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Design, synthesis and anticancer evaluation of 2-Amino pyrimidine linked 7-Azainadzole derivatives

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ABSTRACT

A series of 2-Aminopyrimidine linked 7-Azainadzole derivatives were designed, synthesized and evaluated for their anticancer activity against MCF-7, A549 and MDA-MB-231 human cancer cell lines by MTT assay method. The compound **11g** showed potent anticancer activity against MCF-7, A549, MDA-MB-231 cell lines with IC_{50} values of 0.34 μM, 0.11 μM and 2.33 μM respectively than the positive control, Adiramycin. Compounds **11b, 11g, 11h** and **11j** also showed more potent activity than control, Adiramycin.

Specifications Table

Subject area	Organic Chemistry, Biochemistry, Spectroscopy
Compounds	2-Aminopyrimidine linked 7-Azainadzole derivatives
Data category	Spectral
Data acquisition format	NMR, IR, MS(ESI)
Data type	Analysed
Procedure	2-Aminopyrimidine linked 7-Azainadzole derivatives as Anticancer agents
Data accessibility	Manuscript and supplementary data enclosed with this article

1. Rationale

Cancer is the second most dangerous disease in all over countries**.** Cancer can be occurred by genetic mutations, plethora of both external and internal factors. Various methods are available to treatment of cancers including chemotherapy, radiotherapy, surgery, biological therapy and various combinations. [1] Among them chemotherapy is the more effective treat-

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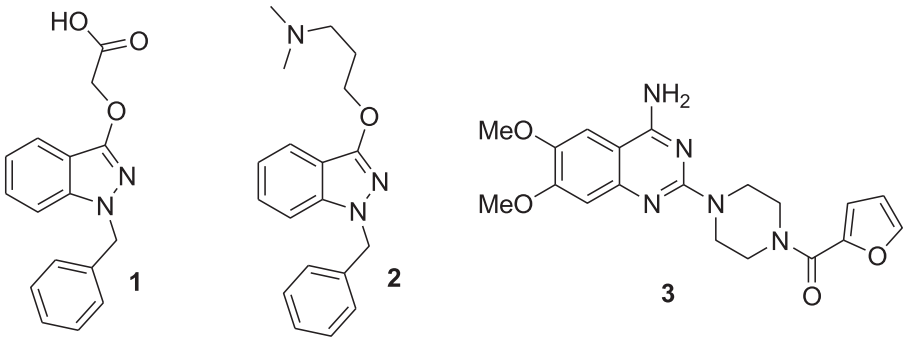


Fig. 1. Structure of bendazac (1), benzydamine (2) and prazosin (3).

ment for cancer. [2] Presently used anticancer drugs are cytotoxic agents and have narrow therapeutic index and frequently acquired resistance. [2] Hence, there is need to development of more efficient and inferior toxicity drugs.

Nitrogen containing heterocyclic compounds are extremely attracted many researchers due to their anticancer activities against a panel of cancer cell lines. [3–26] Indazoles are nitrogen containing fused heterocyclic systems and was displayed different biological activities like antitumor, [27] antidepressant, [28] 5–HT2 receptor agonist, [29] aurora kinase, [30] EGFR inhibitors, [31] antiemetic, [32] dopamine antagonistic, [33] anti-inflammatory, [34] analgesicantipyretic, [35] and anti-HIV activities. [36] Some of indazole core structure containing drugs are available in market such as bendazac (**1,** Fig. 1) [37,38], benzydamine (**2**). [39]

Similarly, pyrimidines are naturally occurring significant pharmacophores and were found application in a wide range of medicinal chemistry due to their pharmacological activities. Pyrimidine scaffold is exhibited remarkable biological activities including antitumour, [40] diuretic, [41] antitubercular, [42] anti-HIV, [43] analgesic, antiviral, anti-inflammatory, [44] cardiovascular agents, [45] antineoplastic, [46] antibacterial, [47] antifilarial, [48] antimicrobial, [49] and anti-malarial. [50] FDA approved anticancer drug such as prazosin (**3)** [51,52] is a pyrimidine nucleus containing compound available in market.

Based on the above findings and continuous of efforts, we have synthesized a series of pyrimidine fused 7-azaindazole (**11a-j**) derivatives. All these derivatives were screened their anticancer activity against human cancer cell lines.

2. Experimental section

2.1. Materials and methods

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. 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. ^{1}H and ^{13}C NMR spectra were recorded on Bruker, Bruker UXNMR/XWIN-NMR (400 MHz, 300 MHz) instrument. Chemical shifts (d) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected.

2.2. 5-(3,4,5-Trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridine (6)

5-bromo-1H-pyrazolo[3,4-b]pyridine (**4**) (20 g, 100.9 mmol), $Pd(PPh_3)_4$ (23 g, 201.8 mmol) and 3,4,5-trimethoxyphenylboronic acid (**5**) (21 g, 100.9 mmol) were dissolved in THF (100 mL). To this solution, was added H_2O (20 ml) and Na_2CO_3 (32 g, 302.7 mmol) was added under stirring and the reaction mixture was reflux for 12 hours. After cooling, Na_2CO_3 was removed by filtration and filtrate was evaporated through rotavapor to get crude product. This crude compound was extracted with ethyl acetate and dried over with $MgSO_4$ to give crude solid. This crude solid obtained was purified by column chromatography with ethyl acetate/hexane (1:1) to afford half white color solid **6**, with 19.2 g in 67% yield. Mp: 178–180 °C, IR (KBr): 3350, 3089, 2961, 828, 759 cm^{-1}; ^{1}HNMR (400, DMSO-d_6): δ 3.87 (s, 6H), 3.90 (s, 3H), 7.20 (s, 2H), 7.83 (s, 1H), 8.10 (s, 1H), 8.65 (s, 1H), 9.18 (bs, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 53.8, 55.4, 55.6, 109.3, 109.6, 123.5, 124.6, 124.8, 125.6, 126.2, 128.1, 133.5, 136.3, 154.1, 155.8; MS (ESI): 286 $[M+H]^+$; Anal. Calcd for $C_{15}H_{15}N_3O_3$: C, 63.15; H, 5.30; N, 14.73. Found: C, 63.02; H, 5.41; N, 14.59.

2.3. 1-(5-(3,4,5-Trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)ethanone (8)

The compound **6** (18 g, 63.1 mmol) was dissolved in 30 mL of dried CH_2Cl_2, followed by addition of acetyl chloride (**7**) (4.5 mL, 63.1 mmol) and TEA (26 ml, 189.3 mmol). The reaction mixture was stirred at room temperature for 6 hours. After completion of the reaction. The reaction mixture was washed with aq$NaHCO_3$ and the solvent was evaporated under

vacuum to afford crude product. The crude product was purified by column chromatography with ethyl acetate/hexane (4:6) to afford yellow color solid **8**, 19.6 g in 95%. Mp: 164–166 °C, IR (KBr): 3046, 2948, 1719, 814, 768 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 2.94 (s, 3H), 3.87 (s, 6H), 3.90 (s, 3H), 7.20 (s, 2H), 7.84 (s, 1H), 8.10 (s, 1H), 8.65 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 34.3, 57.9, 58.4, 61.2, 108.7, 124.1, 129.3, 131.6, 134.7, 138.4, 154.2, 154.9, 193.2; MS (ESI): 328 $[M+H]^+$; Anal. Calcd for $C_{17}H_{17}N_3O_4$: C, 62.38; H, 5.23; N, 12.84. Found: C, 62.54; H, 5.15; N, 12.70.

2.4. 4-(5-(3,4,5-Trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)-6-phenylpyrimidin-2-amine (11a)

The mixture of 1-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)ethanone (**8**) (500 mg, 1.5 mmol), benzaldehyde (**10a**) (1.5 ml, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) in ethanol (15 ml) was reflux for 6 hours. After completing the reaction, the reaction mixture was poured into water, and then washed with water thoroughly. The product was filtered, dried, and recrystallized from ethanol to afford yellow color solid **11a**, 280 mg in 40% yield. Mp: 198–200 °C, IR (KBr): 3470, 3365, 3064, 2938, 811, 751 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.87 (s, 6H), 3.91 (s, 3H), 6.32 (s, 2H), 7.20 (s, 2H), 7.84 (s, 1H), 8.10 (s, 1H), 8.20-8.27 (m, 3H), 8.35 (d, 2H, J = 8.10 Hz), 8.65 (s, 1H), 8.71 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.5, 61.8, 97.4, 117.5, 118.4, 129.6, 130.5, 131.7, 131.5, 133.7, 142.6, 144.7, 150.5, 151.6, 152.7, 157.8, 159.7, 160.3, 161.6; MS (ESI): 455 $[M+H]^+$; Anal. Calcd for $C_{25}H_{22}N_6O_3$: C, 66.07; H, 4.88; N, 18.49. Found: C, 65.93; H, 4.79; N, 18.63.

2.5. 4-(3,4,5-Trimethoxyphenyl)-6-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)pyrimidin-2-amine (11b)

This compound **11b** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 3,4,5-trimethoxybenzaldehyde (**10b)** (294 mg, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11b**, 289 mg in 35% yield. Mp: 209–211 °C, IR (KBr): 3455, 3349, 3089, 2941, 832, 765 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 3.87 (s, 6H), 3.89 (s, 6H), 3.91 (s, 3H), 3.94 (s, 3H), 6.32 (s, 2H), 7.20 (s, 2H), 7.30 (s, 2H), 7.83 (s, 1H), 8.10 (s, 1H), 8.64 (s, 1H), 8.71 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.5, 58.7, 61.7, 61.9, 102.5, 113.6, 117.6, 118.7, 129.4, 131.4, 133.5, 137.4, 144.5, 147.8, 150.7, 151.4, 153.6, 156.6, 157.5, 159.3, 162.5, 165.7; MS (ESI): 545 $[M+H]^+$; Anal. Calcd for $C_{28}H_{28}N_6O_6$: C, 61.76; H, 5.18; N, 15.43. Found: C, 61.61; H, 5.06; N, 15.57.

2.6. 4-(4-Methoxyphenyl)-6-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)py rimidin-2-amine (11c)

This compound **11c** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 4-methoxybenzaldehyde (**10c)** (0.18 ml, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11c**, 293 mg in 40% yield. Mp: 206–208 °C, IR (KBr): 3468, 3345, 3044, 2953, 804, 753 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 3.74 (s, 3H), 3.87 (s, 6H), 3.91 (s, 3H), 6.32 (s, 2H), 6.96 (d, 2H, J = 8.10 Hz), 7.20 (s, 2H), 7.83 (s, 1H), 8.10 (s, 1H), 8.19 (d, 2H, J = 8.10 Hz), 8.64 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.4, 57.9, 61.8, 97.4, 116.4, 117.4, 118.4, 129.3, 131.5, 132.6, 133.7, 144.5, 146.7, 150.4, 151.3, 152.6, 157.6, 158.5, 160.5, 161.6, 162.8; MS (ESI): 485 $[M+H]^+$; Anal. Calcd for $C_{26}H_{24}N_6O_4$: C, 64.45; H, 4.99; N, 17.35. Found: C, 64.60; H, 5.07; N, 17.48. Found: C, 64.47; H, 5.19; N, 17.34.

2.7. 4-(4-Chlorophenyl)-6-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)pyri midin-2-amine (11d)

This compound **11d** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 4-chlorobenzaldehyde (**10d)** (0.18 ml, 1.5 mmol), guanidine hydrochloride (**9**) (286, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11d**, 357 mg in 48% yield. Mp: 220–222 °C, IR (KBr): 3469, 3354, 3076, 2954, 814, 727, 619 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 3.88 (s, 6H), 3.91 (s, 3H), 6.33 (s, 2H), 7.20 (s, 2H), 7.65 (d, 2H, J = 8.14 Hz), 7.83 (s, 1H), 8.10 (s, 1H), 8.20 (d, 2H, J = 8.14 Hz), 8.65 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.5, 61.8, 97.4, 117.5, 118.5, 129.4, 131.4, 131.7, 132.5, 133.5, 134.8, 141.6, 144.7, 150.2, 151.3, 152.7, 157.5, 159.3, 161.4, 162.8; MS (ESI): 489 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}ClN_6O_3$: C, 61.41; H, 4.33; N, 17.19. Found: C, 61.55; H, 4.39; N, 17.32.

2.8. 4-(4-Bromophenyl)-6-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)pyri midin-2-amine (11e)

This compound **11e** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 4-bromobenzaldehyde (**10e)** (277 mg, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11e**, 406 mg in 50% yield. Mp: 226–228 °C, IR (KBr): 3487, 3362, 3076, 2954, 818, 754, 634 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 3.88 (s, 6H), 3.91 (s, 3H), 6.33 (s, 2H), 7.20 (s, 2H), 7.83 (s, 1H), 8.10 (s, 1H), 8.16 (d, 2H, J = 8.14 Hz), 8.21 (d, 2H, J = 8.14 Hz), 8.65 (s, 1H), 8.73 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.5, 61.4, 97.5, 117.4, 118.6, 124.5, 129.4, 131.5, 132.4, 133.5, 133.7, 142.3, 144.6, 150.4, 151.4, 152.6, 157.5, 159.5, 161.5, 162.9; MS (ESI): 534 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}BrN_6O_3$: C, 56.30; H, 3.97; N, 15.76. Found: C, 56.41; H, 3.91; N, 15.86.

2.9. 4-(4-Fluorophenyl)-6-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)pyri midin-2-amine (11f)

This compound **11f** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 4-fluorobenzaldehyde (**10f)** (0.16 mL, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11f**, 378 mg in 52% yield. Mp: 213–215 °C, IR (KBr): 3487, 3376, 3067, 2937, 1185, 812, 756 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 3.87 (s, 6H), 3.90 (s, 3H), 6.33 (s, 2H), 7.20 (s, 2H), 7.68 (d, 2H, J = 8.12 Hz), 7.83 (s, 1H), 8.10 (s, 1H), 8.20 (d, 2H, J = 8.12 Hz), 8.65 (s, 1H), 8.71 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.6, 61.8, 97.5, 117.6, 118.6, 118.9, 129.4, 131.4, 132.7, 132.7, 142.4, 144.5, 150.5, 151.4, 152.6, 157.6, 159.6, 160.4, 161.4, 162.8; MS (ESI): 473 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}FN_6O_3$: C, 63.55; H, 4.48; N, 17.79. Found: C, 63.70; H, 4.60; N, 17.70.

2.10. 4-(5-(3,4,5-Trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)-6-(4-nitrophenyl)pyri midin-2-amine (11g)

This compound **11g** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 4-nitrobenzaldehyde (**10g)** (226 mg, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11g**, 467 mg in 61% yield. Mp: 229–231 °C, IR (KBr): 3473, 3358, 3072, 2916, 1539, 814, 771 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 3.88 (s, 6H), 3.92 (s, 3H), 6.34 (s, 2H), 7.21 (s, 2H), 7.84 (s, 1H), 8.10 (s, 1H), 8.21 (d, 2H, J = 8.14 Hz), 8.25 (d, 2H, J = 8.14 Hz), 8.66 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.6, 61.8, 97.4, 117.5, 118.5, 125.5, 129.5, 131.5, 132.7, 133.6, 144.5, 144.9, 147.4, 150.4, 151.5, 152.8, 157.6, 159.5, 161.3, 162.9; MS (ESI): 500 $[M+H]^+$; Anal. Calcd for $C_{25}H_{21}N_7O_5$: C, 60.12; H, 4.24; N, 19.63. Found: C, 59.99; H, 4.36; N, 19.49.

2.11. 4-(2-Amino-6-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)pyrimidin-4-yl)benzonitrile (11h)

This compound **11h** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 4-cyanobenzaldehyde (**10h)** (197 mg, 3 mmol), guanidine hydrochloride (**5**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11h**, 510 mg in 70% yield. Mp: 234–236 °C, IR (KBr): 3487, 3364, 3076, 2937, 2248, 812, 763 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 3.88 (s, 6H), 3.92 (s, 3H), 6.34 (s, 2H), 7.21 (s, 2H), 7.84 (s, 1H), 8.11 (s, 1H), 8.22 (d, 2H, J = 8.15 Hz), 8.26 (d, 2H, J = 8.15 Hz), 8.66 (s, 1H), 8.72 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.5, 61.8, 97.4, 111.5, 117.5, 118.4, 120.4, 129.4, 130.4, 131.5, 133.4, 133.8, 142.4, 144.5, 150.5, 151.4, 152.4, 157.6, 159.5, 161.5, 162.9; MS (ESI): 480 $[M+H]^+$; Anal. Calcd for $C_{26}H_{21}N_7O_3$: C, 65.13; H, 4.41; N, 20.45. Found: C, 65.00; H, 4.52; N, 20.58.

2.12. 4-(5-(3,4,5-Trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)-6-p-tolylpyrimidin-2-amine (11i)

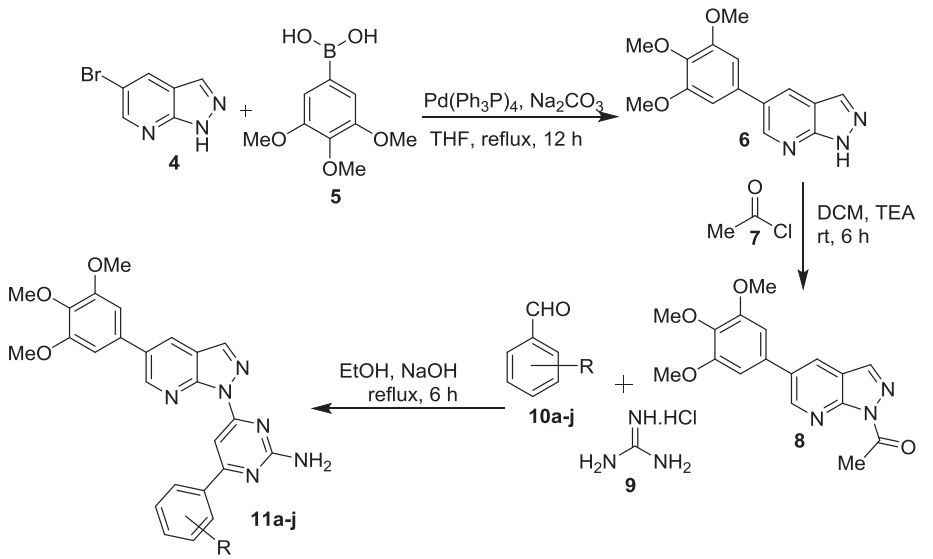
This compound **11i** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 4-methylbenzaldehyde (**10i)** (0.18 ml, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11i**, 345 mg in 48% yield. Mp: 204–206 °C, IR (KBr): 3476, 3365, 3063, 2932, 810, 773 cm^{-1}; ^1H NMR (400 MHz, DMSO- d_6): δ 2.67 (s, 3H), 3.87 (s, 6H), 3.90 (s, 3H), 6.32 (s, 2H), 7.20 (s, 2H), 7.45 (d, 2H, J = 8.10 Hz), 7.83 (s, 1H), 8.10 (s, 1H), 8.18 (d, 2H, J = 8.10 Hz), 8.64 (s, 1H), 8.71 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 23.5, 57.5, 61.8, 97.5, 117.6, 118.4, 127.4, 129.5, 131.4, 132.6, 133.6, 137.6, 144.3, 145.6, 150.5, 151.8, 152.8, 157.5, 159.7, 161.5, 162.9; MS (ESI): 469 $[M+H]^+$; Anal. Calcd for $C_{26}H_{24}N_6O_3$: C, 66.65; H, 5.16; N, 17.94. Found: C, 66.51; H, 5.27; N, 18.07.

2.13. 4-(3,5-Dimethoxyphenyl)-6-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)pyrimidin-2-amine (11j)

This compound **11j** was prepared following the method described for the preparation of the compound **11a**, employing **8** (500 mg, 1.5 mmol) with 3,5-dimethoxybenzaldehyde (**10j)** (249 mg, 1.5 mmol), guanidine hydrochloride (**9**) (286 mg, 3 mmol) and NaOH (2 mL) to afford yellow color solid **11j**, 355 mg in 45% yield. Mp: 211–213 °C, IR (KBr): 3458, 3373, 3076, 2919, 831, 746 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ 3.78 (s, 6H), 3.87 (s, 6H), 3.90 (s, 3H), 6.32 (s, 2H), 6.78 (s, 1H), 7.09 (s, 2H), 7.20 (s, 2H), 7.83 (s, 1H), 8.10 (s, 1H), 8.63 (s, 1H), 8.70 (s, 1H); ^{13}C NMR (75 MHz, DMSO- d_6): δ 57.5, 58.7, 61.8, 102.4, 109.5, 117.5, 118.5, 120.5, 129.5, 131.4, 133.6, 134.6, 144.5, 150.5, 151.4, 152.5, 157.5, 159.4, 161.5, 162.8, 163.8; MS (ESI): 515 $[M+H]^+$; Anal. Calcd for $C_{27}H_{26}N_6O_5$: C, 63.03; H, 5.09; N, 16.33. Found: C, 63.19; H, 4.99; N, 16.20.

2.14. MTT assay

The cytotoxic activity of the compounds was determined using MTT assay. 1×10^4 cells/well were seeded in 200 ml DMEM, supplemented with 10% FBS in each well of 96-well microculture plates and incubated for 24 hours at 37 °C in a CO_2 incubator. Compounds, diluted to the desired concentrations in culture medium, were added to the wells with respective vehicle control. After 48 hours of incubation, 10 ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (5 mg/ml) was added to each well and the plates were further incubated for 4 hours. Then the supernatant from each well was carefully removed, formazon crystals were dissolved in 100 ml of DMSO and absorbance at 540 nm wavelength was recorded.



Where, R = H (10a, 11a), 3,4,5-trimethoxy (10b, 11b), 4-methoxy (10c, 11c), 4-chloro (10d, 11d), 4-bromo (10e, 11e), 4-fluoro (10f, 11f), 4-nitro (10g, 11g), 4-cyano (10h, 11h), 4-methyl (10i, 11i), 3,5-dimethoxy (10j, 11j)

Scheme 1. Synthesis of 2-Aminopyrimidine linked 7-Azainadzole derivatives. Where, R = H (10a, 11a), 3,4,5-trimethoxy (10b, 11b), 4-methoxy (10c, 11c), 4-chloro (10d, 11d), 4-bromo (10e, 11e), 4-fluoro (10f, 11f), 4-nitro (10g, 11g), 4-cyano (10h, 11h), 4-methyl (10i, 11i), 3,5-dimethoxy (10j, 11j).

Table 1
In vitro anticancer activity of compounds (**11a-j**) in IC_{50} µM.

Compound	MCF-7	A549	MDA MB-231
11a	3.78	-	-
11b	1.89	1.44	1.23
11c	12.8	9.33	-
11d	24.7	8.23	7.30
11e	13.6	-	-
11f	15.2	17.2	-
11g	0.34	0.11	2.33
11h	1.02	0.19	1.90
11i	4.67	34.2	-
11j	2.67	1.56	1.45
Adriamycin	3.12	2.10	3.41

3. Results and discussion

3.1. Chemistry

Synthesis of newly compounds (**11a-j**) was outlined and showed in Scheme 1. Starting compound 5-bromo-1H-pyrazolo[3,4-b]pyridine (**4**) was undergo Suzuki coupling reaction with 3,4,5-trimethoxyphenylboronic acid (**5**) in THF, $Pd(Ph_3P)_4$, Na_2CO_3 at reflux for 12 hours to afford pure 5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridine (**6**) in good yield. Then this intermediate **6** was acylated with acetyl chloride (**7**) in CH_2Cl_2, triethyl amine at room temperature for 6 hours to gave 1-(5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-1-yl)ethanone (**8**). After intermediate **8** was cyclized with guanidine hydrochloride (**9**) and substituted aromatic aldehydes (**10a-j**) in ethanol, NaOH at reflux for 6 hours to gave pure target compounds (**11a-j**).

3.2. Biological evaluation

3.2.1. In vitro *anticancer activity*

These newly compounds (**11a-j**) were tested their anticancer activity against different human cancer cell lines such as (MCF-7, A549 and MDA MB-231) by MTT assay and results are summarized in Table 1, adriamycin used as a positive control. The compound **11g** showed potent anticancer activity against MCF-7, A549, MDA-MB-231 cell lines with IC_{50} values of 0.34

μM, 0.11 μM and 2.33 μM respectively. The compound **11h** exhibited promising anticancer activities against MCF-7, A549, MDA-MB-231 cell lines with IC_{50} values of 1.02 μM, 0.19 μM and 1.90 μM respectively. The compound **11b** showed better anticancer activities against MCF-7, A549, MDA-MB-231 cell lines with IC_{50} values of 1.89 μM, 1.44 μM and 1.23 μM. While, another compound **11b** exhibited selective activity against MCF-7 cell line with IC_{50} values of 3.78 μM. The another compound **11j** showed comparable anticancer activities with standard drug against MCF-7, A549, MDA-MB-231 cell lines with IC_{50} values of 2.67 μM, 1.56 μM and 1.45 μM respectively. The remaining compounds **11c, 11d, 11e** and **11f** showed less activities against specified cell lines.

4. Conclusion

In conclusion, we have synthesized a series of pyrimidine linked azaindazole (**11a-j**) derivatives. All these new derivatives were evaluated for their anticancer activity against three human cancer cell lines including MCF-7, MDA MB-231 (Lung cancer) and A549 (Breast cancer). Here adriamycin used as positive control. Among them, compounds **11b, 11g, 11h** and **11j** were exhibited more potent activity than control drug. In future, the most active compounds **11g** and **11h** may allow for *in vivo* and clinical studies.

Declaration of Competing Interest

It is informed to your kind notice that We submit our original research article entitled .Synthesis and biological evaluation of 2-Aminopyrimidine linked 7-Azainadzole derivatives as anticancer agents" for your Journal. This article having no conflict of interest.

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