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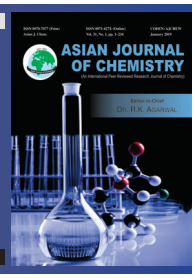

Microwave Assisted One-Pot Three-Component Synthesis of Novel Pyranocarbazole Derivatives as Antiproliferative Agents and Molecular Docking Studies

BOBBALA RAMANA REDDY* and PEDAVENKATAGARI NARAYANA REDDY

Department of Chemistry, School of Technology, GITAM University, Hyderabad-502102, India

*Corresponding author: E-mail: bobbala.ramana91@gmail.com

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A new series of pyrano[3,2-*c*]carbazole and pyrano[2,3-*a*]carbazole derivatives have been synthesized by one-pot three-component coupling of 4-hydroxycarbazole or 2-hydroxycarbazole, aromatic substituted aldehydes and (E)-N-methyl-1-(methylthio)-2-nitroethenamine under microwave irradiation. This transformation presumably occurs *via* Knoevenagel condensation-Michael addition–tautomerism intramolecular O-cyclization-elimination sequence of reactions creating one C-O bond and two C-C bonds. The significant features of this one-pot reaction include catalyst free, solvent free, atom-economy, no column chromatographic purification, short reaction time and good yield. Further, the synthesized pyranocarbazole derivatives were evaluated for their antiproliferative activity against four cancer cell lines such as DU 145 (prostate cancer), MDA-MB-231 (breast cancer), SKOV3 (ovarian cancer) and B16-F10 (skin cancer). The results clearly demonstrated that trimethoxyphenyl substituted pyrano[3,2-*c*]carbazole (**9h**) exhibited most potent antriproliferative activity against tested cell lines. Compounds **9a**, **9b** and **11a** were also displayed pronounced antriproliferative activity. In addition, molecular docking studies revealed that the lead compounds bind to the colchicine binding site of the tubulin effectively.

Keywords: Microwave, Pyranocarbazole, Antiproliferative, Molecular docking.

INTRODUCTION

Carbazole is the central core of a large family of alkaloids [1-4] exhibiting promising biological activities such as antibacterial, antifungal, antitumor, anti-inflammatory, anti-histaminic and neuroprotective properties [5-9]. Among these, pyranocarbazoles formed a prominent group due to their occurrence in various plants and useful biological activities including mosquitocidal, antimicrobial, anti-inflammatory and antioxidant activities [10,11].

Girinimbine (**1**) and isomahanine (**3**) have been reported to possess significant cytotoxicity against lung cancer (NCI-522) [12-14], whereas, koenimbine (**2**) showed rapid scavenging activities against the 1,1-diphenyl-2-picrylhydrazyl radical [15]. Clausamines **4** and **5** have been reported to inhibit EBV activation in Raji cells [16-18]. Furthermore, pyranocarbazole derivatives have also used in the field of medicinal chemistry as a pharmacophore for different therapeutic indications.

The pharmacological potential of the pyranocarbazole derivatives has led to a strong interest in their synthesis and diverse synthetic methods have been developed [19-31]. Methods using domino aldol type/6π-electrocyclization, palladium catalyzed intramolecular C-O and C-C cross-coupling reaction [20] and iron-mediated oxidative cyclization [21] are common. Palladium(II)-catalyzed carbazole construction followed by pyran ring annulations [23,25-28], hexahydro-Diels-Alder reaction [32] and other methods have also been used [33-37]. Recently, we have developed a simple and efficient one-pot synthetic approach to pyrano[3,2-*c*]carbazole derivatives [38]. We also reported another approach for the synthesis of novel pyrano[3,2-*c*]carbazole derivatives *via* domino Knoevenagel-hetero-Diels-Alder reaction [39,40]. These derivatives showed significant anticancer activity by inhibiting tubulin polymerization. Recently, we utilized carbazole Mannich bases for the construction of pyrano[3,2-*c*]carbazole derivatives *via ortho*-quinone methide formation [41]. Despite of these synthetic methods, development of new method for the diversity oriented synthesis of pyranocarbazoles from simple starting materials is of great interest.

Based on the important biological profile of pyranocarbazoles and our continuing interest on carbazole heterocycles

[38-44], here we wish to report a new series of pyrano[3,2-*c*] and pyrano[2,3-*a*]carbazole derivatives by one-pot three component coupling reaction of 4-hydroxycarbazole or 2-hydroxycarbazole, aromatic substituted aldehydes and (E)-Nmethyl-1-(methylthio)-2-nitroethenamine under microwave irradiation.

EXPERIMENTAL

All chemicals were purchased from Lancaster (Alfa Aesar, Johnson Matthey Co, Ward Hill, MA, USA), Sigma-Aldrich (St Louis, MO, USA) and Spectrochem Pvt Ltd (Mumbai, India). Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254 and visualization on TLC was achieved by UV light or iodine indicator. 1H NMR spectra were recorded on Avance (300 MHz); Bruker, Fallanden, Switzerland instruments. Chemical shifts were reported in ppm, downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage of 3.98 kV and ESI mode positive ion trap detector.

General procedure for the preparation of compounds (9a-j and 11a-f): A mixture of 4-hydroxycarbazole (**6**) (1 mmol) or 2-hydroxycarbazole (**10**) (1 mmol), aromatic aldehydes (**7**) (1 mmol) and (E)-N-methyl-1-(methylthio)-2-nitroethenamine (**8**) (1 mmol) were heated under microwave irradiation (360 W) for 5 min. After completion of the reaction (confirmed by TLC), the reaction mixture was cooled to room temperature. The precipitated solid was filtered, washed with ethanol (5 mL) and dried to afford the pure products **9a-j** or **11a-f** (yields: 66-89 %).

***N*-Methyl-3-nitro-4-phenyl-4,7-dihydropyrano[3,2-*c*]carbazol-2-amine (9a):** Pale brown solid. m.p. >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.59 (s, 1H, –N*H*–), 10.61 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.25 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.55 (d, 1H, *J* = 8.1 Hz, Ar–*H*), 7.45 (t, 1H, *J* = 8.1 Hz, Ar–*H*), 7.37-7.20 (m, 7H, Ar–*H*), 7.15 (d, 1H, *J* = 6.9 Hz, Ar–*H*), 5.49 (s, 1H, – C*H*–), 3.47 (d, 3H, *J* = 4.8 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 145.4, 141.9, 140.0, 139.7, 128.3, 127.2, 126.3, 126.1, 125.7, 122.1, 119.7, 119.3, 114.0, 111.2, 109.9, 108.7, 108.2, 41.1, 28.9; ESIMS: *m/z* 372 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{22}H_{18}N_3O_3$: 372.1403, found: 372.1408.

4-(4-Fluorophenyl)-*N*-methyl-3-nitro-4,7-dihydropyrano[3,2-*c*]carbazol-2-amine (9b): Dark brown solid. m.p. 218–220 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.59 (s, 1H, –N*H*–), 10.60 (d, 1H, *J* = 4.8 Hz, –N*H*–), 8.24 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.55 (d, 1H, *J* = 8.7 Hz, Ar–*H*), 7.45 (t, 1H, *J* = 7.5 Hz, Ar–*H*), 7.41-7.21 (m, 5H, Ar–*H*), 7.06 (t, 2H, *J* = 8.7 Hz, Ar–*H*), 5.52 (s, 1H, –C*H*–), 3.46 (d, 3H, *J* = 4.8 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0, 141.9, 141.6, 141.6, 140.0, 139.7, 129.2, 129.1, 126.2, 125.8, 122.1, 119.7, 119.4, 115.1, 114.8, 75 113.8, 111.2, 109.9, 108.8, 108.1, 41.1, 28.9; ESIMS: *m/z* 390 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{22}H_{17}N_3O_3F$: 390.1117, found: 390.1122.

***N*-Methyl-3-nitro-4-(3-nitrophenyl)-4,7-dihydropyrano-[3,2-*c*]carbazol-2-amine (9c):** Pale brown solid. m.p. 185–187 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.62 (s, 1H, –N*H*–), 10.64 (d, 1H, *J* = 5.1 Hz, –N*H*–), 8.26 (d, 2H, *J* = 8.7 Hz, Ar–*H*), 8.04 (d, 1H, *J* = 8.4 Hz, Ar–*H*), 7.78 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.68-7.35 (m, 3H, Ar–*H*), 7.32-7.22 (m, 3H, Ar–*H*), 5.73 (s, 1H, –C*H*–), 3.48 (d, 3H, *J* = 4.5 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 158.9, 145.4, 141.9, 140.0, 139.8, 128.2, 126.2, 125.8, 122.2, 119.7, 119.3, 119.0, 114.0, 113.8, 111.2, 111.0, 109.9, 108.9, 108.1, 41.1, 28.9; ESIMS: *m/z* 417 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{22}H_{17}N_4O_5$: 417.1912, found: 417.1919.

4-(2-(Methylamino)-3-nitro-4,7-dihydropyrano[3,2-*c*]carbazol-4-yl)benzonitrile (9d): Dark brown solid. m.p. 188–190 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.62 (s, 1H, –N*H*–), 10.63 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.24 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 7.73 (d, 2H, *J* = 8.1 Hz, Ar–*H*), 7.54 (t, 2H, *J* = 8.1 Hz, Ar–*H*), 7.46 (t, 1H, *J* = 7.5 Hz, Ar–*H*), 7.36-7.20 (m, 4H, Ar–*H*), 5.61 (s, 1H, –C*H*–), 3.47 (d, 3H, *J* = 4.8 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 155.8, 145.4, 141.9, 140.0, 139.8, 135.6, 128.2,126.2, 125.7, 125.5, 125.2, 125.0, 122.2, 119.7, 119.3, 113.2, 111.3, 109.9, 108.9, 108.1, 41.0, 28.9; ESIMS: *m/z* 397 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{23}H_{17}N_4O_3$: 397.1801, found: 397.1805.

***N*-Methyl-3-nitro-4-(4-(trifluoromethyl)phenyl)-4,7-dihydropyrano[3,2-*c*]carbazol-2-amine (9e):** Pale brown solid. m.p. >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.61 (s, 1H, –N*H*–), 10.63 (d, 1H, *J* = 4.8 Hz, –N*H*–), 8.25 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.72-7.55 (m, 5H, Ar–*H*), 7.46 (t, 1H, *J* = 7.5 Hz, Ar–*H*), 110 7.38-7.20 (m, 3H, Ar–*H*), 5.62 (s, 1H, –C*H*–), 3.47 (d, 3H, *J* =5.1 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.9, 150.8, 150.1, 141.9, 140.1, 139.7, 128.2, 126.1, 125.8, 125.3, 125.2, 125.2, 122.2, 119.7, 119.4, 112.9, 111.2, 109.9, 108.9, 107.6, 41.0, 29.0; ESIMS: *m/z* 440 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{23}H_{17}F_3N_3O_3$: 440.1405, found: 440.1412.

4-(2-(Methylamino)-3-nitro-4,7-dihydropyrano[3,2-*c*]carbazol-4-yl)phenol (9f): Dark brown solid. m.p. >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.56 (s, 1H, –N*H*–), 10.57 (d, 1H, *J* = 4.8 Hz, –N*H*–), 9.20 (s, 1H, –OH), 8.23 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.54 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.45 (t, 1H, *J* = 7.8Hz, Ar–*H*), 7.36-7.18 (m, 3H, Ar–*H*), 7.07 (d, 2H, *J* = 8.1 Hz, Ar–*H*), 6.62 (d, 2H, *J* = 8.1 Hz, Ar–*H*), 5.37 (s, 1H, –C*H*–), 3.45 (d, 3H, *J* = 4.8 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 155.8, 145.9, 141.9, 139.8, 139.7, 135.8, 128.2, 126.2, 125.7,122.1, 119.7, 119.3, 115.0, 114.7, 113.8, 111.2, 108.7, 108.1,41.1, 28.9; ESIMS: *m/z* 388 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{22}H_{18}N_3O_4$: 388.1303, found: 388.1307.

4-(3-Methoxyphenyl)-N-methyl-3-nitro-4,7-dihydropyrano[3,2-*c*]carbazol-2-amine (9g): Dark brown solid. m.p. 218–220 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.58 (s, 1H, –N*H*–), 10.60 (d, 1H, *J* = 4.8 Hz, –N*H*–), 8.25 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 7.54 (d, 1H, *J* = 8.1 Hz, Ar–*H*), 7.45 (t, 1H, *J* = 7.8 Hz, Ar–*H*), 7.38-7.20 (m, 3H, Ar–*H*), 7.15 (t, 1H, *J* = 8.1 Hz, Ar– *H*), 6.92 (s, 1H, Ar–*H*), 6.83 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 6.72 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 5.47 (s, 1H, –C*H*–), 3.70 (s, 3H, –OC*H*$_3$), 3.46 (d, 3H, *J* = 4.8 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2, 159.1, 147.0, 141.9, 140.0, 139.7, 129.5, 126.1, 125.7,122.1 119.7, 119.3, 119.2, 113.9, 113.7, 111.2, 111.0, 109.9,108.7, 108.0, 54.9, 41.0, 28.9; ESIMS: *m/z* 402 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{23}H_{20}N_3O_4$: 402.1213, found: 402.1216.

***N*-Methyl-3-nitro-4-(3,4,5-trimethoxyphenyl)-4,7-dihydropyrano[3,2-c]carbazol-2-amine (9h):** Dark brown solid. m.p. >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.12 (s, 1H, –N*H*–), 10.75 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.25 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 7.57 (d, 1H, *J* = 8.1 Hz, Ar–*H*), 7.45 (t, 2H, *J* = 7.8 Hz, Ar–*H*), 7.30 (m, 3H, Ar–*H*), 7.21 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 5.47 (s, 1H, –C*H*–), 3.79 (s, 9H, –OC*H*$_3$), 3.76 (d, 3H, *J* = 4.8 Hz, – C*H*$_3$); ^{13}CNMR (75 MHz, $CDCl_3$): δ 159.2, 159.1, 145.4, 141.9, 140.1, 139.9, 139.7, 128.3, 127.1, 126.2, 125.8, 122.8, 122.0, 119.8, 119.1, 113.8, 111.2, 109.9, 108.5, 108.0, 58.6,54.9, 41.1, 28.9; ESIMS: *m/z* 462 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{25}H_{24}N_3O_6$: 462.1604, found: 462.1611.

4-(4-Isopropylphenyl)-*N*-methyl-3-nitro-4,7-dihydropyrano[3,2-*c*]carbazol-2-amine (9i): Dark brown solid. m.p. 178–180 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.57 (s, 1H, –N*H*–), 10.59 (d, 1H, *J* = 4.8 Hz, –N*H*–), 8.25 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 7.54 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.45 (t, 1H, *J* = 7.2 Hz, Ar–*H*), 7.35-7.17 (m, 5H, Ar–*H*), 7.10 (d, 2H, *J* = 8.1 Hz, Ar–*H*), 5.46 (s, 1H, –C*H*–), 3.46 (d, 3H, *J* = 4.8 Hz, –C*H*$_3$), 2.79 (m, 1H, –C*H*$(CH_3)_2$), 1.13 (s, 3H, –C*H*$_3$), 1.11 (s, 3H, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 154.3, 153.9, 146.4, 142.8, 139.9, 139.7, 128.3, 127.1, 126.2, 125.8, 122.8, 122.2, 119.7,119.4, 115.0, 114.3, 111.2, 108.8, 108.1, 40.7, 33.0, 29.0, 23.8; ESIMS: *m/z* 414 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{25}H_{24}N_3O_3$: 414.1509, found: 414.1514.

***N*-Methyl-3-nitro-4-(pyridin-3-yl)-4,7-dihydropyrano-[3,2-*c*]carbazol-2-amine (9j):** Pale brown solid. m.p. 238–240 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.62 (s, 1H, –N*H*–), 10.63 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.64 (s, 1H, Ar–*H*), 8.36 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 8.25 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 7.55 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.46 (t, 1H, *J* = 7.5 Hz, Ar–*H*), 7.40-7.20 (m, 3H, Ar– *H*), 7.19-7.00 (m, 2H, Ar–*H*), 5.55 (s, 1H, –C*H*–), 3.47 (d, 3H, *J* = 4.5 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.5, 161.8, 159.1, 145.4, 141.9, 140.0, 139.7, 128.3, 127.3, 126.2, 125.7,122.1, 119.7, 119.3, 114.0, 111.2, 109.9, 108.7, 108.2, 41.1, 28.9; ESIMS: *m/z* 373 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{21}H_{17}N_4O_3$: 373.2143, found: 373.2152.

***N*-Methyl-3-nitro-4-phenyl-4,11-dihydropyrano[2,3-*a*]carbazol-2-amine (11a):** Dark brown solid. m.p. 190–192 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.41 (s, 1H, –N*H*–), 10.38 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.08 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.46(d, 2H, *J* = 7.8 Hz, Ar–*H*), 7.42-7.32 (m, 2H, Ar–*H*), 7.30-7.00 (m, 5H, Ar–*H*), 5.89 (s, 1H, –C*H*–), 3.25 (d, 3H, *J* = 4.8 Hz, – C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 145.4, 144.4, 142.2, 139.1, 131.0, 128.7, 126.6, 125.7, 123.5, 122.5, 119.7, 119.3,115.3, 111.2, 109.9, 108.2, 98.3, 41.1, 28.9; ESIMS: *m/z* 372$(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{22}H_{18}N_3O_3$: 372.1373, found: 372.1379.

4-(4-Bromophenyl)-*N*-methyl-3-nitro-4,11-dihydropyrano[2,3-*a*]carbazol-2-amine (11b): Dark brown solid. m.p. 212–214 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.41 (s, 1H, –N*H*–), 10.38 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.10 (d, 1H, *J* =7.8 Hz, Ar–*H*), 7.45 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.42-7.30 (m, 4H, Ar–*H*), 7.28-7.00 (m, 4H, Ar–*H*), 5.88 (s, 1H, –C*H*–), 3.23 (d, 3H, *J* = 5.1 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.0, 145.4, 143.5, 142.6, 141.4, 139.1, 128.7, 125.7, 123.5, 122.5, 122.2, 119.5, 119.0, 118.8, 115.5, 111.4, 109.3, 108.1, 98.5, 41.0, 28.9; ESIMS: *m/z* 451 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{22}H_{17}N_3O_3Br$: 451.2141, found: 451.2149.

***N*-Methyl-3-nitro-4-(4-nitrophenyl)-4,11-dihydropyrano[2,3-*a*]carbazol-2-amine (11c):** Dark brown solid. m.p. 180–182 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.65(s, 1H, –N*H*–), 10.60 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.12 (d, 1H, *J* = 7.8 Hz, Ar–*H*), 7.48 (d, 1H, *J* = 7.6 Hz, Ar–*H*), 7.43-7.35 (m, 3H,100 Ar–*H*), 7.28-7.10 (m, 5H, Ar–*H*), 5.81 (s, 1H, –C*H*–), 3.30 (d,3H, *J* = 4.8 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1,148.5, 145.2, 141.9, 141.1, 139.3, 129.2, 128.5, 125.7, 124.8,123.5, 122.2, 119.5, 119.3, 115.1, 111.1, 109.5, 107.1, 108.4,98.3, 41.4, 28.7; ESIMS: *m/z* 417 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$calcd. for $C_{22}H_{17}N_4O_5$: 417.1815, found: 417.1823.

4-(2-(Methylamino)-3-nitro-4,11-dihydropyrano[2,3-*a*]carbazol-4-yl)benzonitrile (11d): Dark brown solid. m.p. 198–200 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.40 (s, 1H, –N*H*–),110 10.32 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.08 (d, 1H, *J* = 7.8 Hz, Ar–*H*),7.50 (d, 2H, *J* = 7.8 Hz, Ar–*H*), 7.45-7.40 (m, 3H, Ar–*H*), 7.35-7.10 (m, 4H, Ar–*H*), 5.76 (s, 1H, –C*H*–), 3.26 (d, 3H, *J* = 4.5 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.1, 146.5, 141.8, 141.3, 139.6, 128.7, 126.0, 125.1, 122.4, 119.6, 119.4, 119.2, 117.4, 115.4, 111.0, 109.9, 109.6, 109.4, 108.8, 107.3, 98.7, 41.2, 28.9; ESIMS: *m/z* 397 $(M+H)^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{23}H_{17}N_4O_3$: 397.1309, found: 397.1315.

4-(3-Methoxyphenyl)-N-methyl-3-nitro-4,11-dihydropyrano[2,3-*a*]carbazol-2-amine (11e): Pale brown solid. m.p. 190–192 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.46(s, 1H, –N*H*–), 10.38 (d, 1H, *J* = 4.8 Hz, –N*H*–), 8.08 (d, 1H, *J* = 8.4 Hz, Ar–*H*), 7.50 (d, 1H, *J* = 7.5 Hz, Ar–*H*), 7.35 (t, 1H, *J* =7.8 Hz, Ar–*H*), 7.30-7.00 (m, 4H, Ar–*H*), 6.88 (d, 2H, *J* = 6.9 Hz, Ar–*H*), 6.71 (d, 1H, *J* = 6.9 Hz, Ar–*H*), 5.87 (s, 1H, –C*H*–), 3.69 (s, 3H, –OC*H*$_3$), 3.24 (d, 3H, *J* = 4.8 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.2, 159.0, 141.9, 141.4, 141.0, 139.8, 130.2, 126.5, 125.4, 122.6, 119.8, 119.6, 119.4, 119.2, 115.7, 110.5,110.0, 109.7, 108.6, 107.3, 54.9, 41.0, 28.9; ESIMS: *m/z* 402(M+H)$^+$; HRMS: *m/z* $(M+H)^+$ calcd. for $C_{23}H_{20}N_3O_4$: 402.1406, found: 402.1414

***N*-Methyl-3-nitro-4-(pyridin-3-yl)-4,11-dihydropyrano-[2,3-*a*]carbazol-2-amine (11f):** Dark brown solid. m.p. >250 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.61 (s, 1H, –N*H*–), 10.63 (d, 1H, *J* = 4.5 Hz, –N*H*–), 8.64 (s, 1H, Ar–*H*), 8.36 (d, 1H, *J* = 4.2 Hz, Ar–*H*), 8.25 (d, 1H, *J* = 7.8 Hz, Ar–*H*),7.66 (d, 1H, *J* = 8.1 Hz, Ar–*H*), 7.55 (d, 1H, *J* = 7.8 Hz, Ar–*H*),7.46 (t, 1H, *J* = 7.8 Hz, Ar–*H*), 7.35-7.18 (m, 4H, Ar–*H*), 5.55 (s,1H, –C*H*–), 3.47 (d, 3H, *J* = 4.5 Hz, –C*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.0, 161.3, 159.1, 146.4, 141.5, 138.7, 136.4, 134.1, 126.5, 126.2, 122.6, 119.7, 119.4, 119.1, 117.3, 114.3, 109.5, 108.8, 107.1, 41.1, 28.9; ESIMS: *m/z* 373 $(M+H)^+$; HRMS: *m/z*$(M+H)^+$ calcd. for $C_{21}H_{17}N_4O_3$: 373.1609, found: 373.1617

Cell cultures, maintenance and antiproliferative evaluation: All cell lines used in this study were purchased from the American Type Culture Collection (ATCC, United States). DU 145, MDA-MB-231, SKOV3, B16-F10 were grown in Dulbecco's modified Eagle's medium (containing 10 % FBS in ahumidified atmosphere of 5 % CO_2 at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/ 60 mm dishes and seeded in 96-wel plates. The synthesized

test compounds were evaluated for their *in vitro* antiproliferative activity in four different cancer cell lines. A protocol of 24 h continuous drug exposure was used and a MTT cell proliferation assay was used to estimate cell viability or growth. Individual cell lines were grown in their respective media containing 10 % fetal bovine serum and were seeded into 96-well microtiter plates in 200 µL aliquots at plating densities depending on the doubling time of individual cell lines. 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. Aliquots of 2 µL of the test compounds were added to the wells already containing 198 µL of cells, resulting in the required final drug concentrations. For each compound, five concentrations (0.01, 0.1, 1, 10 and 100 µM) were evaluated and each was done in triplicate wells. Plates were incubated further for 24 h and the assay was terminated by the addition of 10 µL of 5 % MTT and incubated for 60 min at 37 °C. Later, the plates were air-dried. Bound stain was subsequently 60 eluted with 100 µL of DMSO and the absorbance was read in multimode plate reader (Varioscan Flash) at a wavelength of 560 nm. Percent growth was calculated on a plate by plate basis for test wells relative to control wells. The above determinations were repeated thrice. The growth inhibitory effects of the 65 compounds were analyzed by generating dose response curves as a plot of the percentage surviving cells *versus* compound concentration. The sensitivity of the cancer cells to the test compound was expressed in terms of IC_{50}, a value defined as the concentration of compound that produced 50 % reduction as 70 compared to the control absorbance. IC_{50} values are indicated as means ± SD of three independent experiments.

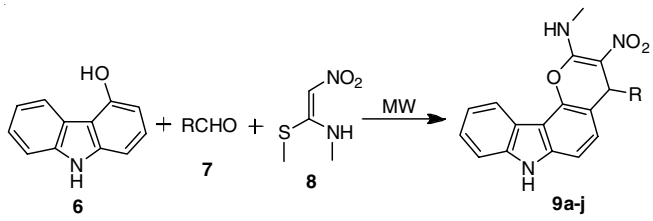
Molecular modelling: The docking studies were performed using Glide (Schrodinger) and the favourable interactions between ligand molecule with receptor were analyzed. The PDB ID 3E22 was used for docking study which was downloaded from protein data bank site (www.rcsb.org). The protein was refined by using protein 80 preparation wizard procedure available with maestro suite of Schrodinger software (Maestro, Schrodinger, LLC, New York, NY, 2017). This is carried out to release any unwanted strain in crystal structure of the protein and to add the missing hydrogens in the protein and assign all bond orders in correct format.

Ligand preparation: All ligands were drawn by using maestro interface of Schrodinger software by edit-build structure protocol. The valency of all ligands cross checked and minimized by macromodel minimization and optimized the structures. Those 90 optimized structures further processed by using ligprep protocol to get stable confirmation, which will be ready for docking. The output structure was used for docking study.

Grid generation and docking process: The grid generation 95 and docking performed through preprocess optimization and minimization. All ligands were drawn by using maestro software and prepared for docking by ligprep module (LigPrep, Schrodinger, LLC, New York, NY, 2017). Docking of the ligands performed through Glide program by applying XP scoring 100 function. The docking protocol in this study was obtained using OPLS 2005 all-atom force field. Glide uses an explicit water model for modelling salvation effects. The demonstrated research output and results are the structural model exploration and involved methodology is very effective. In this methodology we 105 taken G-score, which is a modified and extended empirical scoring function based on Chem-Score the docking method that is XP descriptors been used to score (G-score) for ligand includes drug like properties such as Lipo-EvdW, H-bond and RotPenal. Lipo-EvdW is the Chem-Score lipophilic pair term and fraction 110 of the total receptor-ligand vdw's energy.

RESULTS AND DISCUSSION

Initially, a one-pot three-component reaction using equimolecular amount of 4-hydroxycarbazole (**6**), aromatic aldehyde (**7**) and (E)-N-methyl-1-(methylthio)-2-nitro-ethenamine (**8**) were chosen as the model reaction for the construction of pyranocarbazole derivatives (**9a**) (**Scheme-I**).



Scheme-I: Synthesis of pyrano[3,2-*c*]carbazole derivatives (**9a-9j**)

In order to find optimized conditions the model reaction was performed in ethanol which failed to yield the product even after 24 h either at ambient temperature or reflux conditions (Table-1, entry 1-2). Then the reaction was then tested in the presence of bases such as triethylamine, pyridine and DMAP in different solvents (Table-1, entries 3-5) but these bases could not promote the reaction efficiently even at prolonged reaction times. However, when the reaction carried out under neat conditions at 100 °C without any base, it was gratifying to discover that reaction completed within 3 h with the yield of N-methyl-3-nitro-4-phenyl-4,7-dihydropyrano-[3,2-*c*]carbazol-2-amine (**9a**) (64 %, Table-1, entry 6). Inspired by this result, the model reaction was further investigated in the absence of solvent under microwave irradiation at 360 W for 5 min, which led to formation of product **9a** in 89 % yield (Table-1, entry 7). Next we tried 540 W and the product **9a** was obtained in 70 % yield (Table-1, entry 8). Further increment of reaction time or radiation power to 720 W led to a 40 decrease in yield (Table-1, entry 9, 10). Hence, we selected 360 W for all subsequent reactions. Further, the method does not require column chromatographic purification for isolation of the products. After completion of the reaction, the precipitated solid was filtered and washed with ethanol to give the pure pyranocarbazole without the need for column chromatography.

With optimized conditions in hand we focused on the scope of this method with various substituted aromatic aldehydes (Table-2). It was observed that benzaldehydes bearing electron withdrawing groups such as F, CN, CF_3 and NO_2 groups exhibited good reactivity in the reaction and gave the corresponding pyrano[3,2-*c*]carbazole derivatives in good yields (Table-2). In addition, electron-donating OMe group was also favourable for the transformation (**9g-h**). The reaction

TABLE-1
OPTIMIZATION OF REACTION CONDITIONS FOR THE SYNTHESIS OF PYRANO[3,2-*c*]CARBAZOLE DERIVATIVES (**9a**)

Entry	Solvent	Base	Temp. (°C)	Time	Yield (%)
1	Ethanol	–	RT	24 h	–
2	Ethanol	–	Reflux	24 h	85
3	Ethanol	TEA	Reflux	24 h	56
4	Toluene	Pyridine	Reflux	12 h	94
5	CH_3CN	DMAP	Reflux	10 h	–
6	–	–	100 °C	3 h	64
7	–	–	MW. 360 W	5 min	89
8	–	–	MW. 540 W	5 min	70
9	–	–	MW. 720 W	5 min	49
10	–	–	MW. 360 W	10 min	74

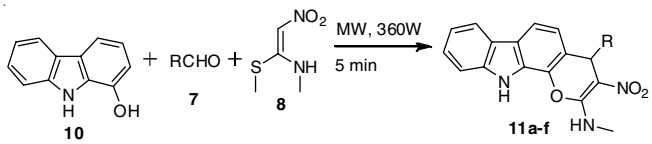
TABLE-2
SYNTHESIS OF PYRANO[2,3-*a*]CARBAZOLE DERIVATIVES (**9a-j**) UNDER OPTIMIZED CONDITIONS

Compound No.	R	Yield (%)
9a	C_6H_5	89
9b	4-F-C_6H_4	82
9c	2-NO_2-C_6H_4	83
9d	4-CN-C_6H_4	78
9e	4-CF_3-C_6H_4	81
9f	4-OH-C_6H_4	75
9g	4-OCH_3-C_6H_4	69
9h	3,4,5-$(OCH_3)_3$-C_6H_2	76
9i	4-Isopropyl-C_6H_4	79
9j	3-Phenylpyridine	77

was also quite successful with heteroaryl 3-pyridinecarboxaldehyde afford the corresponding pyrano[3,2-*c*]carbazole derivative in 76 % yield (Table-2, entry 9j). However, aliphatic aldehydes such as acetaldehyde, propionaldehyde did not participate in the reaction. The products were characterized by ^1H, ^{13}C NMR, IR and mass spectroscopic analyses.

Initially, Knoevenagel condensation reaction of 4-hydroxy carbazole (**6**) and benzaldehyde (**7**) affords adduct (**A**). Then the adduct **A** upon Michael-type addition with (E)-N-methyl-1-(methylthio)-2-nitroethenamine (**8**) affords the open-chain intermediate (**B**). The intermediate (**B**) apparently tautomerized to another intermediate (**C**) *via* an imine-enamine tautomerism. Finally, the intermediate (**C**) undergoes intramolecular ocyclization to form (**9a**) through the elimination of MeSH.

Encouraged by the above results, next we have focused our attention on synthesis of pyrano[2,3-*a*] derivatives between the 2-hydroxycabazole (**10**) aromatic substituted aldehydes (**7**) and (E)-N-methyl-1-(methylthio)-2-nitroethenamine (**8**) under optimized conditions. For instance, three component coupling of 2-hydroxycarbazole (**10**), benzaldehyde (**7**) and (E)-N-methyl-1-(methylthio)-2-nitroethenamine (**8**) under MW irradiation afford. The corresponding N-methyl-3-nitro-4-phenyl-4,11-dihydropyrano [2,3-*a*]carbazol-2-amine (**11a**) in 76 % yield. Bromo, nitro-, cyano- and methoxy substituted aromatic aldehydes also participated well in the one-pot three component coupling reactions and the corresponding pyrano-[2,3-*a*]carbazoles (**11b-e**) were obtained in good yields (**Scheme-II**). The reaction was also quite successful with heterocyclic 3-pyridine carboxaldehyde (Table-3, entry **11f**).



Scheme-II: Synthesis of pyrano[2,3-*a*]carbazole derivatives (**11a-f**) under optimized conditions

TABLE-3
SYNTHESIS OF PYRANO[2,3-*a*]CARBAZOLE DERIVATIVES (**11a-f**) UNDER OPTIMIZED CONDITIONS

Compound No.	R	Yield (%)
11a	C_6H_5	76
11b	4-Br-C_6H_4	68
11c	4-NO_2-C_6H_4	70
11d	4-CN-C_6H_4	67
11e	3-OCH_3-C_6H_4	71
11f	3-Phenylpyridine	66

Antiproliferative activity: To investigate the anticancer activities of the synthesized compounds, we have evaluated antiproliferative activities of all synthesized compounds **9a-j** and **11a-f** by an *in vitro* assay on four cancer cell lines belonging to different tumor types such as DU 145 (prostate cancer), MDA-MB-231 (breast cancer), SKOV3 (ovarian cancer) and B16-F10 (skin cancer). Results are expressed as IC_{50} values (Table-4) the concentration at which a compound causes 50 % cell death with respect to a control culture. A well known anticancer agent Doxorubicin was used as a reference compound in this assay [45].

As illustrated in Table-4, pyrano[3,2-c]carbazoles (**9a-j**) exhibited more potent antiproliferative activity than the pyrano-[2,3-a]carbazoles (**11a-f**) against the four tested cancer cell lines. Further studies reveal that trimethoxyphenyl substituted pyrano[3,2-c]carbazole (**9h**) exhibited most potent antriproliferative activity in comparison with other compounds in all the four cancer cell lines (Table-4). From the *in vitro* antipro-

TABLE-4
ANTIPROLIFERATIVE ACTIVITY OF PYRANO[3,2-*c*]CARBAZOLE DERIVATIVES(**9a-j**) AND PYRANO[2,3-*a*]CARBAZOLE (**11a-f**) DERIVATIVES ON VARIOUS CANCEROUS CELL LINES

Compd.	IC_{50} (μM)			
	DU145	MDAMB231	SKOV3	B16-F10
9a	7.2 ± 0.38	7.9 ± 0.42	11.8 ± 0.46	19.4 ± 0.49
9b	14.5 ± 0.56	6.8 ± 0.37	6.3 ± 0.35	8.4 ± 0.66
9c	23.0 ± 0.42	18.6 ± 0.54	15.9 ± 0.59	10.0 ± 0.58
9d	27.1 ± 0.51	NA	11.8 ± 0.61	22.4 ± 0.47
9e	17.8 ± 0.47	11.3 ± 0.65	12.6 ± 0.48	21.0 ± 0.66
9f	NA	NA	21.9 ±0.53	19.8 ± 0.58
9g	13.7 ± 0.56	23.5 ± 0.54	13.7 ± 0.72	23.7 ± 0.49
9h	8.4 ± 0.44	5.7 ± 0.41	5.1 ± 0.37	9.5 ± 0.48
9i	17.1 ± 0.57	9.9 ± 0.40	17.0 ± 0.68	25.5 ± 0.66
9j	16.3 ± 0.53	59.8 ± 0.62	19.2 ± 0.75	13.2 ± 0.57
11a	9.1 ± 0.39	14.0 ± 0.38	9.2 ± 0.88	18.3 ± 0.62
11b	47.1 ± 0.58	33.2 ± 0.55	23.4 ± 0.64	22.5 ± 0.83
11c	37.8 ± 0.62	44.9 ± 0.62	19.1 ± 0.54	30.3 ± 0.76
11d	61.1 ± 0.73	53.3 ± 0.87	17.3 ± 0.67	22.3 ± 0.48
11e	29.4 ± 0.68	NA	17.1 ± 0.48	34.5 ± 67
11f	41.2 ± 0.56	29.9 ± 0.67	10.8 ± 0.76	NA
DA	0.7 ± 0.19	0.7 ± 0.25	0.8 ± 0.24	2.0 ± 0.53

DA = Doxorubicin a

liferative activity data, a preliminary structure-activity relationship of the synthesized compounds was studied.

Among the pyrano[3,2-*c*]carbazole compounds, compound **9a** exhibited significant growth inhibitory activity against DU145 and MDA-MB-231 with IC_{50} values of 7.2 μM and 7.9 μM. To study the effect of substitution on the phenyl ring, flouro, nitro, cyano, hydroxy, isopropyl and methoxy groups were introduced in the *ortho*, *para* and *meta* positions. Compound **9b** with fluoro substitution on phenyl ring exhibited good inhibition with IC_{50} values of 6.8 μM against breast cancer, 6.3 μM against ovarian cancer and 8.4 μM against skin cancer cell lines. This could be attributed to the presence of huge electron density around the fluorine substituent on the phenyl ring. Compound **9c** with strong electron-withdrawing substituent (NO_2) exhibited its efficacy against skin cancer cells. Whereas, in case of compounds **9d** and **9e** the presence of cyano and trifluoromethyl groups on the phenyl ring might responsible for hindering their cytotoxicity when compared with compounds **9a** and **9b**. On the other hand, hydroxyphenyl substituted compound **9f** exhibited lower activities than others. Interestingly, trimethoxyphenyl substituted pyrano[3,2-*c*]-carbazole compound **9h** was more active with IC_{50} values of 8.4 μM against DU145, 5.7 μM against MDAMB231, 5.1 μM against SKOV3, 9.5 μM against B16-F10, respectively in their antiproliferative activity. It is obvious that the 3,4,5-trimethoxy groups in the phenyl ring of the compound **9h** are responsible for increase the cytotoxicity. However, mono methoxy substituted compound **9g** displayed moderate activities with IC_{50} values of 13.7 μM against both prostate cancer and ovarian cancer cell lines. The effect of isopropyl group on phenyl ring of compound **9i** was observed significant inhibition of the growth of breast cancer cell line with IC_{50} value of 9.9 μM. These data indicated that compounds containing electron donating methoxy substituent on phenyl ring showed enhanced activity compared to compounds containing electron withdrawing substituents (NO_2, CN, CF_3). To further elucidate the structure activity relationships, the phenyl ring was extensively modified with pyridine ring in compound **9j**, which exhibited reduced cytotoxicity in all the tested cell lines.

Among pyrano[2,3-*a*]pyranocarbazole derivatives compound **11a** displayed significant IC_{50} value of 9.1 μM 5 against prostate cancer and 9.2 μM against ovarian cancer cell lines. All other substitutions on the phenyl ring that were tested, including bromo, nitro, cyano, methoxy groups (**11b-e**) decreased the activity substantially compared to the corresponding compound **11a.**Overall, our current activity data suggested that compounds **9a**, **9b, 9h** and **11a** presented in this study have good anti-cancer properties. Trimethoxy groups in the phenyl ring are essential for potent cytotoxicity. These initial studies will definitely help in identifying the lead molecules with anticancer properties and these studies are currently underway in our laboratory.

Molecular modeling: In order to rationalize the experimental results obtained, molecular docking studies were perfor-

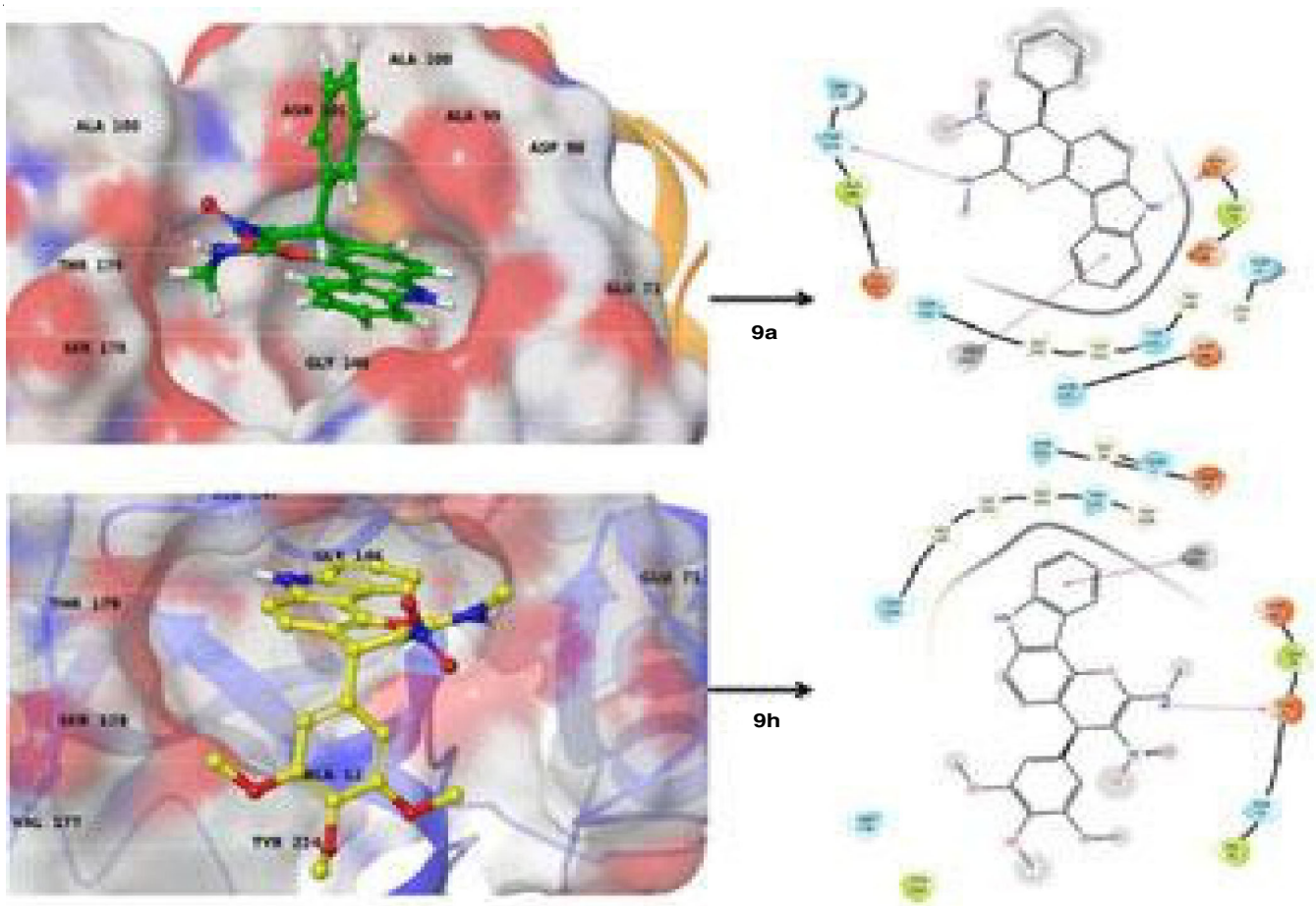


Fig. 1.

med on representative compounds like **9a**, **9b** and **9h** against tubulin structures. Glide docking has been employed and the lead compounds docked in the colchicines binding pocket of tubulin (PDB code: 3E22).

The results of molecular docking are depicted in Fig. 1. It is well studied that colchicine binding site is generally present at the interface of α,β-tubulin heterodimers [46,47]. The lead compounds used for the docking study are shown as different colours of ball and stick model and hydrogen bonds are shown as blue coloured dots.

Docking simulations showed that compound **9a** was surrounded by the amino acid residues like Ser178, Thr 179, Ala 180, Glu 183, Ser 140, Gly 143, Gly 144, Thr 145, Gly146, Ash69, Leu70 and Glu71. In addition, the carbazole ring NH involved in hydrogen bonging with Glu71 residue and methyl substituted NH formed hydrogen bonding interaction with Thr179 residue. Binding is seen to be stabilized by hydrogen bonding. Similar results were observed in compound **9b**. Compound **9b** also shows two significant hydrogen bonding interactions with amino acid residues Glu71 and Thr179.

Whereas, compound **9h** made hydrophobic interactions with amino acid residues like Ser140, Gly142, Gly143, Gly144, Thr145, Gly146, Ash69, Leu70, Glu71, Thr73, Thr73, Val74, Tyr224 and Ser178. Additionally, there is hydrogen bonding interaction between compound **9h** with Glu71 residue of the target protein. Indeed, Ser178 and Tyr 224 amino acid residues of β-tubulin formed significant hydrophobic interactions with the trimethoxyphenyl moiety of **9h** appeared to stabilize the ligand in the active site. Therefore, these docking simulations suggested that the ligands bind to the active site effectively. The results obtained are in agreement and further strengthened with *in vitro* antiproliferative activity.

Conclusion

In conclusion, the present work reports a simple and efficient one-pot three-component protocol for the expedient synthesis of novel pyrano[3,2-*c*]carazoles and pyrano[2,3-*a*]carbazole derivatives in good yields. It is noteworthy that this reaction results in the formation of three new bonds (two C–C and one C–O) in a single operation. The significant advantages of this protocol include catalyst free, solvent free, readily available substrates, short reaction time, atom-economy and no column chromatographic purification. These advantages make this methodology facile and appropriate to create diverse libraries. Screening of all sixteen new pyranocarbazole derivatives against cancer cell lines DU 145, MDA-MB-231, SKOV3 and B16-F10 revealed that synthesized compounds displayed moderate to potent antiproliferative activity. Structure-activity studies indicated that trimethoxyphenyl substituted pyrano[3,2-*c*]carbazole derivative **9h** displayed the most potent antiproliferative activity against the four-cell lines. In addition,the *in silico* molecular docking results supported the *in vitro* studies. These results may be helpful for the future design of structurally related tubulin inhibitors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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