Benzothiazine based acetohydrazides and acetamides as anticancer agents

Sana Aslam[1], Fang Wang[2], Matloob Ahmad[3]*, Ameer Fawad Zahoor[3], Asim Mansha[3], Azhar Rasul[4] and Liwu Fu[2]*

[1]Department of Chemistry, Government College Women University, Faisalabad, Pakistan
[2]State Key Laboratory of Oncology in South China, Cancer Center, Sun Yat-Sen University, Guangzhou, China
[3]Department of Chemistry, Government College University, Faisalabad, Pakistan
[4]Department of Zoology, Government College University, Faisalabad, Pakistan

Abstract: Four series of pyrazolobenzothiazine derivatives were evaluated for their anticancer activity against six different cancer cell lines *i.e.,* KB (human oral carcinoma cells), MCF-7(human breast carcinoma cells), A549 (human alveolar adenocarcinoma cells), Hep-G2 (liver carcinoma cells), SGC-7901(human gastric carcinoma cells) and S1 (human colon carcinoma cells) using MTT assay. Among eighteen compounds tested, six compounds *i.e.,* 1a, 1b, 1d, 4a, 4d and 4e were more active than 5-florouracil against human oral carcinoma cells (KB). Moreover, compounds 2b and 2c showed activity comparable to 5-FU against KB cell line. In addition, eight compounds were non-toxic to human PBM cells and thus exhibit selective anticancer activity.

Keywords: Pyrazolobenzothiazine ring system, 1,2-Benzothiazine 1,1-dioxide, anticancer activity.

INTRODUCTION

Cancer is a continued threat for humanity on the globe both in advanced countries as well as in developing ones. The burden is expected to grow more in less developed countries due to various factors, like poor diet, smoking and poor medical facilities. According to the estimates, 4.3 million new cancer cases were diagnosed and 2.9 million people died of cancer in China in 2018 (Feng *et al*., 2019). On the other hand, American Cancer Society estimated that 1,762,450 new cancer cases will be registered in united states alone and 606,880 cancer deaths are expected to occur in the country in 2019 (Siegel *et al*., 2019). The situation is expected to be more severe in low income countries. These facts indicate that there is a continued need for the development of more effective anticancer drugs.

Thiazine family is among the most significant bioactive heterocycles. This ring plays a vital role in a number of anti-inflammatory and analgesic drugs, such as, meloxicam(Bekker *et al*., 2018), piroxicam (Akogwu *et al*., 2017) etc. Moreover, it has exhibited excellent ability as template for a wide range of bioactive derivatives possessing activities, for example, antimicrobial(Patel *et al*., 2016), anti-viral (Cannalire *et al*., 2018) and as enzyme inhibitors(Ahmad *et al*., 2019). Various 3,6-diazaphenothiazines were recently reported for their anticancer activities against glioblastoma SNB-19 with efficacy even ten times better than cisplatin(Morak-Młodawska *et al*., 2019). 1,4-Benzothiazine-2-carbohydrazide derivatives have been observed as moderately active against HT-29 human colorectal cancer cell line (Rai *et al*., 2017). A series of 1,6-diazaphenothiazines has shown high effectivity against MCF-7 cell line and some of the compounds appeared to be more effective than cisplatin with $IC_{50} < 5$ µg/mL (Morak-Młodawska *et al*., 2016). 2-Amino-1,3-thiazines exhibited their potential for inhibition of human breast cancer MCF-7 and human esophageal cancer EC-9706 cell lines (Nagendra Prasad *et al*., 2019). 9-Anilinoacridines substituted with thiazines showed significant in-vitro anti-tumour activity with CTC_{50} value of 0.20 to 0.39µM (Kalirajan *et al*., 2019). Bis-1,3-thiazine derivatives exhibited activity against breast and liver cancer cell lines while remaining safe for normal cells(El Malah *et al*., 2018). Ruthenium complexes of 1,2-benzothiazine derivatives appeared as highly effective inhibitors for HCT116 cells (Aman *et al*., 2014).

Pyrazolobenzothiazine has recently been proved as an effective heterocyclic template for bioactive molecules. Its derivatives are known for their antiviral (Khalid *et al*., 2015), cholinesterase inhibitors (Aslam *et al*., 2014b) and monoamine oxidase inhibitors (Ahmad *et al*., 2019). In this research article, we present anticancer activity of eighteen compounds belonging to four series of pyrazolobenzothiazine derivatives (fig. 1) i.e. Pyrazolobenzothiazine based hydrazides (1a-g) (Aslam *et al*., 2014a), (2a-c)(Ahmad *et al*., 2010) and acetamides (3a-c) & (4a-e) (Ahmad *et al*., 2013b).

**Corresponding author:* e-mail: matloob.ahmad@gcuf.edu.pk; matloob_123@yahoo.com

MATERIALS AND METHODS

Chemistry

The synthesis of acetohydrazides (1a-g and 2a-c) was carried out by similar routes *i.e.,* starting with corresponding pyrazolobenzothiazine precursors and coupling it with methyl chloroacetate to get ester derivative (Step-1), ester derivative from step-1 was converted to respective hydrazide by reflux with hydrazine hydrate and finally, treatment of this acetohydrazide with a series of substituted benzaldehydes resulted in the formation of targeted derivatives 1a-g and 2a-c (table 1). Acetamides 3a-c and 4a-e were prepared by condensation of parent pyrazolobenzothiazine ring system with chloroacetic acid and the resultant carboxylic acid moiety was converted to corresponding benzyl amides (3a-c) and anilides (4a-e) by using the amide coupling agents; boran-THF complex for 3a-c and thionyl chloride for 4a-e (table 1).

Anticancer activity and cytotoxicity against human cancer and normal cells

The *in vitro* cytotoxicity of the synthesized compounds against different cancer cell lines was performed with the MTT assay according to Mosmann'smethod (Mosmann, 1983). The detailed procedure is as reported by Liwu Fu in literature (Jin *et al*., 2013).

STATISTICAL DATA

All experiments were conducted in replicate. *Mean of experiments are presented, n = 3 and $p < 0.05$ were considered statistically significant.

RESULTS

Chemistry

The synthesis of all these compounds is carried out from saccharine as a starting material. The series 2a-c, 3a-c and

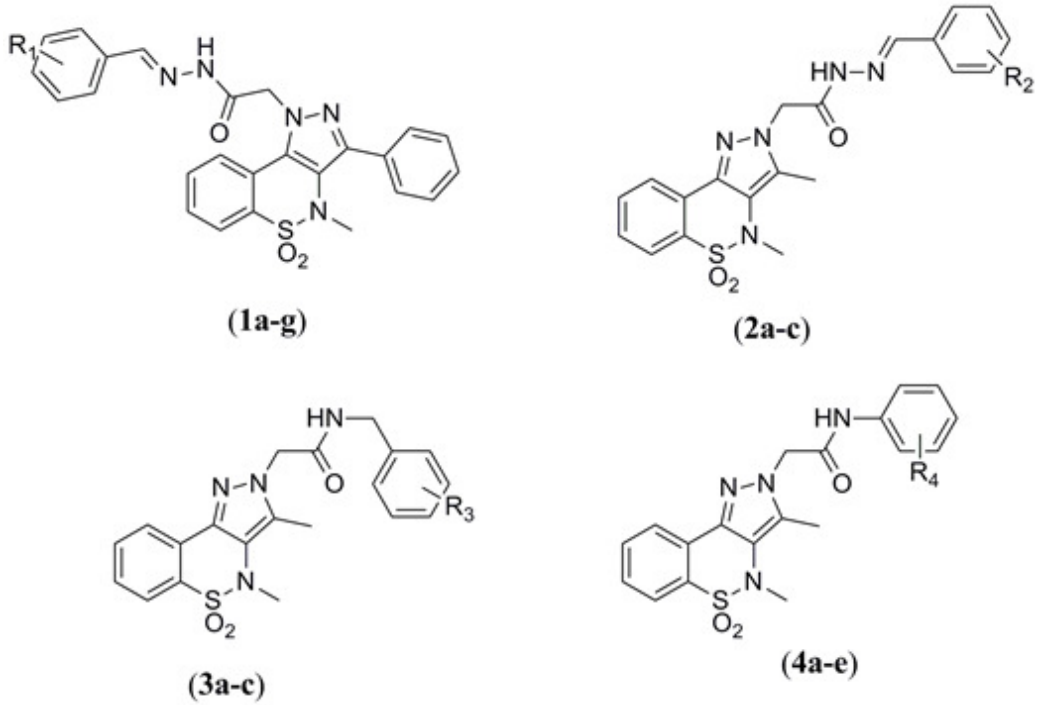

Fig. 1: Structures of the titled compounds screened for anticancer activity

Table 1: Structural Parameters for Compounds 1a-g, 2a-c, 3a-c and 4a-e

Sr. No.	Compound Code	R	Sr. No.	Compound Code	R
1	1a	4-Br	10	1e	3,4-$(Cl)_2$
2	1b	4-Cl	11	1f	4-NO_2
3	1c	4-OMe	12	1g	3-Cl
4	1d	2-F	13	2a	2-NO_2
5	2b	2,4-$(Cl)_2$	14	2c	3,4-$(Cl)_2$
6	3a	4-OMe	15	3b	3-Br
7	3c	2,4-$(OMe)_2$	16	4a	H
8	4b	3-OMe	17	4c	2-F
9	4d	2-Br	18	4e	3-Br

Table 2: Anticancer Screening of the Titled Compounds 1a-g, 2a-c, 3a-c and 4a-e

S No.	Code Number	Cell Lines: IC_{50} (μM)*						Normal Human Cell Line
		KB	MCF-7	A549	S1	Hep-G2	SGC-7901	PBM
1	1a	7.90	>50	24.32	18.37	41.76	>50	97.0
2	1b	13.74	>50	27.82	21.89	>50	>50	>100
3	1c	22.75	>50	>50	>50	>50	>50	>100
4	1d	10.72	>50	41.58	27.18	>50	>50	>100
5	1e	19.68	>50	20.52	21.89	>50	>50	38.0
6	1f	23.47	>50	>50	>50	>50	>50	>100
7	1g	>50	>50	24.96	22.70	24.20	41.56	51.6
8	2a	34.44	>50	>50	>50	35.04	>50	-
9	2b	16.91	>50	>50	>50	26.52	>50	>100
10	2c	16.92	>50	>50	30.52	>50	>50	37.3
11	3a	20.78	>50	>50	>50	>50	>50	>100
12	3b	>50	>50	>50	18.88	>50	>50	-
13	3c	17.14	>50	>50	>50	>50	>50	-
14	4a	6.83	>50	>50	>50	>50	>50	7.7
15	4b	19.44	>50	>50	>50	>50	>50	10.5
16	4c	17.11	>50	>50	>50	>50	>50	18.0
17	4d	14.36	>50	>50	>50	>50	>50	2.4
18	4e	10.84	>50	>50	>50	23.62	>50	8.1
19	5-FU	16.26	12.47		5.55	3.07	5.54	

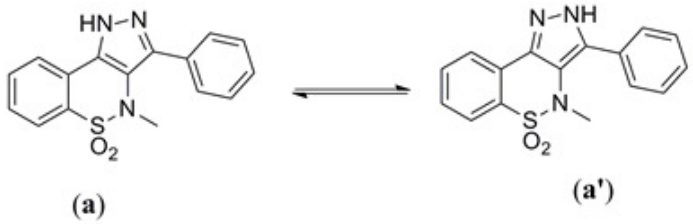

Fig. 2: Tautomeric Forms of Precursor Pyrazolobenzothiazine

4a-e contain 3-methyl substitution on pyrazole moiety whereas series 1a-g bears 3-phenyl substitution at the same position which is relatively a bulky group. This feature facilitates the existence of the two tautomeric forms in equilibrium (fig. 2) and this hypothesis is supported by ^{1}H NMR data as well as by XRD (Aslam *et al*., 2014a). In the rest of series 2a-c, 3a-c and 4a-e, the ^{1}H NMR data indicates the existence of no tautomeric forms. The rest of structural parameters are described in the form of table 1.

Anticancer activity

Benzothiazine scaffold has previously been reported for its anticancer properties (Xia *et al*., 2007, Rai *et al*., 2017). In our pursuit of synthesizing more selective and potent anti-cancer agents, benzothiazine based acetohydrazides and acetamides. Compounds were synthesized and evaluated for their anticancer activity against six different cancer cell lines *i.e.,* KB (human oral carcinoma cells), MCF-7(human breast carcinoma cells), A549 (human alveolar adenocarcinoma cells), Hep-G2 (liver carcinoma cells), SGC-7901(human gastric carcinoma cells) and S1 (human colon carcinoma cells). The results are presented in table 2.

Our results demonstrated that sixteen of our synthesized compounds have potency to inhibit the growth of KB cells with IC_{50} value ranging from (6-34μM). Compounds 4a and 1a were observed to be the most potent compounds with significantly lower IC_{50} values 6.83 and 7.90 μM respectively against KB cells (fig. 3). Compound 1a exhibited selectivity in targeting KB cells while being non-cytotoxic to human PBM cells with EC_{50} value 97.0 μM. Compounds 1b, 1c, 1d, 1f and 2b being moderately active against KB cells with IC_{50} values <20μM with no cytotoxic effects to normal human PBM cells with EC_{50} values >50μM. None of the synthesized compounds were active against MCF-7 cell line. Compounds 1a, 1b, 1e and 1g inhibited the growth of A549 cells with IC_{50} values 24.32, 27.82, 20.52 and 24.96μM respectively. Compounds 1a and 3b have potential to inhibit S1 cells growth with IC_{50} values of 18.37 and 18.88μM respectively which are non-toxic to human PBM cells with EC_{50} values 97 and >100μM respectively. Three compounds, 1g, 2b and 4e inhibited the growth of HepG2 cells with IC_{50} values 24.20, 26.52 and 23.62μM. Compounds 1g and 2b were selective against HepG2 cells having no cytotoxic effects on normal PBM cells. Only one compound 1g has potential to inhibit the proliferation

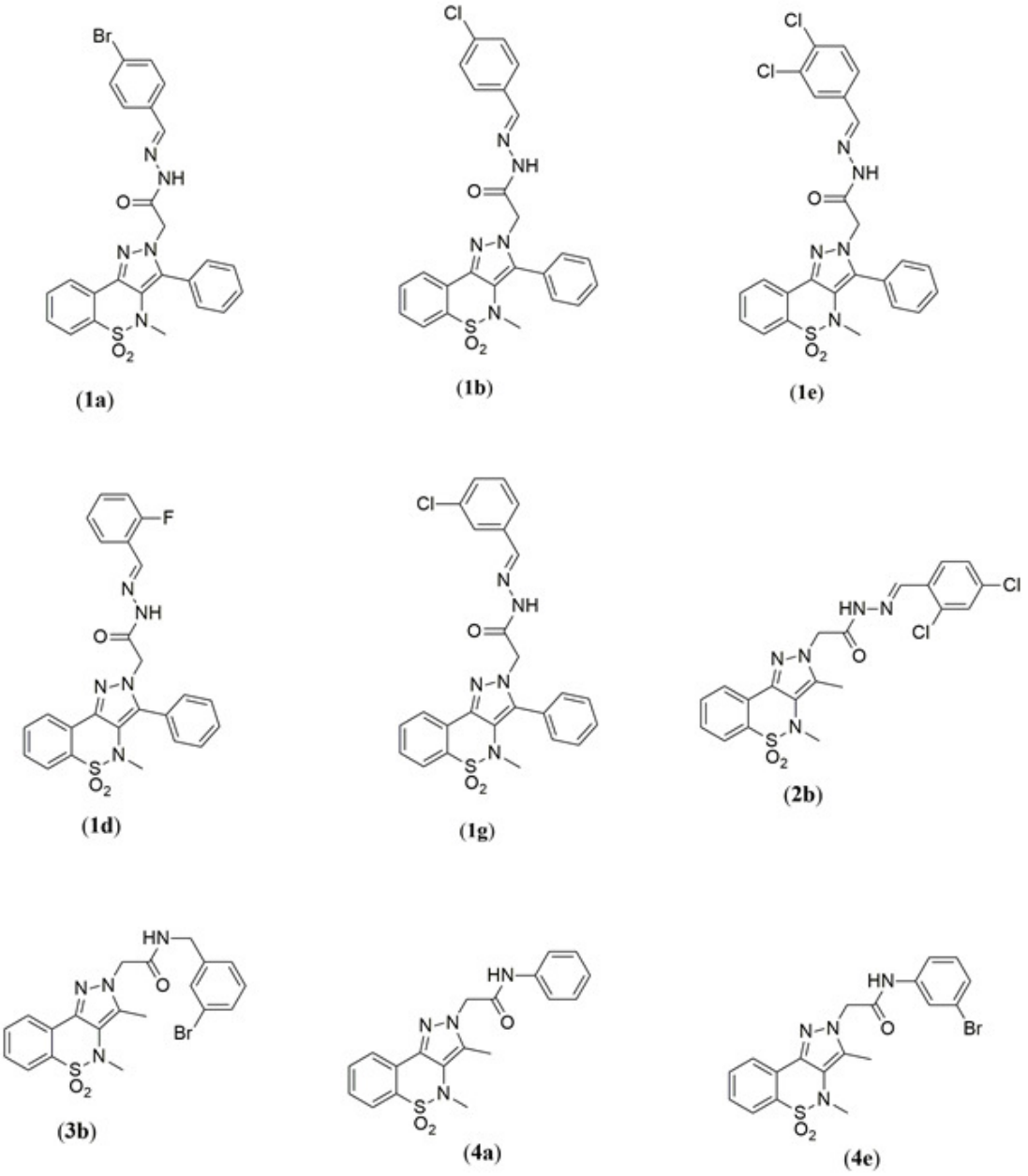

Fig. 3: Structures of active inhibitors of cancer cell growth

of SGC-7901 cells with IC_{50} value of 41.56μM. Eight of the compounds 1a-c, 1f, 2b, and 3a-c were observed to be non-toxic and it is interesting that most of these non-toxic compounds were toxic to various cancer cell lines as discussed earlier. Finally, the cytotoxicity these compounds was compared with their toxicity values to normal PBM cells as described (1a-g) (Aslam *et al*., 2014a), (2a-c)(Ahmad *et al*., 2010) and (3a-c) & (4a-e) (Ahmad *et al*., 2013b). It is indicated that most of the compounds displayed selectivity in inhibiting growth of cancer cells.

DISCUSSION

Our study has indicated the anticancer potential of pyrazolobenzothiazine based acetohydrazides and acetamides. The incorporation of these functionalities to benzothiazine ring system is planned on the bases of literature reports for the anticancer character of acetohydrazides and acetamides. Recently, acetohydrazide derivatives of 3-benzyl-4,8-dimethylbenzopyrone were reported as anticancer agents (El-Ansary *et al*., 2017). The anti-cancer activity of pyrazole-based hydrazides has been documented against

A549 cells with IC_{50} value 23- 67µM (Xia *et al*., 2007). Moreover, 1,4-benzothiazine based carbohydrazides have been reported as inhibitors of HT-29 human colorectal cancer cell line and among the carbohydrazides, 3,4-dichlorobenzylidene substituted molecules emerged as the best anticancer agent (Rai *et al*., 2017). Similar results are received in our studies where compound 1e bearing the same substitution showed moderately good inhibitory activity for KB cell line. Overall, six compounds showed effectiveness greater than 5-FU for against KB cells while some others were moderately active. Compounds 2a-c, 3a-c and 4a-e containing 3-methylsubstitution on pyrazole moiety represented cytotoxicity towards KB and S1 cells with more potency. Compound 2b and 2c presented most superior cytotoxicity from all the evaluated products. Interestingly, the presence of the chloro group at 2,4 and 3, 4 position of pyrazole moiety resulted in a dramatic increase in the overall *in vitro* anticancer potency. The chloro-substitution, with efficient electron withdrawing properties might be the crucial for the inhibition of tumor cells.

Moreover, non-toxic nature of these benzothiazine based hydrazides and acetamides towards PBM cells present their selective potential as well as best binding scaffold towards cancer targets. It was represented that the cytotoxic potency was highly dependent, as expected, on the patterns and substitution types on pyrazole moiety and the most potent compounds against cancers have halogen substitutions. Among acetohydrazides, 4-bromo and 4-chloro substitutions presented the most potent compounds with significant cytotoxicities against the KB, A549 and S1 cells while from acetamides, 2,4 and 3,4-dichloro substitutions represented potency towards KB and S1 cells.

CONCLUSION

It is concluded that most of the tested pyrazolobenzothiazine compounds exhibited anticancer activity. The most active compounds are observed to have halogen substitutions in their structures. Six compounds 1a-c, 1f, 2b and 3a-c exhibited selectivity in targeting cancer cells and were non-toxic to human PBM cells. Among various substituents, the halo-substituted compounds and dihalo ones displayed greater activity. The data indicates that pyrazolobenzothiazine derivatives express cytotoxicity preferable to KB cell lines. Interestingly, many of the synthesized compounds displayed better cytotoxic activity as compared to 5-FU. Further structural modifications of these compounds are expected to result in more selective and potent anticancer agents.

ACKNOWLEDGMENTS

The authors (SA & MA) are grateful to Higher Education Commission, Pakistan for financial assistance.

REFERENCES

Ahmad M, Siddiqui H, Gardiner J, Parvez M and Aslam S (2013a). Synthesis and antioxidant studies of novel N-substituted benzyl/phenyl-2-(3,4-dimethyl-5,5-dioxido-pyrazolo[4,3-c][1,2]benzothiazin-2(4H)-yl)acetamides. *Med. Chem. Res.,* **22**(2): 794-805.

Ahmad M, Siddiqui HL, Zia-Ur-Rehman M and Parvez M (2010). Anti-oxidant and anti-bacterial activities of novel N′-arylmethylidene-2-(3, 4-dimethyl-5, 5-dioxidopyrazolo[4,3-c][1,2]benzothiazin-2(4H)-yl) acetohydrazides. *Eur. J. Med. Chem.,* **45**(2): 698-704.

Ahmad S, Jalil S, Zaib S, Aslam S, Ahmad M, Rasul A, Arshad MN, Sultan S, Hameed A, Asiri AM and Iqbal J (2019). Synthesis, X-ray crystal and monoamine oxidase inhibitory activity of 4,6-dihydrobenzo [c]pyrano[2,3-e][1,2]thiazine 5,5-dioxides: *In vitro* studies and docking analysis. *Eur. J. Pharm. Sci.,* **131**: 9-22.

Akogwu EI, Saganuwan SA and Onyeyili PA (2017). Comparative pharmacokinetics of piroxicam in male and female West African Dwarf goats. *Cogent Food & Agriculture,* **3**(1): 1294444.

Aman, F, Hanif, M, Siddiqui, WA, Ashraf, A, Filak, LK, Reynisson, J, Söhnel, T, Jamieson, SMF and Hartinger, CG (2014). Anticancer Ruthenium(η6-p-cymene) complexes of nonsteroidal anti-inflammatory drug derivatives. *Organometallics,* **33**(19): 5546-5553.

Aslam S, Ahmad M, Zia-Ur-Rehman M, Montero C, Detorio M, Parvez M and Schinazi RF (2014a). Synthesis and anti-HIV-1 screening of novel N′-(1-(aryl)ethylidene)-2-(5,5-dioxido-3-phenylbenzo[e] pyrazolo[4,3-c][1,2]thiazin-4(1H)-yl)acetohydrazides. *Arch. Pharm. Res.,* **37**(11): 1380-1393.

Aslam S, Zaib S, Ahmad M, Gardiner JM, Ahmad A, Hameed A, Furtmann N, Gutschow M, Bajorath J and Iqbal J (2014b). Novel structural hybrids of pyrazolobenzothiazines with benzimidazoles as cholinesterase inhibitors. *Eur. J. Med. Chem.,* **78**: 106-117.

Bekker A, Kloepping C and Collingwood S (2018). Meloxicam in the management of post-operative pain: Narrative review. *J. Anaesthesiol. Clin. Pharmacol.,* **34**(4): 450-457.

Cannalire R, Tarantino D, Astolfi A, Barreca ML, Sabatini S, Massari S, Tabarrini O, Milani M, Querat G, Mastrangelo E, Manfroni G and Cecchetti V (2018). Functionalized 2,1-benzothiazine 2,2-dioxides as new inhibitors of Dengue NS5 RNA-dependent RNA polymerase. *Eur. J. Med. Chem.,* **143**: 1667-1676.

El-Ansary SL, Abdel Rahman DE and Abdel Ghany LMA (2017). Synthesis and anticancer evaluation of some new 3-Benzyl-4,8-Dimethylbenzopyrone derivatives. *Open Med. Chem. J.,* **11**: 81-91.

El Malah T, Ishak EA, Nour HF, Shaker RM, Ali MM, Mahmoud AE and Soliman SM (2018). Synthesis and *In vitro* anticancer evaluation of symmetrically bridged

1,3-thiazine derivatives. *J. Heterocycl. Chem.,* **55**(7): 1746-1755.

Feng RM, Zong YN, Cao SM and Xu RH (2019). Current cancer situation in China: Good or bad news from the 2018 global cancer statistics? *Cancer Commun*., **39**: 22.

Jin C, Liang YJ, He H and Fu L (2013). Synthesis and antitumor activity of novel chalcone derivatives. *Biomed. Pharmacother.,* **67**(3): 215-217.

Kalirajan R, Gaurav K, Pandiselvi A, Gowramma B and Sankar S (2019). Novel Thiazine Substituted 9-Anilinoacridines: Synthesis, Antitumour activity and Structure-Activity Relationships. *Anticancer Agents Med Chem.,* **19**: 1-9.

Khalid Z, Aslam S, Ahmad M, Munawar MA, Montero C, Detorio M, Parvez M and Schinazi RF (2015). Anti-HIV activity of new pyrazolobenzothiazine 5,5-dioxide-based acetohydrazides. *Med. Chem. Res.,* **24**(10): 3671-3680.

Morak-Młodawska B, Pluta K, Latocha M and Jelen M (2016). Synthesis, spectroscopic characterization, and anticancer activity of new 10-substituted 1,6-diazaphenothiazines. *Med. Chem. Res.,* **25**(11): 2425-2433.

Morak-Mlodawska B, Pluta K, Latocha M, Jelen M and Kusmierz D (2019). Synthesis, anticancer activity, and apoptosis induction of novel 3,6-diazaphenothiazines. *Molecules,* **24**(2): 267.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods,* **65**(1-2): 55-63.

Prasad NT, Eeda KR, Gudise VB, Basha, SF & Anwar, S (2019). Design, synthesis and biological evaluation of substituted 2-amino-1,3-thiazine derivatives as antituberculosis and anti-cancer agents. *Synth. Commun.,* **49**(10): 1277-1285.

Patel, C, Bassin, JP, Scott, M, Flye, J, Hunter, AP, Martin, L and Goyal M (2016). Synthesis and antimicrobial activity of 1,2-benzothiazine derivatives. *Molecules* (Basel, Switzerland), **21**(7): 861.

Rai A, Raj V, Singh AK, Keshari AK, Kumar U, Kumar D and Saha S (2017). Design and synthesis of 1,4-benzothiazine derivatives with promising effects against colorectal cancer cells. *Cogent Chemistry,* **3**(1): 1303909.

Siegel RL, Miller KD and Jemal A (2019). Cancer statistics *CA Cancer J. Clin.,* **69**(1): 7-34.

Xia Y, Dong ZW, Zhao BX, Ge X, Meng N, Shin DS and Miao JY (2007). Synthesis and structure–activity relationships of novel 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide derivatives as potential agents against A549 lung cancer cells. *Bioorg. Med. Chem.,* **15**(22): 6893-6899.