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Anticancer activity of structural hybrids of various 5/6-membered-heterocycles with pyrazolobenzothiazine 5,5-dioxide

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Abstract: Thiophene, furan, coumarin and pyrazolobenzothiazine are well familiar for their biological activities. In this research, pyrazolobenzothiazine ring system is hybridized with various S, N & O-containing heterocycles and the resulting compounds were screened 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. Most of the compounds exhibited good activity against KB, S1 and A549 cancer cell lines. **5k** and **5p** appeared as potent inhibitors of KB cell line with IC_{50} values 2.78 and 4.39 μM respectively, **5q** was a potent inhibitor of MCF-7 (IC_{50} value = 13.64 μM) and **5j** an excellent inhibitor of A549 cell line having IC_{50}value of 1.03 μM. **5p** and **5q** were inhibitors of S1 cell line (IC_{50} values of 8.29 μM and 7.69 μM respectively), whereas, **5o** and **5q** as inhibitors of Hep-G2 cell line were discovered. A number of compounds show activity exceeding that of 5-fluoruracil in different cell assays. The most potently active compounds, **5j, 5p** and **5q**, exhibited selectivity in targeting cancerous cells as compared to normal human PBM cells while, **5k** and **5o** displayed significant toxicity in normal cells.

Keywords: Pyrazolobenzothiazine, anti-cancer activity, cytotoxicity, selective inhbition.

INTRODUCTION

Cancer remains a major cause of death and a major health issue worldwide. American Cancer Society has estimated that around 1.81 million new cases of cancer will be registered in USA alone in the year 2020 and around 0.61 million people will die of cancer in 2020 (Siegel *et al.,* 2020). FDA has approved a number of drugs for the treatment of various types of cancer but due to their side effects, these drugs have limited applications. The development of new drugs may reduce these problems. Natural products derived from plants, marine sources and recently from microorganisms are a good source for lead compounds to develop as new anticancer agents (Xiao *et al.,* 2016). Alongside these natural products, generation of new synthetic structures remains an important approach for medicinal chemists to develop new medicines targeting cancer. There is a continued need for the drugs that target cancer cells selectively without having toxicity to normal cells. Thiophene has emerged as an excellent heterocyclic scaffold as being a part of many marketed drugs (Zhao *et al.,* 2018; Mohareb *et al.,* 2013). The functionalized 2,3-fusedthiophene scaffolds were recently studied against HeLa and Hep G2 cellswhich resulted in a promising candidate having IC50 value of 12.61 μg/mL in HeLa cell line and 33.42 μg/mL in Hep G2 cells (Zhao *et al.,* 2019). Thiophene based chalcone derivatives were observed as good anticancer agents against lung cancer cell line (A549) with IC50 values of 6.3±0.9 mM and with GI50 values of 1.9±0.3 mM on A549 cells (Duddukuri *et al.,* 2018). On the other hand, benzothiazine ring system is an excellent template for pharmaceutically applicable molecules. Oxicam drugs are being clinically used for the treatment of inflammatory disorders. Various other derivatives have been reported to show antimicrobial (Tamatam *et al.,* 2019), anti-inflammatory (Shabbir *et al.,* 2016) and antitumor activities (Rai *et al.,* 2017). We have reported the synthesis of benzothiazine and pyrazolobenzothiazine derivatives as monoamine oxidase inhibitors (Ahmad *et al.,* 2019; Saddique *et al.,* 2018; Ahmad *et al.,* 2018), anti-HIV agents (Aslam *et al.,*2014) and as alpha-glucosidase inhibitors (Saddique *et al.,* 2019). In our recent study, we have reported pyrazolobenzothiazine based acetohydrazides as anticancer agents (Aslam *et al.,* 2019). The aim of the work was to screen the pyrazolobenzothiazine hybrid derivatives for their anticancer activity and thus, to display the cytotoxic potential of these compounds.

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MATERIALS AND METHODS

All the chemicals used in this research work were purchased from Alfa Aesar (Germany) and or Sigma Aldrich (USA).

Chemistry

The precursor compound, 3-benzoyl-4-hydroxy-2*H*-1,2-benzothiazine 1,1-dioxide (**2**) was synthesized by the Gabriel-Coleman rearrangement of *N*-phenacyl saccharine (**1**). This was *N*-alkylated with various alkyl chloroacetates and the resulting esters (**3**) were reacted with hydrazine monohydrate to form pyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide derivative (**4**). Finally, the title compounds were obtained by condensing **4** with a range of acetylthiophenes and substituted heterocyclic ketones (**Scheme 1**) (Aslam *et al.,* 2014). Structural modifications were made on hydrazone moiety by using different substituted thiophenes and other heterocycles like thiazole, pyridine, furan and coumarin. In order to establish structure-activity relationship and the role of methyl group at N=CCH_3 in biological activity, an aldimine (**5v**) having N=CH moiety was prepared by using thiophen-2-carboxaldehyde.

Anticancer activity and cytotoxicity against human pbm cells

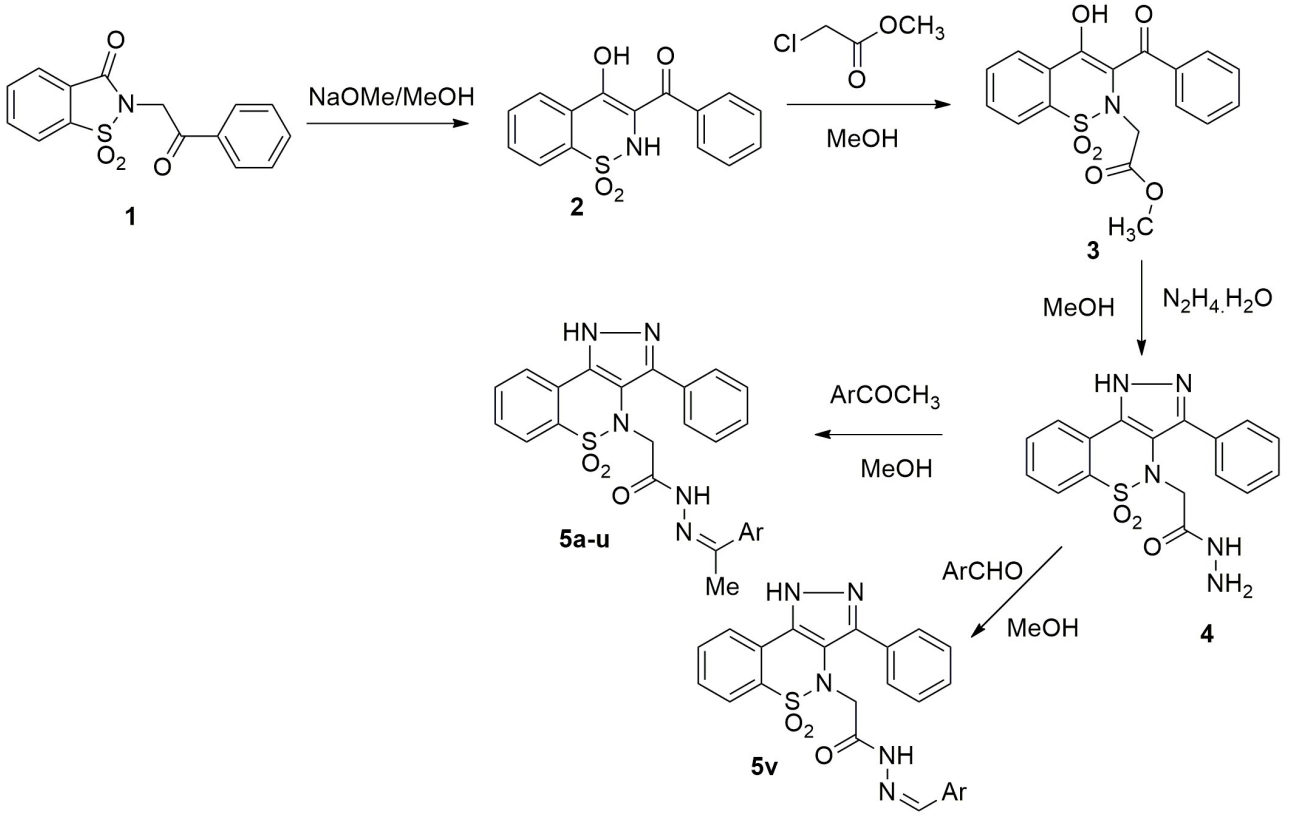
The MTT assay was performed for the *in vitro* cytotoxicity of the synthesized compounds against different cancer cell lines according to Mosmann's method (Mosmann T, 1983). The detailed procedure is as reported by us in literature (Jin *et al.,* 2013). The work is done in *Sun Yat-sen University Cancer Center, Guangzhou, China.* In addition, cytotoxicity of the synthesized compounds was checked according to the reported method (Stuyver *et al.,* 2002). The cytotoxicity work against human PBM cells is reported in our earlier work (Aslam *et al.,* 2014).

STATISTICAL ANALYSIS

All experiments were conducted in replicate. *Mean of experiments are presented, n = 3 and $p < 0.05$ were considered statistically significant. The statistical analysis were performed through SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

All the compounds were tested for their cytotoxicity in six



Scheme 1: Synthetic scheme of pyrazolobenzothiazine derivatives (**5a-v**)[Aslam *et al.,* 2014]

drug-sensitive cancer cell lines::KB, MCF-7, A549, Hep-G2, SGC-7901 and S1 cell lines. Cytotoxic assay was performed using MTT method (Experimental protocols (Mosmann T,1983) and the results expressed as IC_{50} (μM) are summarized in table 1. 5-Fluorouracil was also evaluated against five cell lines. The results for normal human PBM cell lines are described in our previous studies (Aslam *et al.,* 2014). Among the tested compounds, **5k** and **5p** appeared as best active against KB cell line, **5j** against A549 cell line, and **5p** and **5q** against S1 cell line displayed promising anticancer activity.

DISCUSSION

Anticancer Activity

The results described in table 1 have indicated that the titled compounds emerged as good anticancer agents. The synthesis of these compounds were designed with the help of literature evidences indicating the anticancer potential of thiophene derivatives (Said and Elshihawy, 2014), coumarin derivatives (Garazd *et al.,* 2017) and benzothiazine derivatives (Rai *et al.,* 2017). The cytotoxicity assays showed that most of the tested pyrazolobenzothiazine hybrid derivatives (**5a-v**) inhibited *in vitro* growth of KB cells. Among the twenty-three compounds, thirteen exhibited greater activity than 5-florouracil *i.e.,* 16.26μM and eight other compounds exhibited moderate activity. Compound **5k** having naphthalene-2-yl substitution exhibited activity about 6-fold greater than 5-florouracil with IC_{50} value of 2.78 μM. Similarly, **5p** bearing 1,4-benzodioxane-6-yl substitution exhibited activity about 4-fold greater than 5-FU with an IC_{50} of 4.39 μM (fig. 1). It is worth mentioning that compound **5p** is considerably non-toxic to normal human PBM cells with EC_{50} value of 67.1 μM. The methylthiophen-2-yl containing compounds, the activity was observed in following order; 5-Me>4-Me >3-Me with IC_{50}s of 13.13, 26.2 and 26.69 μM. These results resemble the recent literature report on benzothiazine derivatives especially acetohydrazides that exhibited potency to inhibit the growth of KB cells with IC_{50} value ranging from (6-34μM) (Aslam *et al.,* 2019).

MCF-7 Cancer Cell Line: Overall, eight compounds exhibited weak inhibitory activity against the MCF-7 cell line. Among them, **5q** bearing 5-nitrothiophen-2-yl substitution had comparable activity to 5-FU with an IC_{50} value of 13.64 μM. Interestingly, **5q** had no toxicity at all to the normal human PBM cells *i.e.,* EC_{50} value of 85 μM (table 1 and fig. 1).

Table 1: Anti-cancer activity of novel series of pyrazolobenzothiazine derivatives **(5a-v)**

Sr. No.		Products	Cell Lines: IC_{50} (μM)					
		Ar	KB	MCF-7	A549	S1	Hep-G2	SGC-7901
1	**5a**	Thiophen -2-yl	20.56	>50.00	41.30	24.65	48.99	>50.00
2	**5b**	3-Methylthiophen -2-yl	26.69	>50.00	>50.00	31.62	47.00	>50.00
3	**5c**	Thiophen -3-yl	16.66	>50.00	36.84	21.10	49.02	>50.00
4	**5d**	2,5-Dimethylthiophen-3-yl	14.19	41.58	>50.00	**11.09**	49.92	>50.00
5	**5e**	3-Chlorothiophen-2-yl	21.10	>50.00	>50.00	31.01	49.16	>50.00
6	**5f**	3-Bromothiophen-2-yl	22.16	>50.00	>50.00	22.16	>50.00	>50.00
7	**5g**	5-Chlorothiophen-2-yl	**11.00**	27.94	21.79	16.55	33.58	35.24
9	**5h**	2,5-Dichlorothiophen-3-yl	12.62	39.13	>50.00	17.10	38.61	>50.00
10	**5i**	4-Methylthiophen-2-yl	26.20	ND	ND	ND	ND	ND
11	**5j**	Coumarin-3-yl	**11.30**	>50.00	**1.03**	20.79	>50.00	>50.00
12	**5k**	Naphthalen-2-yl	**2.78**	28.54	22.39	**12.13**	**31.14**	>50.00
13	**5l**	5-Methylthiophen-2-yl	13.13	34.38	38.53	15.64	43.03	>50.00
14	**5m**	Benz[*b*]furan-2-yl	12.26	>50.00	>50.00	14.55	>50.00	>50.00
15	**5n**	Pyrrole-2-yl	>50.00	>50.00	>50.00	>50.00	>50.00	>50.00
16	**5o**	5-Bromothiophen-2-yl	10.52	34.38	24.11	14.24	19.64	>50.00
17	**5p**	1,4-Benzodioxane-6-yl	4.39	42.97	27.32	8.29	46.51	>50.00
18	**5q**	5-Nitrothiophen-2-yl	11.24	13.64	20.67	7.64	18.23	24.96
19	**5r**	5-Methylfuran-2-yl	42.66	>50.00	>50.00	22.23	>50.00	>50.00
20	**5s**	2,5-Dimethylfuran-3-yl	26.18	41.93	>50.00	29.28	41.17	>50.00
21	**5t**	Thiazolo -2-yl	12.11	>50.00	>50.00	22.45	40.26	>50.00
22	**5u**	Pyridino-4-yl	14.76	>50.00	>50.00	22.62	>50.00	>50.00
23	**5v**	Thiophen-2-yl	11.74	>50.00	>50.00	>50.00	39.16	>50.00
		5-FU	16.26	12.47		5.55	3.07	5.54

*All experiments were conducted in replicate.

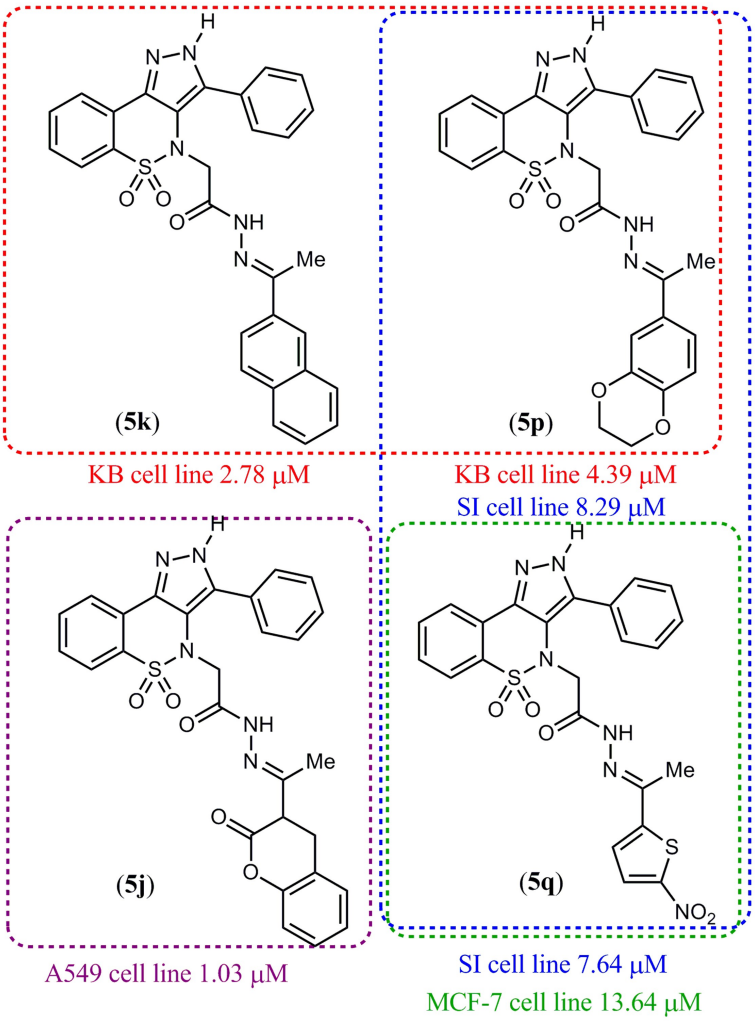


Fig. 1: Structures of potent inhibitors of cancer cell growth

A549 Cancer Cell Line: Among the series, eight compounds exhibited anticancer activity against the A549 cell line. Compound **5j** being the most potent inhibitor with an IC_{50} value of 1.03 µM, Moreover, it is significantly non-toxic to normal human PBM cells with an EC_{50} value of 82.7 µM (fig. 1).

S1 Cancer Cell Line: Two compounds- **5p** and **5q** exhibited good inhibition activity with IC_{50} values of 8.29 and 7.64 µM respectively. These two compounds were non-toxic to human PBM cells with EC_{50} values of 67.1 and 85.0 µM respectively (fig. 1). Among the series, nineteen compounds appeared as inhibitors of S1 cancer cell line (IC_{50} values <50µM). Compounds **5d**, **5g**, **5h**, **5k**, **5l**, **5m** and **5o** appeared as moderate inhibitors with IC_{50} values <20 µM, among them **5l** and **5m** were non-toxic to human PBM cells with EC_{50} values >50 µM.

GC-7901 Cancer Cell Line: Most compounds showed weak antiproliferative activity against human gastric carcinoma cells tumor cell line. Two representative compounds **5g** and **5q** displayed moderate antitumor activity with IC_{50} values of 35.24 and 24.96 µM respectively.

Hep-G2 Cancer Cell Line: Fifteen compounds were observed as weakly active against Hep-G2 cell line with **5o** and **5q**, the most active inhibitors of all with IC_{50} values of 19.64 and 18.23 µM respectively. As discussed earlier, **5q** is non-toxic to normal human PBM cells. Most of the derivatives with strong electron-withdrawing substituent groups such as bromo and nitro on thiophene ring had significantly higher cytotoxicities than those with electron-donating groups or with no substitution. The work leads for the further development of new anticancer molecules, especially new structural hybrids of substituted pyrazolobenzothiazine with thiophene, coumarin, naphthalene and benzodioxane ring system.

CONCLUSION

In summary, we report that a number of synthetic pyrazolobenzothiazine derivatives show useful levels of inhibition *i.e.,* <10µM across six human cancer cell lines.

Among these, **5k** and **5p** are potent inhibitors of the KB cell line and **5j** is potent inhibitors of A549 cell lines respectively. The same group of lead compounds showed excellent inhibition against various other cell lines: **5p** and **5q** against S1 cell line were discovered. Moreover, the potent active compounds **5j, 5p** and **5q** exhibited selectivity in targeting cancerous cell as compared to normal human PBM cells and thus offer promise as a new scaffold for development of these leads for anticancer activity.

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