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Synthesis and Cytotoxic Studies of Pyrrolopyrimidine Derivatives

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The synthesis and *in vitro* cytotoxicity of new pyrrolopyrimidine derivatives is reported in this work. All the compounds were characterized by IR, NMR and MS. They are examined for cytotoxic activity against HeLa. Pyrrolopyrimidine derivatives of benzyl amine (**8g**) and 4-bromoaniline (**8k**) showed a potent activity, which is comparable to that of standard Sorafenib.

Keywords: Cytotoxic, Pyrrolopyridines, *in vitro*, HeLa.

INTRODUCTION

Cervical cancer is top-ranked cancer in women contributing to the high mortality rate in the developing countries [1]. The risk of cervical cancer is highly associated with human papillomavirus infections (HPV) and approximately 90% of cervical cancer patients are the victims of HPV infections [2]. About 40 types of HPV have been known to infect genital tract and at least 15 types of them are marked as potential carcinogens [3]. Other factors linked with initiation of cervical cancer are smoking and consumption of oral contraceptives [4]. Initiation of cervical cancer can be easily detected at the early stages using Pap smear test and detection at advanced stages may lead to poor progression and increased mortality [5]. Several antibiotics, vaccines, small molecule derivatives have been approved by FDA and implemented in treating cervical cancer. Cervarix is a recombinant bivalent vaccine which targets HPV and administered as a preventive measure against cervical cancer. Bleomycin (antibiotic) and topotecan (small molecule) are the FDA approved drugs against cervical cancer [6,7].

Pyrrolopyrimidine is a bicyclic nitrogen containing compound where electron deficient six membered pyrimidine ring is fused to the electron rich five membered pyrrole ring. It is a class of 7-deazapurine analogs, known to exhibit various significant biological activities like antiviral [8], antibacterial and antifungal [9,10], enzyme inhibitor [11], antitumor [12] and antifolate activity [13]. All these pharmacological properties of pyrrolopyrimidines make it a unique scaffold in the field of medicinal chemistry. Pyrrolopyrimidines are the renowned for their anticancer activity due to their action on broad spectrum of targets including tyrosine kinases [14-16], cell cycle regulators (CDKs, aurora kinase) [17], growth factor receptors (VEGFR, FGFR) [18] and other metabolically significant proteins (dihydrofolatereductases, adenosine antagonist receptor) [19]. In addition, cyclopentylpyrrolopyrimidine has been attributed as the tie-2 kinase inhibitor which is entangled with angiogenesis in tumor [20]. Herein, we report the synthesis and antiproliferative potential of novel cyclopentylpyrrolopyrimidine derivatives against cervical cancer cell lines as a part of our ongoing research on cancer therapy [21-28].

EXPERIMENTAL

The melting points were determined on Selaco melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT-IR model 8300 spectrophotometer. ^{1}H NMR

spectra were recorded on an NMR spectrometer operating at 400 MHz using TMS as internal standard. Mass spectra were recorded using electronspray ionization mass spectrometry. The C, H and N analysis were performed using CE-400 CHN analyzer. Reactions were monitored by TLC using pre-coated sheets of silica gel G/UV-254 of 0.25 mm thickness (Merck 60F254) using UV light for visualization. All the chemicals were obtained from the reputed commerical sources like Sigma-Aldrich, Fluka and Merck Chemicals. Human cervical cancer line HeLa was obtained from National Centre for Cell Science, Pune, India.

Synthesis of 5-bromo-2-chloro-*N*-cyclopentylpyrimidin-4-amine (3): To a solution of 5-bromo-2,4-dichloropyrimidine (**1**) (20 mmol) in ethanol (50 mL), cyclopentylamine (**2**) (22 mmol) and diisopropylethylamine (40 mmol) was added at room temperature and the reaction mixture was stirred for 15 h. The progress of the reaction was monitored by TLC. After the completion of reaction, content was concentrated under vacuum and the residue was purified by column chromatography using hexane-ethyl acetate (7:3 v/v) as eluent to get pure product **3** [29]. Yield 86%; m.p.: 95-96 ºC; IR (KBr, ν_{max}, cm^{-1}): 1279, 1287, 1571, 1726; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.46-1.51 (m, 4H, $(CH_2)_2$), 1.81-2.02 (m, 4H, $(CH_2)_2$), 3.74 (m, 1H, CH), 4.41 (s, 1H. N-H), 8.86 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.3, 160.2, 157.1, 103.0, 52.8, 34.9, 24.6; MS (ESI): *m/z* 276 (M+1); Anal. calcd. (found) % for $C_9H_{11}N_3BrCl$: C, 39.09 (39.11); H, 4.01 (4.05); N, 15.09 (15.13).

Synthesis of methyl 2-chloro-7-cyclopentyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine-5-carboxylate (5): A solution of 5-bromo-2-chloro-*N*-cyclopentylpyrimidin-4-amine (**3**, 20 mmol), lithium chloride (20 mmol), K_2CO_3 (50 mmol) and palladium(II) acetate (1 mmol) in DMF (50 mL) was degassed with nitrogen. Methyl propiolate (**4**) (60 mmol) was added under nitrogen atmosphere and the reaction mixture was heated at 120 ºC for 5 h. The progress of the reaction was monitored by TLC. After the completion of reaction, cooled the contents to room temperature, filtered through celite, diluted with water (100 mL) and extracted with ethyl acetate (150 mL × 3). The combined organic extract was washed with brine (150 mL), dried over anhydrous sodium sulphate. The solvent was evaporated under vacuum and the residue was purified by column chromatography using hexane-ethyl acetate (8:2 v/v) as eluent to get pure product **5**. Yield 61%; m.p.: 122-124 ºC; IR (KBr, ν_{max}, cm^{-1}): 1285, 1298, 1574, 1728, 2928, 3027; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.42-1.53 (m, 4H, $(CH_2)_2$), 1.83-2.01 (m, 4H, $(CH_2)_2$), 3.71 (m, 1H, CH), 3.88 (s, 3H, OMe), 7.36 (s, 1H, Ar-H), 8.89 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 165.9, 160.7, 152.9, 151.4, 123.6, 108.2, 105.4, 70.0, 51.5, 34.6, 24.5; MS (ESI): *m/z* 280 (M+1); Anal. calcd. (found) % for $C_{13}H_{14}N_3O_2Cl$: C, 55.82 (55.86); H, 5.04 (5.09); N, 15.02 (15.08).

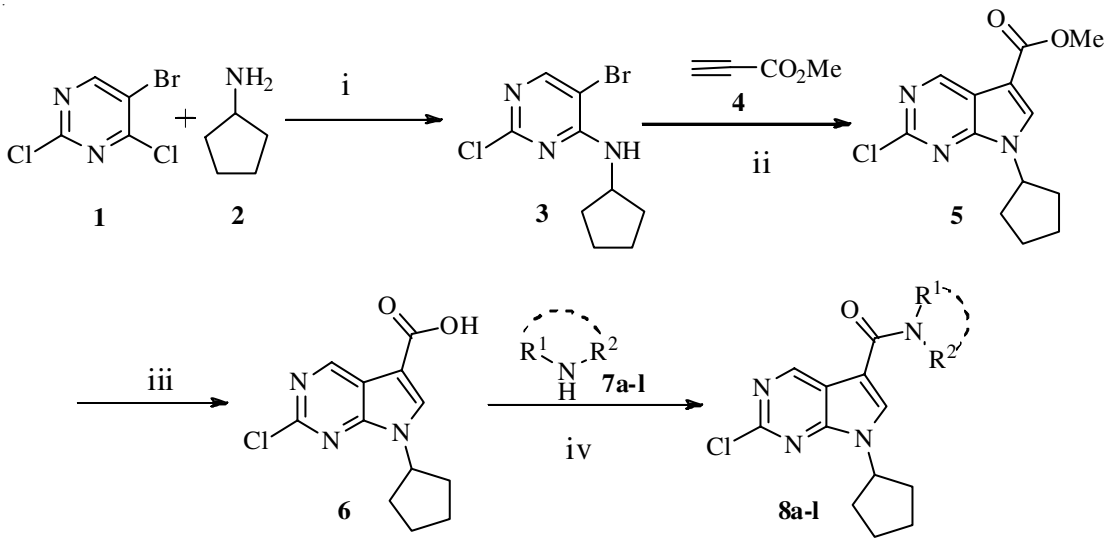
Synthesis of 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine-5-carboxylic acid (6): To a solution of methyl 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylate (**5**) (20 mmol) in ethanol (50 mL), 10% NaOH solution (100 mL) was added at 0 ºC and stirred the reaction mixture for about 10 h at room temperature. The progress of the reaction was monitored by TLC. After the completion of reaction, ice cold water (100 mL) and 2.5 M HCl solution (100 mL) was added dropwise at 0 ºC. The precipitate was filtered using Büchner funnel, washed with ice cold water, drained and dried in vacuum oven for 24 h. Yield 82%; m.p.: 177-179 ºC; IR (KBr, ν_{max}, cm^{-1}): 1296, 1586, 1715, 1738, 2928, 3027, 3285; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.42-1.52 (m, 4H, $(CH_2)_2$), 1.81-1.99 (m, 4H, $(CH_2)_2$), 3.71 (m, 1H, CH), 7.12 (s, 1H, Ar-H), 8.89 (s, 1H, Ar-H), 11.2 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.3, 160.7, 152.9, 151.4, 123.6, 108.2, 105.4, 70.2, 34.5, 24.6; MS (ESI): *m/z* 266 (M+1); Anal. calcd. (found) % for $C_{12}H_{12}N_3O_2Cl$: C, 54.25 (54.31); H, 4.55 (4.62); N, 15.82 (15.89).

Synthesis of 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide (8a-l): To a solution of 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid (**6**) (1 mmol) in dichloromethane (5 mL), triethylamine (2.5 mmol), 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride [EDC·HCl] (1.1 mmol), hydroxybenzotriazole [HOBt] (0.1 mmol) and amines **7a-l** (1.1 mmol) were sequentially added at 0 ºC. The reaction mixture was stirred at room temperature for 4-6 h and monitored by TLC. After the completion, water (20 mL) was added to the mixture, extracted with dichloromethane (20 mL × 3). The combined dichloromethane extract was washed with brine (20 mL) and solvent was removed under vacuum to get crude products **8a-l**, which were purified by column chromatography using hexane-ethyl acetate (8:2 v/v) as eluent (**Scheme-I**).

2-Chloro-7-cyclopentyl-*N,N*-diethyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide (8a): Yield 85%; m.p.: 172-174 ºC; IR (KBr, ν_{max}, cm^{-1}): 558, 1291, 1295, 1579, 1722, 2921, 3022; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.28 (m, 6H, $(CH_3)_2$), 1.68 (m, 2H, CH_2), 2.05 (m, 4H, $(CH_2)_2$), 2.33 (m, 2H, CH_2), 3.35 (d, 2H, J = 6.8 Hz, NCH_2), 3.60 (d, 2H, J = 7.2 Hz, NCH_2), 4.83 (m, 1H, CH), 7.27 (s, 1H, Ar-H), 8.79 (s, 1H, Ar- H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 169.9, 154.3, 149.3, 136.0, 117.1, 106.6, 98.3, 57.7, 39.5, 31.0, 24.7, 13.1; MS (ESI): *m/z* 321 (M+1); Anal. calcd. (found) % for $C_{16}H_{21}N_4OCl$: C, 59.90 (59.97); H, 6.60 (6.69); N, 17.46 (17.51).

2-(Chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)piperidin-1-yl)methanone (8b): Yield 83%; m.p.: 185-186 ºC; IR (KBr, ν_{max}, cm^{-1}): 547, 1282,1284, 1571, 1706, 3012; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.42 (m, 10H, $(CH_2)_{10}$), 2.04 (m, 2H, CH_2), 2.31 (m, 2H, CH_2), 4.52 (m, 5H, CH, $(CH_2)_2$), 7.26 (s, 1H, Ar-H), 8.76 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 171.6, 161.2, 152.5, 151.7, 123,6, 122.9, 108.4, 70.3, 47.9, 35.1, 25.7, 24.9, 23.7; MS (ESI): *m/z* 333 (M+1); Anal. calcd. (found) % for $C_{17}H_{21}N_4OCl$: C, 61.35 (61.85); H, 6.36 (6.51); N, 16.83 (16.96).

(2-Chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)(morpholino)methanone (8c): Yield 80%; m.p.: 189-191 ºC; IR (KBr, ν_{max}, cm^{-1}): 542, 1275, 1287, 1568, 1709, 3008; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.62 (m, 4H, $(CH_2)_2$), 2.03 (m, 2H, CH_2), 2.35 (m, 2H, CH_2), 3.74 (m, 4H, $(CH_2)_2$), 4.82 (m, 5H, $(CH_2)_2$, CH), 7.29 (s, 1H, Ar-H), 8.78 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 169.1, 161.3, 152.7, 151.3,



Scheme-I: Reagents and reaction conditions: (i) DIPEA/EtOH, RT, 15 h; (ii) LiCl/K_2CO_3, $Pd(OAc)_2$/DMF; (iii) 10% aq. NaOH/EtOH, RT, 5 h; (iv) EDC.HCl/HOBt, Et_3N/CH_2Cl_2

124.9, 108.3, 107.1, 69.1, 67.7, 47.5, 34.9, 24.9; MS (ESI): *m/z* 335 (M+1); Anal. calcd. (found) % for $C_{16}H_{19}N_4O_2Cl$: C, 57.40 (57.46); H, 5.72 (5.76); N, 16.73 (16.79).

2-Chloro-7-cyclopentyl-N-(4-methylbenzyl)-7*H*-pyrrolo-[2,3-*d*]pyrimidine-5-carboxamide (8d): Yield 78%; m.p.: 181-183 ºC; IR (KBr, ν_{max}, cm^{-1}): 549, 1289, 1292, 1564, 1727, 3017, 3378; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.67 (m, 4H, $(CH_2)_2$), 2.01 (m, 2H, CH_2), 2.37(m, 2H, CH_2), 2.60 (s, 3H, CH_3), 4.58 (s, 2H, CH_2), 4.60 (m, 1H, CH), 7.20 (m, 5H, Ar-H), 8.33 (s, 1H, N-H), 8.77 (s, 1H, Ar-H).^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 167.8, 161.4, 152.8, 152.4, 135.8, 134.3, 128.2, 127.9, 127.8, 116.4, 101.5, 57.4, 43.7, 31.0, 24.9, 21.0; MS (ESI): *m/z* 369 (M+1); Anal. calcd. (found) % for $C_{20}H_{21}N_4OCl$: C, 65.12 (65.19); H, 5.74 (5.81); N, 15.19 (15.23).

2-Chloro-7-cyclopentyl-N-(4-methoxybenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8e): Yield 81%; m.p.: 187-189 ºC; IR (KBr, ν_{max}, cm^{-1}): 551, 1291, 1299, 1576, 1723, 3021, 3374; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.68 (m, 4H, $(CH_2)_2$), 2.00 (m, 2H, CH_2), 2.36 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 4.58 (s, 2H, CH_2), 4.79 (m, 1H, CH), 6.77 (s, 1H, Ar-H), 7.29 (m, 4H, Ar-H), 8.39 (s, 1H, N-H), 8.77 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 168.1, 162.7, 158.5, 153.4, 152.5, 130.6, 130.0, 124.7, 116.8, 110.7, 106.9, 69.7, 55.3, 47.6, 39.1, 24.4; MS (ESI): *m/z* 385 (M+1); Anal. calcd. (found) % for $C_{20}H_{21}N_4O_2Cl$: C, 62.42 (62.53); H, 5.50 (5.58); N, 14.56 (14.60).

2-Chloro-*N*-(4-chlorobenzyl)-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8f): Yield 76%; m.p.: 198-200 ºC; IR (KBr, ν_{max}, cm^{-1}): 542, 1299, 1568, 1718, 1292, 3015, 3371; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.69 (m, 4H, $(CH_2)_2$), 2.13 (m, 2H, CH_2), 2.39 (m, 2H, CH_2), 4.64 (m, 1H, CH), 5.50 (s, 2H, CH_2), 6.77 (s, 1H, Ar-H), 7.24 (m, 4H, Ar-H), 8.42 (s, 1H, N-H), 8.75 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 167.6, 161.4, 152.8, 152.2, 149.2, 136.8, 133.9, 132.7, 129.3, 124.6, 108.5, 69.6, 43.9, 33.8, 23.9; MS (ESI): *m/z* 389 (M+1); Anal. calcd. (found) % for $C_{19}H_{18}N_4OCl_2$: C, 58.62 (58.65); H, 4.66 (4.69); N, 14.39 (14.43).

***N*-Benzyl-2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide (8g):** Yield 80%; m.p.: 176-178 ºC; IR (KBr, ν_{max}, cm^{-1}): 554, 1289, 1297, 1578, 1721, 3014; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.67 (m, 4H, $(CH_2)_2$), 2.17 (m, 2H, CH_2), 2.32 (m, 2H, CH_2), 4.67 (m, 1H, CH), 5.54 (s, 2H, CH_2), 6.69 (s, 1H, Ar-H), 7.39 (m, 5H, Ar-H), 8.39 (s, 1H, N-H), 8.78 (s, 1H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 167.9, 162.7, 155.6, 152.1, 137.4, 128.4, 127.3, 126.0, 123.3, 109.8, 106.4, 70.1, 44.5, 34.7, 24.1; MS (ESI): *m/z* 355 (M+1); Anal. calcd. (found) % for $C_{19}H_{19}N_4OCl$: C, 64.31; H, 5.40; N, 15.79; Found: C, 64.36; H, 5.44; N, 15.81.

2-(Chloro-7-cyclopentyl-*N*-(furan-2-ylmethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8h): Yield 83%; m.p.: 180-182 ºC; IR (KBr, ν_{max}, cm^{-1}): 544, 1269, 1567, 1711, 1269, 3011; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.65 (m, 4H, $(CH_2)_2$), 2.15 (m, 2H, CH_2), 2.37 (m, 2H, CH_2), 4.68 (m, 1H, CH), 5.54 (s, 2H, CH_2), 6.30 (t,1H, *J* = 8.0 Hz, Ar-H), 6.47 (m, 1H, Ar-H), 7.19 (s, 1H, Ar-H), 7.43 (m, 1H, Ar-H), 8.46 (s, 1H, N-H), 8.78 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 167.4, 161.3, 152.2, 151.3, 148.2, 142.2, 135.5, 110.5, 110.4, 108.2, 106.5, 57.3, 36.7, 36.5, 24.9; MS (ESI): *m/z* 345 (M+1); Anal. calcd. (found) % for $C_{17}H_{17}N_4O_2Cl$: C, 59.22 (59.28); H, 4.97 (4.99); N, 16.25 (16.30).

2-(Chloro-7-cyclopentyl-*N*-(pyridin-3-ylmethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8i): Yield 70%; m.p.: 190-192 ºC; IR (KBr, ν_{max}, cm^{-1}): 552, 1288, 1295, 1581, 1723, 3028, 3379; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.65 (m, 4H, $(CH_2)_2$), 2.19 (m, 2H, CH_2), 2.38 (m, 2H, CH_2), 4.69 (m, 1H, CH), 5.51 (s, 2H, CH_2), 6.74 (s,1H, Ar-H), 7.39 (m, 1H, Ar-H), 7.84 (t, 1H, *J* = 6.2 Hz, Ar-H), 8.34 (t, 1H, *J* = 6.8 Hz, Ar-H), 8.37 (s, 1H, N-H), 8.67 (s, 1H, Ar-H), 8.75 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 168.1, 161.5, 153.7, 151.5, 149.1, 147.4, 136.9, 136.1, 123.8, 123.3, 108.5, 107.6, 69.0, 43.8, 33.9, 24.7; MS (ESI): *m/z* 356 (M+1); Anal. calcd. (found) % for $C_{18}H_{18}N_5OCl$: C, 60.76 (60.78); H, 5.10 (5.21); N, 19.68 (19.73).

2-(Chloro-7-cyclopentyl-*N*-(4-methoxyphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8j): Yield 68%;

m.p.: 202-204 ºC; IR (KBr, ν_{max}, cm^{-1}): 552, 1295, 1297, 1574, 1721, 3028, 3379; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.62 (m, 4H, $(CH_2)_2$), 2.13 (m, 2H, CH_2), 2.38 (m, 2H, CH_2), 3.68 (s, 3H, OCH_3), 4.79 (m, 1H, CH), 6.77 (s, 1H, Ar-H), 7.21 (d, 2H, *J* = 8.0 Hz, Ar-H),7.58 (d, 2H, *J* = 8.0 Hz, Ar-H), 8.35 (s, 1H, NH), 8.75 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 164.2, 161.6, 158.9, 153.1, 151.8, 131.6, 123.4, 122.9, 115.6, 109.5, 106.3, 69.4, 55.2, 35.1, 24.0; MS (ESI): *m/z* 371 (M+1); Anal. calcd. (found) % for $C_{19}H_{19}N_4O_2Cl$: C, 61.54 (61.59); H, 5.16 (5.24); N, 15.11 (15.16).

***N*-(4-Bromophenyl)benzyl-2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8k):** Yield 65%; m.p.: 213-215 ºC; IR (KBr, ν_{max}, cm^{-1}): 544, 1276, 1281, 1570, 1715; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.69 (m, 4H, $(CH_2)_2$), 2.03 (m, 2H, CH_2), 2.31 (m, 2H, CH_2), 4.67 (m, 1H, CH), 6.77 (s, 1H, Ar-H), 7.24 (m, 4H, Ar-H), 8.23 (s,1H, N-H), 8.76 (s, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 160.9, 160.1, 154.5, 152.7, 151.3, 136.7, 131.4, 122.7, 122.5, 108.2, 107.8, 57.5, 30.9, 25.0; MS (ESI): *m/z* 419 (M+1); Anal. calcd. (found) % for $C_{18}H_{16}N_4OBrCl$: C, 51.51 (51.56); H, 3.84 (3.87); N, 13.35 (13.39).

2-Chloro-*N*-(3-chloropropyl)-7-cyclopentyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine-5-carboxamide (8l): Yield 55%; m.p.: 171-173 ºC; IR (KBr, ν_{max}, cm^{-1}): 561, 1292, 1297, 1583, 1719, 2871, 3019; ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 1.28 (m, 2H, (CH_2), 1.68 (m, 2H, CH_2), 2.05 (m, 6H, $(CH_2)_3$), 3.32 (m, 2H, CH_2), 3.60 (m, 3H, CH_2,CH), 7.27 (s, 1H, Ar-H), 8.36 (s, 1H, N-H), 8.79 (s, 1H, Ar-H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 162.4, 156.1, 153.6, 152.7, 134.9, 107.9, 104.7, 57.0, 40.2, 39.1, 36.0, 30.4, 24.9; MS (ESI): *m/z* 341 (M+1); Anal. calcd. (found) % for $C_{15}H_{18}N_4OCl_2$: C, 52.80 (52.86); H, 5.32 (5.39); N, 16.42 (16.45).

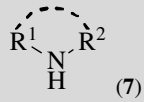
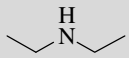
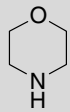
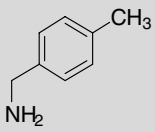
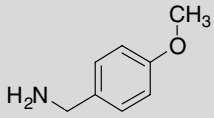
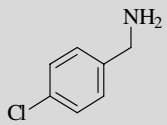
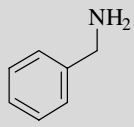
***in vitro* Cytotoxicity assay:** HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 1 X antibiotic-antimycotic solution with 10% FBS. The newly synthesized compounds were tested for their cytotoxic effect by using MTT dye uptake method against the HeLa cell lines as described previously [30,31]. Briefly, the cells (2.5×10^4/mL) were incubated in a 96-well plate in triplicate in the presence or absence of different concentrations of synthesized compounds in a final volume of 0.2 mL for indicated time intervals at 37 ºC. Consequently, 20 µL MTT solution (5 mg/mL in PBS) was added to each well. After a 2 h incubation at 37 ºC, 0.1 mL lysis buffer (20% SDS, 50% DMF) was added; incubation was continued overnight at 37 ºC; later the optical density (OD) at 570 nm was measured by varioskan plate reader.

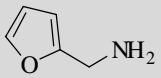
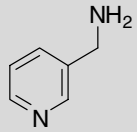
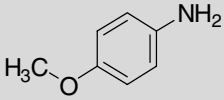
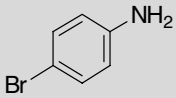
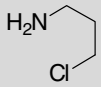
RESULTS AND DISCUSSION

A highly regioselective aromatic nucleophilic substitution of chlorine in 5-bromo-2,4-dichloropyrimidine (**1**) was achieved by cyclopentylamine (**2**) to give 5-bromo-2-chloro-*N*-cyclopentylpyrimidin-4-amine (**3**). Michael addition of amine **3** to methyl propiolate (**4**) followed by palladium catalyzed intramolecular cyclization afforded methyl 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylate (**5**). Later, the methyl ester **5** was hydrolyzed using aqueous alcoholic sodium hydroxide, followed by neutralization of the reaction mixture with calculated amount of dilute hydrochloric acid furnished 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid (**6**) in good yield. The key intermediate **6** was then coupled with various amines **7a-l** using 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride [EDC·HCl] and hydroxybenzotriazole [HOBt] to give 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamides (**8a-l**) in good yields.

Cytotoxicity studies: The antiproliferative effect of newly synthesized pyrrolopyrimidine derivatives by using MTT assay on the HeLa cell lines was examined. Sorafenib was used as positive control which showed the IC_{50} of 4.1 µM. Among the tested compounds, **8g** and **8k** displayed intense activity with the IC_{50} of 7.4 and 7.6 µM, respectively comparable to the standard shown in Table-1. Compound **8h** exhibited remarkable activity (IC_{50} of 9.9 µM) also **8f** showed satisfactory results with IC_{50} of 12.7 µM. Compounds **8j**, **8d**, **8e**, **8l**, **8c** and **8i** exhibited considerable activity while the compounds **8a** and **8b** displayed least activity among the other compounds synthesized. It was clear that the compounds with benzyl amine derivatives contributed better activity than the other derivatives. In addition, unsubstituted benzyl amine derivative and the bromo derivative showed the maximum activity (**8g** and **8k**). Overall compounds bearing the electron-withdrawing group at the *para* substitution flaunted a good activity and the tertiary amine derivatives were found to exhibit the slightest activity.

TABLE-1
SUMMARY OF YIELDS AND IC_{50} VALUES OF PYRROLOPYRIMIDINE DERIVATIVES (**8a-l**)

 (**7**)	**8**	Yield (%)	IC_{50} (µM) ± SD
	**8a**	85	31.8 ± 5.0
	**8b**	83	31.4 ± 5.1
	**8c**	80	26.8 ± 5.3
	**8d**	78	24.7 ± 4.3
	**8e**	81	24.9 ± 5.7
	**8f**	76	12.7 ± 3.5
	**8g**	80	7.4 ± 5.1

	**8h**	83	9.9 ± 3.1
	**8i**	70	27.2 ± 6.7
	**8j**	68	23.4 ± 6.0
	**8k**	65	7.6 ± 5.5
	**8l**	55	25.7 ± 3.4

IC_{50} of Sorafenib is 4.1 ± 1.6 µM

Conclusion

In summary, a series of new 2-chloro-7-cyclopentyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-5-carboxamides was synthesized, characterized and evaluated for their cytotoxic activity against HeLa cell line. Compounds with the unsubstituted benzyl amine derivative and the bromo derivative **8g** and **8k** demonstrated exquisite activity. Also, the substituents at the *para* position with the electron withdrawing groups **8f** and **8h** exhibited a better activity comparable to the standard drug sorafenib.

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CONFLICT OF INTEREST

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

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