Journal of Applied Pharmaceutical Science Vol. 10(06), pp 006-011, June, 2020
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2020.10602
ISSN 2231-3354

Synthesis and biological evaluation of novel substituted pyrrolo[2,3-d]pyrimidines as anticancer agents

Sandip P. Dholakia[1*], Kaushik B. Kanada[2], Ghanshyam R. Parmar[3]

[1]Department of Chemistry, Shankersinh Vaghela Bapu Institute of Pharmacy, Gandhinagar, Gujarat, India.
[2]Department of Chemistry, Saraswati Institute of Pharmaceutical Education & Research, Gandhinagar, Gujarat, India.
[3]Department of Pharmacy, Sumandeep Vidyapeeth, Vadodara, Gujarat, India.

ARTICLE INFO

Received on: 22/11/2019
Accepted on: 15/04/2020
Available online: 05/06/2020

Key words:
Doxorubicin, pyrrolo[2,3-*d*] pyrimidine, anticancer, breast cancer.

ABSTRACT

Pyrrolopyrimidines are well known scaffold, which play a critical role as anticancer agents, so it thought of interest to synthesize a series of novel substituted pyrrolo[2,3-*d*]pyrimidines having diverse groups at position C4 and N7 of the pyrrolo[2,3-*d*]pyrimidine core and performed *in vitro* screening against **MDA-MB-468 (breast cancer cell line)** cell line. The details of the synthetic methods and characterization data of the synthesized compounds have been presented in this study. Compounds **8a, 8h, 8j, 9h, 9i, 9j, 9m, 9n, and 9o** showed the excellent anticancer activity compared to standard doxorubicin with an IC_{50} value of 6.17 µM/ml against **MDA-MB-468 (breast cancer cell line),** which was non-toxic to normal *vero* cell line.

INTRODUCTION

Cancer is characterized by the uncontrolled growth of abnormal cells (Grange *et al*., 2002; Kari and Dave, 2012). The abnormal growth can also be referred to as a neoplasm. Neoplasms can be benign (noncancerous) or malignant (cancerous). Cancerous growths can occur in any organ of the body and are characterized by three distinct properties: the cells replicate rapidly with reduced growth control, the cells lose contact inhibition *in vitro*, and the resulting neoplasm invades surrounding tissues and may spread to other parts of the body (Foye *et al*., 1995; Kleinsmith, 2006). The cells of benign tumors have reduced growth control but do not invade surrounding tissues or spread to other parts of the body. Other important aspects of cancer cells include the ability to be self-sufficient and generate local angiogenesis while resisting antigrowth and apoptosis signals. If the spread of a malignant neoplasm is not controlled, it can result in death (Hadfield *et al*., 2003; Honore *et al*., 2005; Jordan *et al*., 1998; Whitman *et al*., 1995).

Most breast cancers begin either in the breast tissue made up of glands for milk production, called lobules, or in the ducts that connect the lobules to the nipple. The remainder of the breast is made up of fatty, connective, and lymphatic tissues (Edge *et al*., 2010).

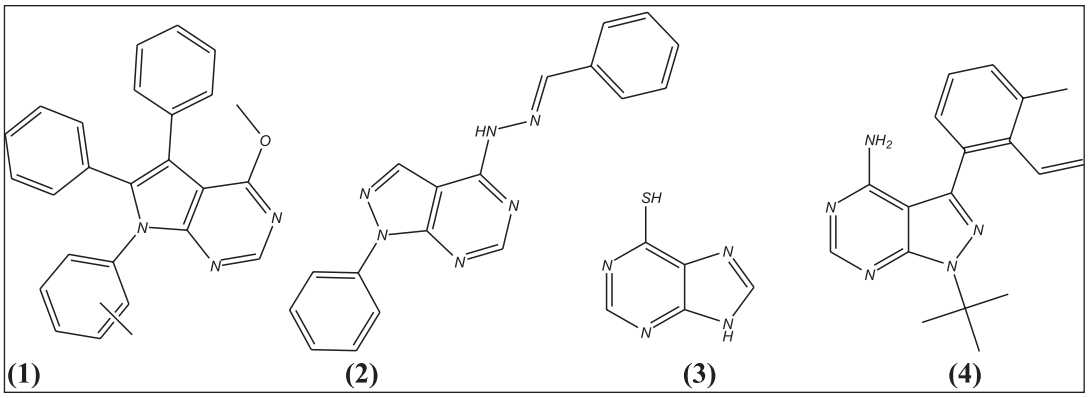
In 2016, an estimated 252,710 new cases of invasive breast cancer will be diagnosed among women and 2,470 cases will be diagnosed in men. Besides, 63,410 cases of *in situ* breast carcinoma will be diagnosed among women. Approximately 40,610 women and 460 men are expected to die from breast cancer in 2016 (Miller *et al*., 2016).

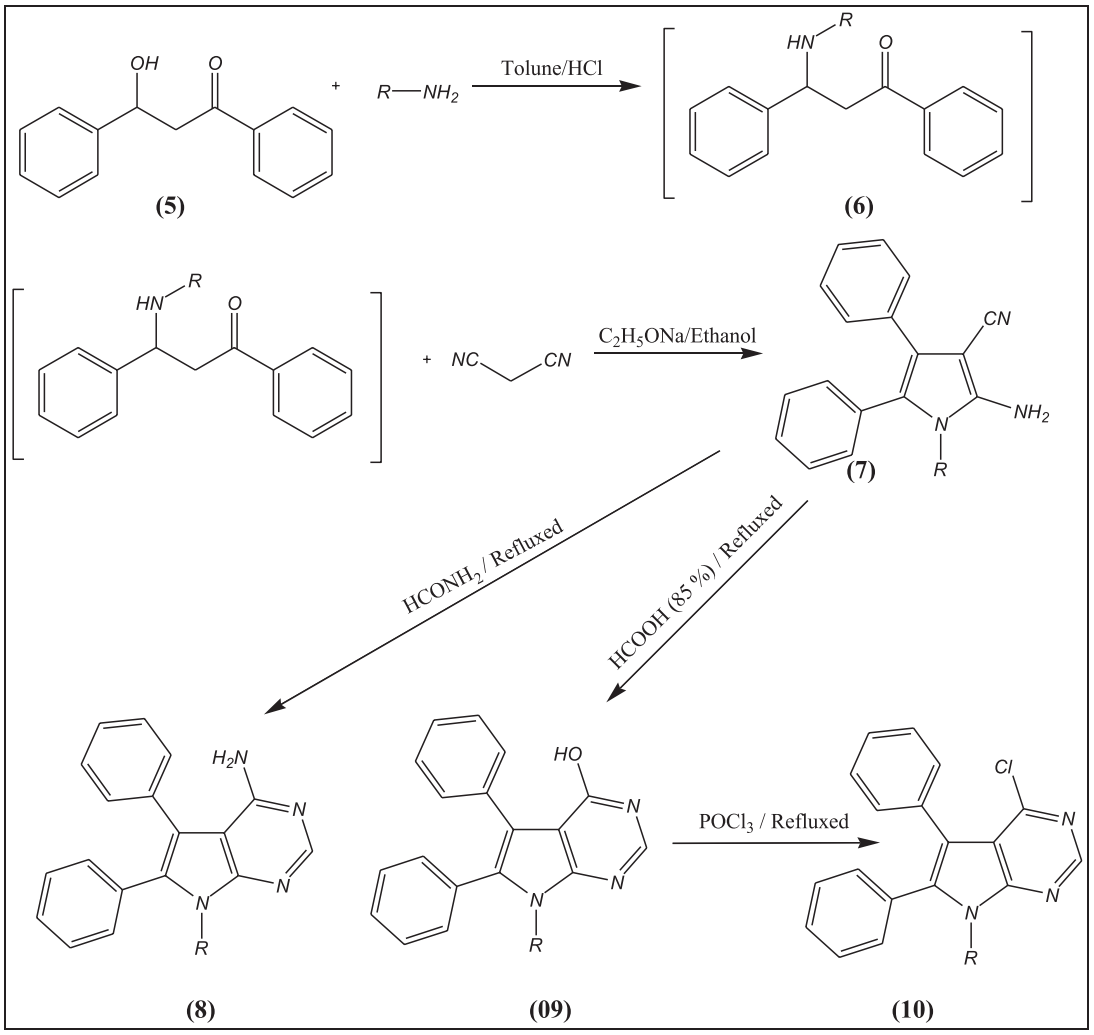
An increasing interest in the biological studies of pyrrolo[2,3-*d*]pyrimidine in the past decade is a consequence of their wide usage as a pharmaceutically important class of compounds. Pyrrolo[2,3-*d*]pyrimidine derivatives have a considerable potential in the field of chemotherapy as they were found to exhibit their antitumor activity by inhibiting different types of enzymes such as cyclin-dependent kinase, *Src* and *Abl* tyrosine kinase, glycogen synthase kinase-3, adenosine deaminase, and epidermal growth factor receptor protein tyrosine kinase (Ghorab *et al*., 2010). The derivatives of pyrrolo[2,3-*d*]

*Corresponding Author
Sandip P. Dholakia, Department of Chemistry, Shankersinh Vaghela Bapu Institute of Pharmacy, Gandhinagar, Gujarat.
E-mail: sandip.chemistry2008 @ gmail.com

pyrimidine have already been discovered as antitumor agents by the NCI (National Cancer Institute, Bethesda, MD) on HCT116 and other cell lines. Some pyrrolo[2,3-*d*]pyrimidines**(1)** structurally related with allopurinol **(2)**, 6-mercaptopurine **(3),** and 1- (1, 1-dimethylethyl)- 3- (1-naphthalenyl)-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-amine (1-NA-PP1) **(4)** have also been reported as potent inhibitors for the growth of several human tumor cell lines (Gangjee *et al*., 2010).

In view of these observations, we undertook the synthesis of three series of novel pyrrolo[2,3-*d*]pyrimidine analogs (Scheme 1) and *in vitro* screening on breast cancer cell line (**8–10**).





Scheme 1. Synthesis of pyrrolo[2,3-*d*]pyrimidine analogs.

MATERIALS AND METHODS

Melting points of all the chemical compounds and solvents were determined in open capillaries and are uncorrected (Table 1). The IR spectra of all compounds were recorded in FT-IR 8400S Shimadzu spectrophotometer using KBr. Mass spectra were obtained using the 2010EV LCMS Shimadzu instrument at 70 eV. ^{1}H NMR spectra were obtained in DMSO on BRUKER Avance-II 400 MHZ instrument, and the chemical shift was measured as parts per million downfield from tetramethylsilane as an internal standard. The target compounds were synthesized as outlined in Scheme 1.

General method for the synthesis of 2-amino-4,5-diphenyl-1-(substituted)-1H-pyrrole-3-carbonitriles (7a–7o)

A mixture of benzoin (2 g, 0.01 mol), substituted amines (0.01 mol), and concentrated HCl (6–8 drops) in toluene (50 ml) was heated under reflux for 6 hours and cooled. The reaction mixture was filtered, and the resulting residue was dissolved in 30 ml of absolute alcohol. A malononitrile (0.66 mg, 0.01 mol) was added to the previous solution followed by sodium ethoxide (2 g sodium metal in 20 ml of absolute alcohol) as a catalyst. The mixture was refluxed until a solid was formed. The solvent was evaporated under reduced pressure, and the residue was recrystallized from methanol to give the pure yellow crystalline product (Dholakia *et al*., 2013).

General method for the synthesis of 5,6-diphenyl-7-substituted-7H-pyrrolo[2,3-d]pyrimidin-4-yl-amines (8a–8o)

A mixture of each of substituted aminopyrroles [**7a–7o**] (0.01 mol) and formamide (30 ml, 0.066 mol) was heated under reflux for 6 hours, cooled, and poured into crush ice to give precipitates which were filtered, dried, and recrystallized from ethanol to yield a yellow crystalline pure product.

General method for the synthesis of 5,6-diphenyl-7-substituted-7H-pyrrolo[2,3-d]pyrimidin-4-ols (09a–09o)

A mixture of the substituted aminopyrrole [**7a–7o**] (0.01 mol) and formic acid (20 ml, 85%) was heated under reflux for 3 hours, cooled, and poured onto crush ice to give precipitates which were filtered, dried, and recrystallized from ethanol to yield a yellow crystalline pure product.

General method for the synthesis of 4-chloro-5,6-diphenyl-7-substituted-7H-pyrrolo[2,3-d]pyrimidines (10a, 10c, 10f, 10m, 10n, and 10o)

An appropriate pyrrolopyrimid-4-ol (**09**, 0.01 mol) in phosphorus oxychloride (30 ml) was heated under reflux for 4 hours, cooled, and poured onto crush ice to give the precipitates which were filtered, dried, and recrystallized from ethanol to yield a yellow crystalline pure product (Gangjee *et al*., 2010).

General method for cell line study (MTT assay)

It is a laboratory test and a standard colorimetric assay for measuring cellular growth. It can also be used to determine the cytotoxicity of potential medicinal agents and other toxic materials.

This assay is a sensitive, quantitative, and reliable colorimetric assay that measures viability, proliferation, and activation of cells. The assay is based on the capacity of mitochondrial dehydrogenase enzymes in living cells to convert

Table 1. Physical properties of 2-amino-4,5-diphenyl-1-(substituted)-1*H*-pyrrole-3-carbonitriles (7a–7o), 5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl-amines (8a–8o), 5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ols (9a–9o), and 4-chloro-5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidines (10a, 10d, 10f, 10m, 10n, and 10o).

Comp. code	Melting point (°C)	Comp. code	Melting point (°C)	Comp. code	Melting point (°C)
7a	170–172	**8c**	235–237	**9e**	233–235
7b	228–230	**8d**	230–232	**9f**	210–212
7c	136–138	**8e**	226–228	**9g**	>300
7d	170–172	**8f**	260–262	**9h**	211–211
7e	174–177	**8g**	>300	**9i**	260–262
7f	164–166	**8h**	273–275	**9j**	>300
7g	123–125	**8i**	235–237	**9k**	240–242
7h	145–147	**8j**	>300	**9l**	269–271
7i	160–122	**8k**	231–333	**9m**	280–282
7j	182–184	**8l**	232–234	**9n**	>300
7k	181–183	**8m**	245–247	**9o**	288–290
7l	160–162	**8n**	>300	**10a**	>300
7m	136–138	**8o**	>300	**10d**	290–292
7n	210–112	**9a**	199–201	**10f**	280–282
7o	200–202	**9b**	213–215	**10m**	>300
8a	262–264	**9c**	180–182	**10n**	>300
8b	218–220	**9d**	190–192	**10o**	291–293

the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in the range of cell lines (Bruce, 1990; Canavan and Doshi, 2000).

2-amino-1,4,5-triphenyl-1H-pyrrole-3-carbonitrile (7a)

Yellow crystalline solid, yield 80%, mass (m/e) 336.15 (M + 1), IR (cm^{-1}) CN—2,201, NH_2—3,385 1H, NMR (δ ppm, DMSO-d6) 5.75 (br-s, 2H, NH_2), 6.93–7.38 (m, 15H, Ar-H).

2-amino-4,5-diphenyl-1-p-tolyl-1H-pyrrole-3-carbonitrile (7d)

Yellow crystalline solid, yield 60%, mass (m/e) 350.16 (M + 1), IR (cm^{-1}) CN—2,207, NH_2—3,322 1H, NMR (δ ppm, DMSO-d6) 2.29 (s, 3H, CH_3), 5.70 (br-s, 2H, NH_2), 6.93–7.26 (m, 14H, Ar-H).

5,6,7-triphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (8a)

Yellow crystalline solid, yield 40%, mass (m/e) 363.15 (M + 1), IR (cm^{-1}) NH_2—3,463 1H, NMR (δ ppm, DMSO-d6) 8.30 (s, 1H, C-2H), 7.06 (br-s, 2H, NH_2), 7.15–7.52 (m, 15H, Ar-H).

5,6-diphenyl-7-m-tolyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (8c)

Yellow crystalline solid, yield 60%, mass (m/e) 378.00 (M + 1) IR (cm^{-1}) NH_2—3,488 1H, NMR (δ ppm, DMSO-d6) 2.36 (s, 3H, CH_3), 8.33 (s, 1H, C-2H), 5.02 (br-s, 2H, NH_2, D_2O Exchangeable), 6.98–7.40 (m, 14H, Ar-H).

7-(2-chlorophenyl)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (8k)

Yellow crystalline solid, yield 30%, mass (m/e) 397.12 (M + 1), 399.11 M + 2 IR (cm^{-1}) NH2—3,465 1H, NMR (δ ppm, DMSO-d6) 8.33 (s, 1H, C-2H), 6.96–7.38 (m, 14H, Ar-H), 5.11 (br-s, 2H, NH_2, D_2O Exchangeable).

5,6,7-triphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ol (9a)

Yellow crystalline solid, yield 40%, mass (m/e) 364.00 (M + 1), IR (cm^{-1}) OH—3,241 1H, NMR (δ ppm, DMSO-d6) 8.20 (s, 1H, C-2H), 10.20 (s, 1H, C-OH), 6.92–7.38 (m, 15H, Ar-H).

5,6-diphenyl-7-m-tolyl-7H-pyrrolo[2,3-d]pyrimidin-4-ol (09c)

Yellow crystalline solid, yield 60%, mass (m/e) 379.0 (M + 1), IR (cm^{-1}) OH—3,234 1H, NMR (δ ppm, DMSO-d6) 2.34 (s, 3H, CH_3), 8.21 (s, 1H, C-2H), 8.60 (s, 1H, C-OH), 6.92–7.37 (m, 14H, Ar-H).

7-(2-chlorophenyl)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ol (9k)

Yellow crystalline solid, yield 30%, mass (m/e) 398.10 (M + 1), 400.10 M + 2 IR (cm^{-1}) OH—3,308 1H, NMR (δ ppm, DMSO-d6) 7.80 (s, 1H, C-2H), 12.25 (s, 1H, C-OH, D_2O exchangeable), 6.90–7.38 (m, 14H, Ar-H).

4-chloro-5,6,7-triphenyl-7H-pyrrolo[2,3-d]pyrimidine (10a)

Yellow crystalline solid, yield 50%, mass (m/e) 382.11 (M + 1), 384.11 (M + 2), IR (cm^{-1}) Cl—695 1H, NMR (δ ppm, DMSO-d6) 6.91–7.20 (m,15H, Ar-H), 7.90 (s, 1H, C-2H).

4-chloro-5,6-diphenyl-7-p-tolyl-7H-pyrrolo[2,3-d]pyrimidine (10d)

Yellow crystalline solid, yield 60%, mass (m/e) 396.11 (M + 1), 398.11 (M + 2), IR (cm^{-1}) Cl—697 1H, NMR (δ ppm, DMSO-d6) 2.51 (s, 3H, CH_3), 7.18–7.84 (m,14H, Ar-H) 8.60 (s, 1H, C-2H)

RESULTS AND DISCUSSION

We synthesized 5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl-amines **(8a–8o),** 5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ols **(9a–9o),** and 4-chloro-5,6-diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*] pyrimidines **(10a, 10c, 10f, 10m, 10n, and 10o)** as depicted in Scheme 1. 2-amino-4,5-diphenyl-1-(substituted)-1*H*-pyrrole-3-carbonitriles **(7a–7o)** were prepared from the reaction of benzoin with respective amines, followed by the treatment of *in situ* generated initially formed intermediate **6** with malononitrile in the presence of sodium ethoxide. 5,6-Diphenyl-7-substituted-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl-amines **(8a–8o)** were prepared by refluxing 2-amino-4,5-diphenyl-1-(substituted)-1*H*-pyrrole-3-carbonitrile **(7a–7o)** with formamide. Another series of compounds containing 4-hydroxy group was prepared by refluxing (**7a–7o)** with formic acid. Some of these 4-hydroxy derivatives (**09a, 09c, 09f, 09m, 09n,** and **09o)** were converted to the corresponding chloro derivatives **10a, 10c, 10f, 10m, 10n, and 10o**, respectively, by refluxing with phosphorus oxychloride.

In the present investigation, all the compounds were evaluated against cell lines named MDA-MB 468 (Breast cancer cell line) and *vero* cell line (normal cell line) for each tested compound as well as standard doxorubicin, and the dose-response curve (DRC) against MDA-MB 468 (Breast cancer cell line) was plotted with 10 analysis point, i.e., with 10 different drug concentrations. The concentration causing 50% cell growth inhibition (IC_{50}) was determined from DRC using GraphPad prism software (Ver. 5.04) (GraphPad Software, Inc., La Jolla, CA) and Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA) application (Table 2).

MDA-MB-468 (breast cancer cell line)—Among all the tested compounds, **8a, 8g, 8h, 8i, 8j, 8k, 8h, 9i, 9j, 9k, 9m, 9n, 9o,** and **10f** showed the highest potential effect on **MDA-MB 468** cell line compared to standard doxorubicin IC50 value. Compounds **8b, 8e, 8l, 8m, 8n, 8o, 9a, 9d, 9e, 9f, 9g, 9l, 10a,** and **10d** possessed a good anticancer/cytotoxicity activity. Compounds **8c, 8d, 8f, 9b, 9c, 10m, 10n,** and **10o** did not show any activity as their IC_{50} values are higher than 50 **µM/ml**. All compounds were also checked for their normal cell line activity with *vero* cell line. Compounds **8a, 8b, 8d, 8e, 8h, 8j, 8l, 8m, 8o, 9a, 9c, 9d, 9e, 9f, 9h, 9i, 9j, 9m, 9n, 9o,** and **10d** were found to be non-toxic to *vero* normal cell line. Compounds **8a, 8h, 8j, 9h, 9i, 9j, 9m, 9n,** and **9o** showed a higher anticancer activity than the standard doxorubicin IC_{50} value on **MDA-MB 468** (breast cancer cell line) cell lines with non-toxic to *vero* normal cell line (Fig. 1).

Table 2. Cell line study of pyrrolo[2,3-*d*]pyrimidine analogs.

Compounds	MDA-MB-468 cell line study		Vero cell line study (normal cell line)	
	IC_{50} (µM/ml)	R^2	IC_{50} (µM/ml)	R^2
8a	2.72	0.9728	254.4	0.9862
8b	18.09	0.9948	631	0.9891
8c	47.2	0.9967	19.04	0.9891
8d	28.45	0.9925	>100	0.9595
8e	14.65	0.9754	>1,000	0.992
8f	37.11	0.9653	15.72	0.9765
8g	06.57	0.9570	93.32	0.9957
8h	2.17	0.9759	>100	0.9738
8i	3.37	0.9839	8.03	0.9767
8j	2.12	0.9909	143.8	0.9778
8k	5.4	0.9750	20.73	0.9726
8l	12.94	0.9628	144.92	0.9869
8m	13.11	0.9834	162	0.9946
8n	12.15	0.9668	133	0.9933
8o	23.69	0.9519	>1,000	0.9823
9a	10.27	0.9878	209.1	0.9986
9b	100.8	0.9892	5.74	0.9905
9c	59	0.9604	>100	0.9764
9d	9.51	0.9987	144.9	0.9978
9e	23.05	0.9884	>100	0.9553
9f	21.11	0.9842	487.4	0.9822
9g	11.55	0.9693	6.9	0.9606
9h	7.24	0.9564	>1,000	0.9955
9i	5.79	0.9449	299	0.8944
9j	4.23	0.9762	>100	0.9988
9k	4.93	0.9556	79.71	0.9902
9l	11.79	0.9535	17.06	0.9783
9m	2.05	0.9897	>100	0.9798
9n	2.11	0.9838	410.4	0.9923
9o	4.2	0.9617	257.5	0.9948
10a	9.66	0.9899	>100	0.9645
10c	25.39	0.9891	94.89	0.9908
10f	4.62	0.9692	23.84	0.9625
10m	247.9	0.9741	57.06	0.9911
10n	89.04	0.9955	69.66	0.9755
10o	100	0.9736	24.43	0.9044
Std.	6.17	0.9229	314.6	0.9921

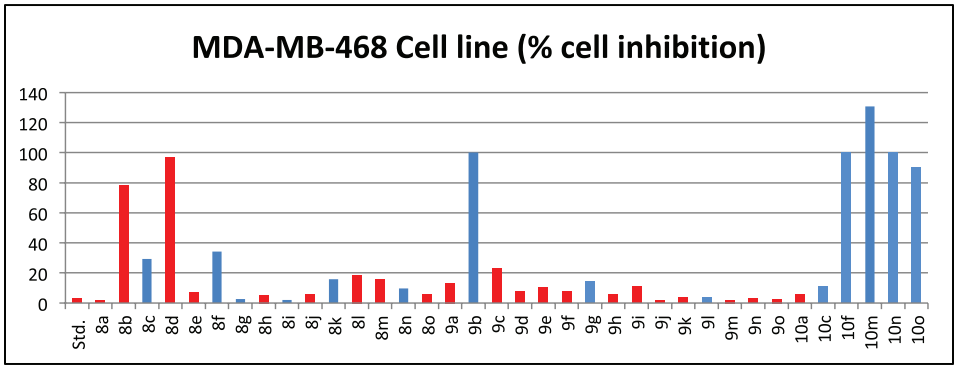


Figure 1. Graphical representation of cell line activity of pyrrolo[2,3-*d*]pyrimidine analogs.
(Blue line indicates that the compounds are toxic to vero normal cell line, and the red line indicates that the compounds are non-toxic to vero normal cell line) against standard doxorubicin.

CONCLUSION

From this cell line study, it can be concluded that compounds series with electron-withdrawing groups on substituent at N7 and electron-donating groups on substituent at C4 give the excellent cytotoxic activity. The compounds with electron-donating or no groups on substituent at N7 and electron-donating groups on substituent at C4 give a good cytotoxic activity, whereas compounds containing electron-withdrawing groups on substituent at N7 and C4 do not give the cytotoxic activity on MDA-MB 468 (breast cancer cell line).

CONFLICT OF INTEREST

Authors declare that they do not have any conflicts of interest.

FUNDING

None.

REFERENCES

Bruce AC. In defense of cell line screening. J Natl Cancer Inst , 1990; 82(13):1107–12.

Canavan TP, Doshi NR. Cervical cancer. Am Fam Phys, 2000; 61(5):1369–76.

Dholakia SP, Patel SA. Synthesis and antimicrobial screening of novel 2-amino-4,5-Diphenyl-1-(Substituted)-1*H*-Pyrrole-3-Carbonitrile. Am J PharmTech Res, 2013; 3(5):447–52.

Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FG, Trotti A. AJCC cancer staging manual. Springer Publisher, New York, NY, 2010.

Foye WO, Lemke TL, William DA. Principles of medicinal chemistry USA. Lippincott Williams & Wilkins Publisher, Philadelphia, PA, 1995.

Gangjee A, Kurup S, Ihnat MA, Thorpe JE, Shenoy SS. Synthesis and biological activity of N^4-phenylsubstituted-6-(2,4-dichlorophenylmethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamines as vascular endothelial growth factor receptor-2 inhibitors and antiangiogenic and antitumor agents. Bioorg Med Chem, 2010; 18(10):3575–87.

Ghorab MM, Ragab FA, Alqasoumi SI, Alafeefy AM, Aboulmagd SA. Synthesis of some new Pyrrolo[2,3-*d*]pyrimidine and pyrazolo[3,4-*d*] pyrimidine derivatives of expected anticancer and radioprotective activity. Eur J Med Chem, 2010; 45:171–8.

Grange JM, Stanford JL, Stanford CA, Morgan CD. Observations on cancer and their relevance today. J R Soc Med, 2002; 6**:**296–302.

Hadfield JA, Ducki S, Hirst N, McGown AT. Tubulin and microtubules as targets for anticancer drugs. Prog Cell Cycle Res, 2003; 5:309–25.

Honore, S, Pasquier E, Braguer D. Understanding microtubule dynamics for improved cancer therapy. Cell Mol Life Sci, 2005; 62:3039–56.

Jordan A, Hadfield JA, Lawrence NJ, McGown AT. Tubulin as a target for anticancer drugs: agents which interact with the mitotic spindle. Med Res Rev, 1998; 18:259–96.

Kari N, Dave W. Rediscovering biology: molecular to global perspectives. Portland Publisher, London, UK, 2012.

Kleinsmith LJ. Principles of cancer biology. Pearson International Publisher, New York, NY, 2006.

Miller KD, Siegel RL, Lin CC. Cancer treatment and survivorship statistics. Cancer J Clin, 2016; 66:271–89.

Whitman W, Coleman D, Wiebe W. Prokaryotes: the unseen majority. Proc Natl Acad Sci, 1995; 12:6578–83.

How to cite this article:
Dholakia SP, Kanada KB, Parmar GR. Synthesis and biological evaluation of novel substituted pyrrolo[2,3-d] pyrimidines as anticancer agents. J Adv Vet Anim Res 2020; 10(06):006–011.