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Research Article

EVALUATION OF ANTICANCER ACTIVITY FOR PYRAZOLE-QUINAZOLINE DERIVATIVES BY TRYPAN BLUE ASSAY METHOD

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ABSTRACT

The mission of medicinal chemistry is to design and synthesize novel chemical entities having potential biological activity. A series of novel substituted pyrazole-quinazoline derivatives were synthesized by condensing Quinazoline and Pyrazole nucleus with various substitutions on aryl ring. A total of 12 derivatives were synthesized. The compounds were initially confirmed by physical properties and chemical test, later characterized with spectral confirmation by UV-VIS, FT-IR, Mass and NMR. The compounds were tested for their anticancer activity using Trypan Blue exclusion assay method using cisplatin as standard reference drug and DMSO as solvent. Trypan blue is an acid azodye of the benzopurine series used as a vital stain. *in*-vitro short term cytotoxic activity of drugs were determined using EAC cells in Phosphate Buffer Saline(PBS) using heamocytometer. The parameters like % cytotoxicity and IC_{50} were determined. The % cytotoxicity of the compounds (1,7,8,9) were found to be considerable at a concentration above 10µg/ml. compounds showing good activity are further proceeded for further studies like SRB assay.

Keywords: Pyrazole- Quinazoline, Anticancer, Trypan blue method and Heamocytometer.

INTRODUCTION

In the world of maladies like cancer and life threatening infections, the primary objective of medicinal chemist is to apply the strategies of drug discovery starting from improvement of existing drugs. Majority of cancers are caused by changes in the cell DNA because of damage due to environment. Cancer is a disease of cells[1]. The search for new anticancer agent is one of the most challenging tasks to the medicinal chemist. Heterocyclic compounds are biologically important class of compounds. This prompted us to synthesize hybrid analogues of two pharmacophores *viz*., Quinazoline and Pyrazole. Quinazoline [2,3] is a bicyclic compound earlier known as benzo-1,3-diazine and Pyrazole [4,5] is a heterocyclic 5-membered ring with 3 carbons and two adjacent nitrogen compounds. The extensive review of literature revealed the compounds have anticancer activity [6,7,8]. Encouraged by the above observations from the literature, it was planned to suitably incorporate the pyrazole in to quinazolinone and to synthesize a better drug with less toxicity to the host, it is observed that chemical modification not only alters physiochemical properties.

MATERIALS AND METHODS

All the chemicals used for the experimental work were commercially procured from various chemical units and are of laboratory grade. The synthesized compounds pyrazole-quinazoline derivatives with their molecular structures were depicted in table-1.

Trypan blue exclusion assay method

Trypan blue /Niagara blue is an acid azodye of the benzopurine series used as a vital stain which is used for staining of the reticuloendothelial system and cells in tissue culture [9]. The main principle involved in assay was live cells or tissues with intact cell membrane will not be colored since cells are very selective in the compounds that pass through the membrane, in a viable cell trypan blue is not absorbed, however, it traverses the membrane in a dead cells hence, dead cells are shown as a distinctive blue colour under microscope [10,11]. All compounds were screened for cytotoxic activity. The viability of the cells was assessed by trypan blue exclusion method.

Procedure

Stock solution of 1x10 [6] cells was prepared in appropriate Phosphate Buffer Saline (PBS) and the viability of the cells was checked by counting with a heamocytometer[12]. DMSO (Dimethyl sulphoxide) is often used since it is cytotoxic at high concentrations, dilutions with medium were made so that final concentration used for treating the cells[13]. Various concentrations of the derivatives 10,25,50 mcg was taken in a clean test tube added 100µl of the EAC cells final volume was adjusted with buffer up to 1ml respectively. Test has been carried out with control and solvent control was also maintained, all the test tubes were incubated at 370C for 3 hrs. After incubation 100µl of 4% trypan blue was added to each test tube. Alive cells and dead cells were calculated by using haemocytometer and checked for the % cytotoxicity. For the assay cisplatin [14] was selected as standard drug for reference. % cytotoxicity [15] was calculated by formula

% Cytotoxicity = (T_{dead}- C_{dead})/T_{total} x 100

Where, T_{dead} =number of dead cells in the drug treated tube

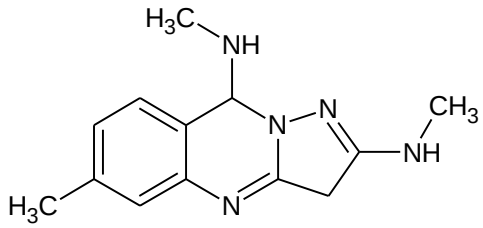
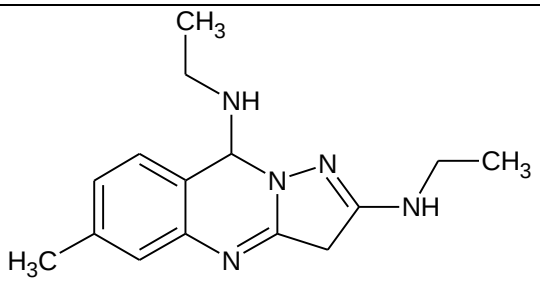
C_{dead} = number of dead cells in control tube

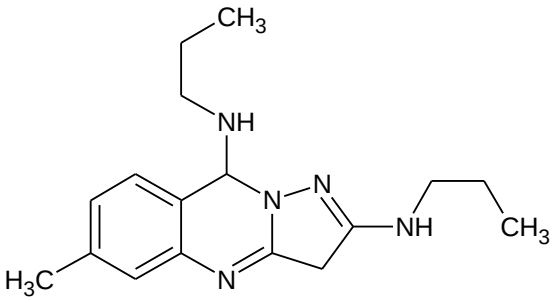
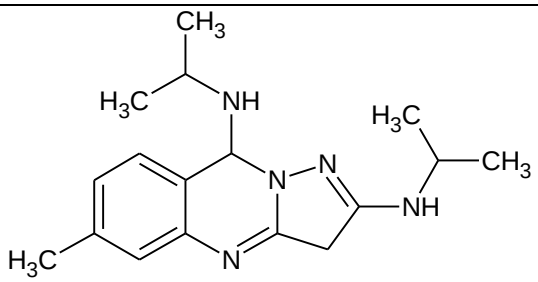
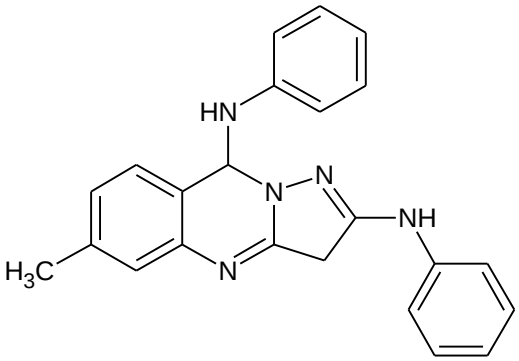
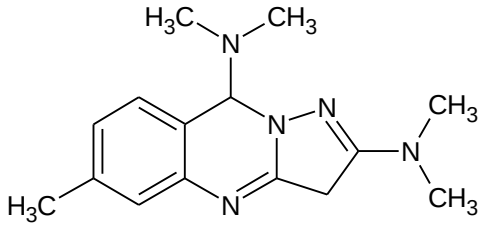
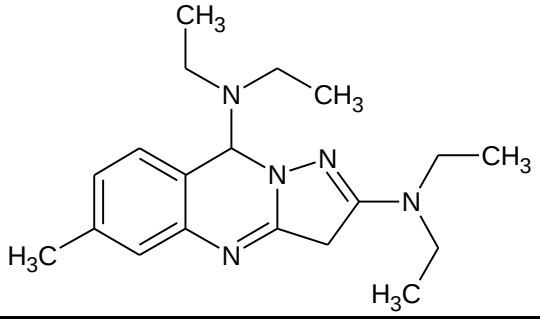
T_{total} = number of dead cells and alive cells in drug treated tube

RESULTS AND DISCUSSION

All the synthesized compounds were screened for their cytotoxicity on EAC cells at 10, 25&50µg/ml. Results for % cytotoxicity of synthesized compounds at different concentrations was shown in table-2.The trypan blue exclusion technique indicates that the IC50 of the standard drug cisplatin is 191.16 and that of control was 500.83. An IC 50 result was depicted in table-3. All the compounds displayed cell necrosis above 10 µg/ml. It reveals that cell necrosis is the main reason for cell death and in some cases morphological changes in cells were found. Compounds of all the synthesized drugs showed the activity. Good activity was shown by compounds of 1,7,8,9.

Table 1: Molecular structures of pyrazole-quinazoline derivatives

S. NO.	MOLECULE NO	MOLECULAR STRUCTURE
1	1	
2	2	

3	3	
4	4	
5	5	
6	6	
7	7	

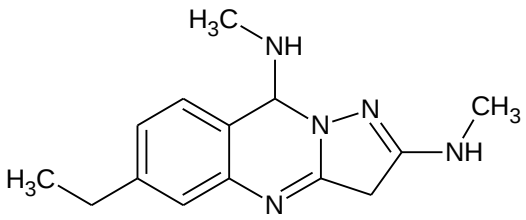
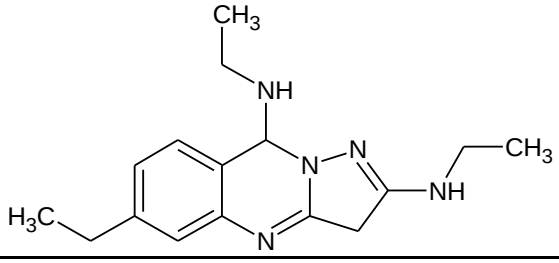
8	8	
9	9	

Table 2: % Cytotoxicity of synthesized compounds at different concentrations

Code	% cytotoxicity		
	10 µg/ml	25 µg/ml	50 µg/ml
1	16.48	17.48	72.51
2	34.59	40.49	65.38
3	19.25	75.33	62.47
4	37.18	57.04	33.39
5	47.23	57.46	68.59
6	25.37	40.14	79.29
7	24.25	74.09	72.30
8	16.37	65.37	96.47
9	15.32	75.18	90.14
Control	10.10	12.03	13.22
Standard (cisplatin)	**16.18**	**16.2**	**23.33**

Table 3: IC 50 of the compounds

Code	IC 50
1	37.87
2	32.12
3	25.49
4	12.62
5	13.01
6	19.84
7	21.75
8	23.28
9	22.41
Control	500.83
Standard (cisplatin)	191.16

CONCLUSION

Synthesis of pyrazole quinazoline derivatives were prepared and their molecular structures were shown in table 1. Based on literature survey compounds were screened for anti-cancer activity, initial evaluation was done for the prepared derivatives by Trypan blue exclusion assay method. Among the prepared compounds all the synthesized drugs showed the activity. Good activity was shown by compounds 1,7,8,9. Further these compounds were evaluated for higher methods of screening for lead discovery.

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