., *p*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.23 (d, J = 8.4 Hz, 2H, Ar-H), 7.81 (d, J = 8.1 Hz, 2H, Ar-H), 7.58 (dd, J = 8.4, 2.4 Hz, 2H, Ar-H), 7.46 (dd, J = 8.7, 2.7 Hz, 2H, Ar-H), 7.28 (dt, 2H, Ar-H), 7.19 (dt, 2H, Ar-H), 3.82 (s, 2H, CH_2), 3.78 (t, J = 7.5 Hz, 2H, CH_2), 3.27 (t, J = 7.5 Hz, 2H, CH_2), 2.62 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 198.26, 163.43, 151.14, 139.42, 130.28, 130.15, 122.34, 121.32, 120.38, 119.65, 118.68, 111.44, 50.41, 46.54, 42.16; EIMS (m/z): **[**M]$^+$ 413.45, [M+1]$^+$ 414.78 ; Fragments: 291.12, 233.08, 166.06, 122.02,114.03, 69.04; Anal. Calcd.For $C_{23}H_{19}N_5O_3$; C, 66.80; H, 4.62; N, 16.93. Found: C, 66.84; H, 4.61; N, 16.9.

2-[4,5-dihydro-2-(4-methoxyphenyl)imidazol-1-ylamino]-1-(9H-carbazol-9-yl)ethanone (3b) Yield 64%; Creamy Crystals; IR (KBr, ν, cm^{-1}): 3417 (N-H), 3051 (C-H, Aromatic), 2868 (C-H, Aliphatic), 1705(C=O),

1585(C=N), 1225(C-O-C, asymmetric), 1045(C-O-C, symmetric), 813 (C-H, def., *p*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 7.55 (dd, *J* = 8.4, 2.4 Hz, 2H, Ar-H), 7.50 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.41 (dd, *J* = 8.4, 2.7 Hz, 2H, Ar-H), 7.21 (dt, 2H, Ar-H), 7.16 (dt, 2H, Ar-H), 6.76 (d, *J* = 7.8 Hz, 2H, Ar-H), 3.77 (s, 2H, CH_2), 3.74 (s, 3H, OCH_3), 3.71 (t, *J* = 7.5 Hz, 2H, CH_2), 3.23 (t, *J* = 7.5 Hz, 2H, CH_2), 2.55 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 198.26, 163.42, 162.92, 130.28, 130.18, 125.45, 122.36, 120.30, 119.57, 118.48, 114.56, 111.45, 56.18, 50.44, 46.51, 42.19; EIMS (m/z): **[**M]$^+$ 398.47, [M+1]$^+$ 399.76; Fragments: 290.92, 233.86, 166.56, 114.26, 107.08, 69.86; Anal. Calcd. for $C_{24}H_{22}N_4O_2$: C, 72.31; H, 5.58; N, 14.08. Found: C, 72.35; H, 5.56; N, 14.07.

2-[4,5-dihydro-2-(4-hydroxyphenyl)imidazol-1-ylamino]-1-(9H-carbazol-9-yl)ethanone (3c) Yield 73%; Light yellow Crystals; IR (KBr, ν, cm^{-1}): 3691 (OH, Phenolic), 3444 (N-H), 3010 (C-H, aromatic), 2978 (C-H, aliphatic), 1704 (C=O), 1602 (C=N), 788 (C-H, def., *p*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 7.52 (dd, *J* = 8.1, 2.4 Hz, 2H, Ar-H), 7.44 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.40 (dd, *J* = 8.4, 2.4 Hz, 2H, Ar-H), 7.21 (dt, 2H, Ar-H), 7.15 (dt, 2H, Ar-H), 6.78 (d, *J* = 7.8 Hz, 2H, Ar-H), 5.01 (s, 1H, OH, D_2O exchangeable), 3.74 (s, 2H, CH_2), 3.69 (t, *J* = 7.5 Hz, 2H, CH_2), 3.21 (t, *J* = 7.5 Hz, 2H, CH_2), 2.51 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 198.23, 163.47, 160.91, 130.84, 130.26, 126.11, 122.38, 120.32, 119.58, 118.48, 116.91, 111.58, 50.47, 46.55, 42.22; EIMS (m/z): **[**M]$^+$ 384.43, [M+1]$^+$ 385.84; Fragments: 292.01, 233.04, 166.19, 114.3693.03, 69.04; Anal. Calcd. for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57. Found: C, 71.85; H, 5.23; N, 14.55.

2-[4,5-dihydro-2-(2-nitrophenyl)imidazol-1-ylamino]-1-(9H-carbazol-9-yl)ethanone (3d) Yield 59%; Yellow crystals; IR (KBr, ν, cm^{-}1): 3415 (N-H), 3047 (C-H, aromatic), 2859 (C-H, aliphatic), 1717 (C=O), 1680 (C=N), 1568 (N-O, asymmetric), 1345 (N-O, symmetric), 752 (C-H, def., *o*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.60-8.28 (m, 4H, Ar-H), 7.57 (dd, *J* = 8.7, 2.7 Hz, 2H, Ar-H), 7.45 (dd, *J* = 8.4, 2.4 Hz, 2H, Ar-H), 7.26 (dt, 2H, Ar-H), 7.18 (dt, 2H, Ar-H), 3.82 (s, 2H, CH_2), 3.74 (t, *J* = 7.5 Hz, 2H, CH_2), 3.26 (t, *J* = 7.5 Hz, 2H, CH_2), 2.58 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 198.27, 163.52, 149.21, 135.21, 132.32, 132.11, 130.11, 130.27, 122.36, 121.28, 120.41, 119.67, 118.71, 111.46, 50.43, 46.58, 42.19; EIMS (m/z): **[**M]$^+$ 413.43, [M+1]$^+$ 414.45; Fragments: 292.04, 232.89, 166.76, 122.02, 114.29, 68.02; Anal. Calcd. for $C_{23}H_{19}N_5O_3$: C, 66.82; H, 4.63; N, 16.94. Found: C, 66.80; H, 4.62; N, 16.93.

2-[4,5-dihydro-2-(3,4,5-trimethoxyphenyl)imidazol-1-ylamino]-1-(9H-carbazol-9-yl)ethanone (3e) Yield 67%; White crystals; IR (KBr, ν, cm^{-1}): 3420 (N-H), 3059 (C-H, aromatic), 2948 (C-H, aliphatic), 1696 (C=O), 1608 (C=N), 1348 (C-N), 1256 (C-O-C, asymmetric), 1090 (C-O-C, symmetric), 813 (C-H, def., tetrasubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.52 (dd, *J* = 8.4, 2.4 Hz, 2H, Ar-H), 7.39 (dd, *J* = 8.1, 2.1 Hz, 2H, Ar-H), 7.20 (dt, 2H, Ar-H), 7.14 (dt, 2H, Ar-H), 6.56 (s, 2H, Ar-H), 3.75 (s, 2H, CH_2), 3.71 (s, 9H, OCH_3), 3.69 (t, *J* = 7.5 Hz, 2H, CH_2), 3.25 (t, *J* = 7.5 Hz, 2H, CH_2), 2.54 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 198.19, 163.41, 151.23, 141.61, 130.21, 127.68, 122.36, 120.25, 119.52, 118.41, 111.38, 106.68, 56.64, 56.12, 50.38, 46.42, 42.14; EIMS (m/z): **[**M]$^+$ 458.54, [M+1]$^+$ 459.55; Fragments: 291.14, 167.08, 166.08, 114.05, 93.08, 69.10; Anal. Calcd. for $C_{26}H_{26}N_4O_4$: C, 68.11; H, 5.72; N, 12.22. Found: C, 68.12; H, 5.73; N, 12.20.

2.2.3 General procedure for the synthesis of 3a'-3j'

To a mixture of 2-(9*H*-carbazol-9-yl)acetohydrazide (**2'**) (0.01 mol) and substituted benzaldehyde (0.01 mol) in 50 ml ethanol, piperazine (0.02 mol) and hydrochloric acid (1 ml) were added in RBF. The resulting mixture was refluxed for 8-12 h. The content was poured into ice-cold water. The solid product thus obtained was filtered, washed with water and recrystallized from ethanol to give compound **3a'-3e'**.

2-(9H-carbazol-9-yl)-N'-[({3,4-dimethoxyphenyl)(piperazin-1-yl)}methyl]acetohydrazide (3a') Yield 59%; Dark brown solid; IR (KBr, ν, cm^{-1}): 3420 (N-H 2°amine), 3349 (N-H 2°amide), 3096 (C-H, aromatic), 2899 (C-H, aliphatic), 1639 (C=O), 1506 (C=C, aromatic), 1333 (C-N),1226(C-O-C, asymmetric),1084(C-O-C, symmetric),941(C-H, def., trisubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.11 (s, 1H, NH, D_2O exchangeable), 7.38 (dd, *J* = 8.1, 2.1 Hz, 2H, Ar-H), 7.29 (dd, *J* = 8.4, 2.1 Hz, 2H, Ar-H), 7.16 (dd, *J* = 8.1, 2.4 Hz, 2H, Ar-H), 7.07 (dd, *J* = 8.1, 2.7 Hz, 2H, Ar-H), 6.47-6.62 (m, 3H, Ar-H), 5.22 (s, 1H, CH), 4.64 (s, 2H, CH_2), 3.83 (s, 1H, NH, D_2O exchangeable), 3.78 (s, 6H, CH_3), 2.82 (t, *J* = 7.8 Hz, 4H, CH_2), 2.61 (t, *J* = 7.8 Hz, 4H, CH_2), 2.11 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 165.10, 149.56, 148.27, 131.82, 130.41, 122.42, 120.24, 119.52, 118.41, 115.23, 113.78, 111.38, 77.78, 56.20, 50.19, 47.13, 45.72; EIMS (m/z): [M]$^+$ 456.60, [M+1]$^+$ 457.23; Fragments: 412.23, 336.14, 238.24, 180.04, 116.73; Anal. Calcd. for $C_{27}H_{31}N_5O_3$: C, 68.48; H, 6.60; N, 14.79. Found: C, 68.47; H, 6.59; N, 14.77.

2-(9H-carbazol-9-yl)-N'-[{(4-bromophenyl)(piperazin-1-yl)}methyl]acetohydrazide(3b') Yield 72%; Brownn solid; IR (KBr, ν, cm^{-1}): 3417 (N-H 2°amine), 3349 (N-H 2°amide), 3045 (C-H, aromatic), 2889 (C-H, aliphatic), 1640 (C=O), 1544 (C=C, aromatic), 1346 (C-N), 1043 (C-Br), 812 (C-H, def., *p*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.11 (s, 1H, NH, D_2O exchangeable), 7.44 (dd, *J* = 8.4, 2.1 Hz, 2H, Ar-H), 7.31 (dd, *J* = 8.1, 2.4 Hz, 2H, Ar-H), 7.35 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.22 (dt, 2H, Ar-H), 7.10 (dt, 2H, Ar-H), 6.98 (d, *J* = 8.1 Hz, 2H, Ar-H), 5.23 (s, 1H, CH), 4.69 (s, 2H, CH_2), 3.86 (s, 1H, NH, D_2O exchangeable), 2.86 (t, *J* = 7.8 Hz, 4H, CH_2), 2.63 (t, *J* = 7.8 Hz, 4H, CH_2), 2.08 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 165.10, 136.54, 131.52, 131.21, 130.42, 122.36, 121.78, 120.26, 119.56, 118.42, 111.40, 77.79, 50.18, 47.10, 45.69; EIMS (m/z): $[M]^+$ 492.42, $[M+1]^+$ 493.43, $[M+2]^+$ 494.30; Fragments: 412.21, 336.81, 238.96, 180.34, 116.53; Anal. Calcd for $C_{25}H_{26}N_5OBr$: C, 60.98; H, 5.32; N, 14.22. Found: C, 60.97; H, 5.31; N, 14.20.

2-(9H-carbazol-9-yl)-N'-{[(3-chlorophenyl)(piperazin-1-yl)]methyl}acetohydrazide(3c') Yield 83%; White solid; IR (KBr, ν, cm^{-1}): 3478 (N-H 2°amine), 3330 (N-H 2°amide), 3122 (C-H, aromatic), 2934 (C-H, aliphatic), 1640 (C=O), 1575 (C=C, aromatic), 1343 (C-N), 1081 (C-Cl), 760 (C-H, def. *m*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.11 (s, 1H, NH, D_2O exchangeable), 7.41 (dd, *J* = 8.1, 2.4 Hz, 2H, Ar-H), 7.33 (dd, *J* = 7.8, 2.1 Hz, 2H, Ar-H), 7.21 (dt, 2H, Ar-H), 7.14 (dt, 2H, Ar-H), 6.98-7.12 (m, 4H, Ar-H), 5.22 (s, 1H, CH), 4.67 (s, 2H, CH_2), 3.85 (s, 1H, NH, D_2O exchangeable), 2.86 (t, *J* = 7.8 Hz, 4H, CH_2), 2.60 (t, *J* = 7.8 Hz, 4H, CH_2), 2.08 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 165.13, 139.82, 133.85, 130.46, 129.82, 128.78, 127.12, 122.37, 120.29, 119.46, 118.47, 111.45, 77.83, 50.23, 47.14, 45.75; EIMS (m/z): $[M]^+$ 447.55, $[M+1]^+$ 448.73, $[M+2]^+$ 449.93; Fragments: 412.29, 336.97, 238.18, 180.25, 116.11; Anal. Calcd. for $C_{25}H_{26}N_5OCl$: C, 67.03; H, 5.85; N, 15.63. Found: C, 67.00; H, 5.83; N, 15.62.

2-(9H-carbazol-9-yl)-N'-{[(2-chlorophenyl)(piperazin-1-yl)]methyl}acetohydrazide (3d') Yield 72%; White crystal; IR (KBr, ν, cm^{-1}): 3445 (N-H 2°amine), 3328 (N-H 2°amide), 3053 (C-H, aromatic), 2893 (C-H, aliphatic), 1633 (C=O), 1554 (C=C, aromatic), 1326 (C-N), 1090 (C-Cl), 748 (C-H, def., *o*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.12 (s, 1H, NH, D_2O exchangeable), 7.40 (dd, *J* = 8.4, 2.1 Hz, 2H, Ar-H), 7.34 (dd, *J* = 8.4, 2.7 Hz, 2H, Ar-H), 7.22 (dt, 2H, Ar-H), 7.15 (dd, *J* = 7.8, 2.4 Hz, 2H, Ar-H), 7.02-7.12 (m, 4H, Ar-H), 5.20 (s, 1H, CH), 4.68 (s, 2H, CH_2), 3.84 (s, 1H, NH, D_2O exchangeable), 2.87 (t, *J* = 7.8 Hz, 4H, CH_2), 2.61 (t, *J* = 7.8 Hz, 4H, CH_2), 2.09 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 165.14, 136.82, 134.34, 130.48, 130.23, 128.81, 126.73, 122.39, 120.31, 119.47, 118.48, 111.48, 77.86, 50.25, 47.16, 45.76; EIMS (m/z): $[M]^+$ 447.52, $[M+1]^+$ 448.82, $[M+2]^+$ 449.51; Fragments: 412.73, 336.29, 238.79, 180.12, 116.15; Anal. Calcd. for $C_{25}H_{26}N_5OCl$; C, 67.03; H, 5.85; N, 15.63. Found: C, 66.99; H, 5.82; N, 15.62.

2-(9H-carbazol-9-yl)-N'-[{(3-nitrophenyl)(piperazin-1-yl)}methyl]acetohydrazide (3e') Yield 70%; White solid; IR (KBr, ν, cm^{-1}): 3413 (N-H 2°amine), 3350 (N-H 2°amide), 3136 (C-H, aromatic), 2877 (C-H, aliphatic), 1659 (C=O), 1563 (C=C, aromatic), 1521 (N-O, asymmetric), 1335 (N-O, symmetric), 1355 (C-N), 703 (C-H, def., *m*-disubstituted); ^{1}H NMR (300 MHz, DMSO-d_6) δ_{ppm}: 8.16 (s, 1H, NH, D_2O exchangeable), 7.52-8.08 (m, 4H, Ar-H), 7.43 (dd, *J* = 8.1, 1.8 Hz, 2H, Ar-H), 7.36 (dd, *J* = 8.4, 2.1 Hz, 2H, Ar-H), 7.25 (dt, 2H, Ar-H), 7.16 (dt, 2H, Ar-H), 5.26 (s, 1H, CH), 4.73 (s, 2H, CH_2), 3.92 (s, 1H, NH, D_2O exchangeable), 2.93 (t, *J* = 7.8 Hz, 4H, CH_2), 2.66 (t, *J* = 7.8 Hz, 4H, CH_2), 2.11 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6) δ_{ppm}: 165.26, 148.21, 139.45, 135.12, 130.51, 129.62, 124.32, 122.45, 120.34, 119.75, 119.67, 118.52, 111.51, 77.94, 50.31, 47.21, 45.83; EIMS (m/z): $[M]^+$ 458.52, $[M+1]^+$ 459.93; Fragments: 412.21, 336.21, 238.86, 180.25, 116.66; Anal. Calcd. for $C_{25}H_{26}N_6O_3$: C, 65.49; H, 5.72; N, 18.33. Found: C, 65.48; H, 5.71; N, 18.32.

2.3 Anticancer activity

2.3.1 Cell culture

The cell lines were grown in RPMI1640 medium containing 10% fetal bovine serum and 2 mmol L-glutamine. In this screening experiment, cells were inoculated into 96 well micro titer plates in 100 µL at plating densitiesas shown in the study details, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs.

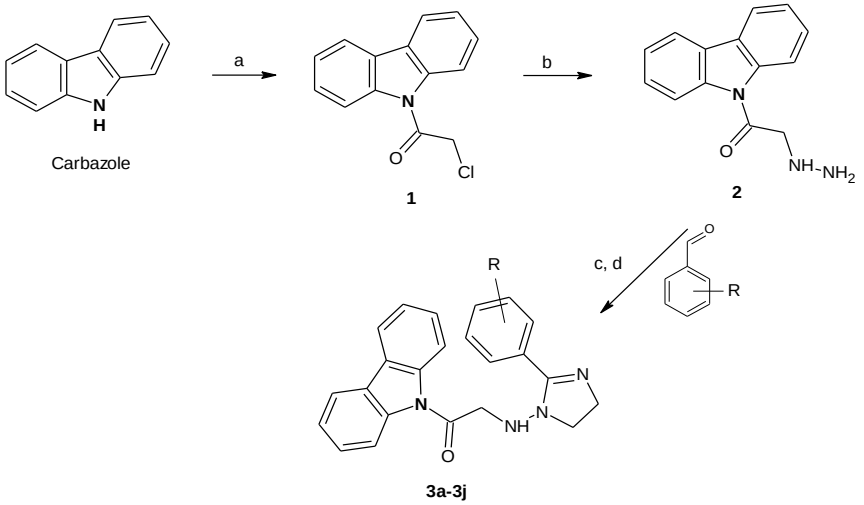
2.3.2 Antitumor activity

After 24 h one 96 well plate containing 5×10^3cells/well was fixed *in situ*with trichloro acetic acid (TCA), to represent a measurement of the cell population at the time of drug addiction (Tz). Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water were frozen and store prior to use.

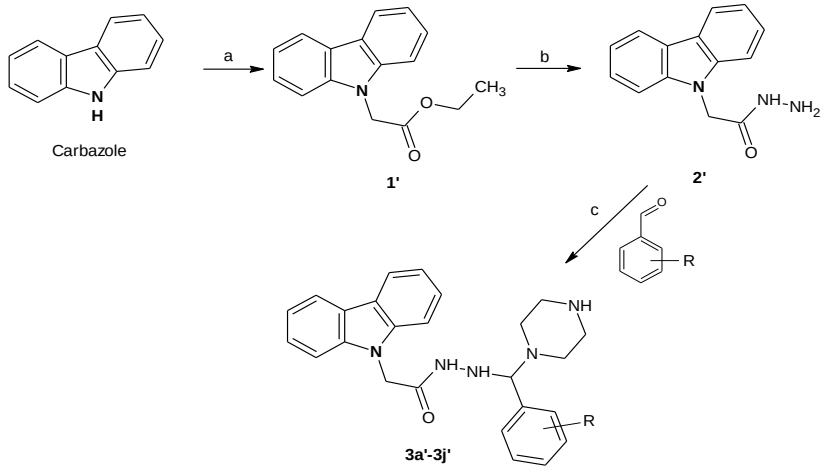
At the time of drug addition, an aliquot of frozen concentrate (1mg/ml) was thawed and diluted to 100µg/ml, 200µg/ml, 400µg/ml and 800µg/ml with complete medium containing test article. Aliquot of 10 µL of these different drug dilutions were added to the appropriate micro -titer wells already containing 90 µL of medium, resulting in the required final drug concentrations i.e.10, 20, 40, 80 µg ml^{-1}. After compound addition, plates were incubated at standard conditions for 48 h and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µL of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant liquid was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µL) at 0.4 % (w/v) in 1 % acetic acid was added to every well, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid, the plates were air dried. Bound stain was subsequently eluted with 10 mmol Tris base (Trizma™), and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percentage growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells × 100. Percentage control growth was measured and a graph was plotted to calculate the GI_{50} value for each compound and was compared with reference drug Adriamycin.

3. Results and Discussion

On the basis of docking results two series of such active compounds i.e. 2-[(4, 5-dihydro-2-substitutedphenyl)imidazol-1-ylamino]-1-(9*H*-carbazol-9-yl)ethanone **(3a-3e)** and 2-(9*H*-carbazol-9-yl)-*N'*-{[(4-substitutedphenyl) (piperazin-1-yl)]methyl}acetohydrazide **(3a'-3e')** were synthesized and identified by spectroscopic studies. The titled compounds were synthesized through multistep synthetic route as shown in Scheme **1** and **2**.



Scheme 1 Synthetic route for compounds **3a-3e**. Reagents: (a) $ClCOCH_2Cl$, (b) NH_2NH_2 (c) NH_4Ac (d) Glyoxal



Scheme 2 Synthetic route for compounds **3a'-3e'.** Reagents: (a) $ClCH_2COOC_2H_5$, (b) NH_2NH_2 (c) piperazine

As mentioned in scheme **1** carbazole on reaction with chloroacetyl chloride afforded N^9-(chloroacetyl)-carbazole (**1**) which on treatment with hydrazine has yielded N^9-(hydrazinoacetyl)-carbazole (**2**), Condensation of (**2**) with various derivatives of benzaldehyde with glyoxal in the presence of ammonium acetate yielded 2-[(4,5-dihydro-2-substitutedphenyl)imidazol-1-ylamino]-1-(9*H*-carbazol-9-yl)ethanone **(3a-3e)**. As depicted in Scheme **2**, series of carbazole derivatives 2-(9*H*-carbazol-9-yl)-*N'*-{[(4-subsitutedphenyl) (piperazin-1-yl)]methyl}acetohydrazide **(3a'-3e')** was synthesized by Mannich reaction. In which carbazole on treatment with ethylchloroacetate yielded 2-(9*H*-carbazole-9-yl)acetate (**1'**) which on further reaction with hydrazine produced 2-(9*H*-carbazole-9-yl)acetohydrazide (**2'**)**.** Finally, Refluxing **2'** with various aryl-aldehydes and piperazine in the presence of acid afforded compounds2-(9*H*-carbazol-9-yl)-*N'*-(substitutedphenyl)(piperazin-1-*yl*)methyl) acetohydrazide (**3a'-3e'**). All the synthesized compounds were analyzed by the TLC, melting point (as given in Table 1) and characterized by spectroscopic studies.

Table 1: Physical characterization of the synthesized compounds (3a-3j) and (3a'-3j')

Compound code	R	R_f value	m.p (°C)
3a	4-NO_2	0.65*	225-226
3b	4-OCH_3	0.56*	213-214
3c	4-OH	0.71*	229-230
3d	2-NO_2	0.60*	218-219
3e	3,4,5-$(OCH_3)_3$	0.67*	231-232
3a'	3,4-$(OCH_3)_2$	0.75#	285-286
3b'	4-Br	0.57#	290-291
3c'	3-Cl	0.64#	256-257
3d'	2-Cl	0.61#	293-294
3e'	3-NO_2	0.69#	248-249

*Solvent system:- Benzene: Chloroform: Methanol (4:3:2);
#Solvent System:- Chloroform: Methanol (8:2)

Characteristic peaks were observed in FTIR, ^{1}H-NMR, ^{13}C-NMR and MASS spectrometry. In the FTIR spectra of compounds (**3a-3e**), derivatives have a strong, characteristic peak in the region 1680-1640 cm^{-1} due to the amide C=O stretching vibration. The N-H stretching vibration band is observed in the region 3400-3300 cm^{-1}. In another series of derivatives (**3a'-3e'**) a characteristic peak was observed at 1690-1650 cm^{-1} due to amide (C=O) stretching vibrations. The ^{1}H-NMR and ^{13}C-NMR spectral data were also consistent with the assigned structures. In the mass spectra of all compounds (**3a-3e**) and (**3a'-3e'**), the $[M+1]^+$ peak was observed. All compounds gave satisfactory elemental analysis.

3.1 Biological Activity

3.1.1 Anticancer Activity

All the newly synthesized compounds were also screened for their *in vitro* anticancer activity against MCF 7 (Human breast cancer cell line) by SRB (Sulphorodamine B) assay method [22] by taking Rebeccamycin as standard. A nondestructive and indefinitely stable colorimetric end point was observed in SRB assay method. Hence, it is an appropriate and sensitive assay to measure percentage growth inhibition. Percentage control growth was measured at four different concentrations (10, 20, 40 and 80 µg/ml) and compared with the standard. A graph was plotted by using the data of percentage control growth (Figure **1**) and the value of GI_{50} was calculated and plotted (as shown in Figure **2**) for each compound. Results for anticancer activity are shown in Table **2.** Results revealed that the tested compounds **3c, 3e and 3a'** are comparable to the reference drug Rebeccamycin with the value of GI_{50}<10µg/ml. Compound **3c** and **3a'** having hydroxyl and dimethoxy group as substituent respectively showed highest activity. Electron donating property of above mentioned group increases the electron density of parent molecule which rationally increases the potency of compound towards anticancer activity. Besides, combination of piperazine or imidazole moiety with carbazole increases the therapeutic value for the same.

Table 2: Results of anticancer activity for compounds (3a-3j) and (3a'-3j')

Human Breast Cancer Cell Line MCF7 %Control Growth Drug Concentrations (µg/mL)					GI_{50} Value (µg/mL)
Compound no.	10	20	40	80	
3a	53.4	38.7	27.2	13.3	12.3
3b	61.6	50.2	41.9	36.0	20.1
3c	16.4	8.9	1.5	-12.2	<10
3d	58.2	49.3	37.0	31.3	19.2
3e	39.5	26.8	16.8	8.3	<10
3a'	22.8	3.6	-10.5	-20.9	<10
3b'	50.2	35.8	24.8	13.8	>10
3c'	55.6	32.5	17.9	9.8	12.4
3d'	54.8	33.8	18.9	9.2	12.2
3e'	55.6	41.5	25.7	14.5	13.9
Adriamycin (Std.)	0.3	-10.7	-33.6	-59.7	<10

4. Conclusion

In conclusion, we have developed topoisomerase-I inhibitors by the combination of heterocycles like piperazine and imidazole with carbazole moiety. Hydrogen attached with nitrogen atom was found to be involved in the formation of hydrogen bond with active site of the target i.e. GLN397 of topoisomerase I. The presence of an electron releasing group on the benzene ring also increases the potency. Potency of the newly synthesized compounds was determined on the basis of their GI_{50} value and percentage control growth. Study stated that carbazole in combination with other heterocycles might be used as a lead for finding of the potent anticancer agents. Substitution at 9^{th} position also increases the therapeutic value of carbazole toward the treatment of cancer.

Acknowledgement

Authors thank management of Rajiv Academy for Pharmacy, Mathura, India for providing research facilities. Research is also supported by Tata Memorial Research Center, Navi Mumbai, India by performing anticancer activity. IIT, Delhi is acknowledged for providing the spectral data of the synthesized compounds.

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