Research Article

COMBINATION OF HETEROCYCLIC COMPOUNDS: AS ANTICANCER AGENTS

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ABSTRACT

Benzimidazole and Pyrazole both are heterocyclic aromatic organic compounds. These two heterocyclic compounds are occupied prominent place among various classes of aromatic organic compounds. Heterocyclic compounds having a variety of therapeutic uses, including antitumor, antifungal, antiparasitic, analgesics, antiviral, antihistamine, as well as use in cardiovascular disease, neurology, endocrinology, and ophthalmology. Both Benzimidazole and Pyrazole are also effective in anticancer. All the newly synthesized heterocyclic compounds were screened for the in-vivo cytotoxic activity by XTT assay methods using various cell lines like VERO, NCI, MDA-MB-453 and MDA-MB-468 using doxorubicin as standard drugs for comparison of results. Aims: To design and identify new heterocyclic compounds to potent anticancer activity. Methods: XTT assay method. Results: By using different cell lines for 10 compounds we find positive results for one or two compounds. From the discussion, we conclude that compound which contain o-nitrobenzene as attachment gives better anticancer activity in all cell lines, whereas compound contain p-fluorobenzene as attachment gives better anticancer activity in cell line like NCI, MDA-MB-453 and MDA-MB-468. The compound contains m-nitrobenzene gives positive results in VERO cell line.

KEY WORDS: XTT, VERO, NCI, MDA-MB-453, MDA-MB-468

INTRODUCTION

Benzimidazole and Pyrazole both are heterocyclic aromatic organic compounds. These two heterocyclic compounds are occupied prominent place among various classes of aromatic organic compounds. Heterocyclic compounds having a variety of therapeutic uses, including antitumor, antifungal, antiparasitic, analgesics, antiviral, antihistamine, as well as use in cardiovascular disease, neurology, endocrinology, and ophthalmology¹. Benzimidazole and Pyrazole are also effective in anticancer. All the newly synthesized heterocyclic compounds were screened for the in-vivo cytotoxic activity by XTT assay methods using various cell lines like VERO, NCI, MDA-MB-453 and MDA-MB-468².

AIMS AND OBJECTIVE: To design and identify new heterocyclic compounds to potent anticancer activity.

PHARMACOLOGICAL SCREENING METHODS

XTT assay Method³

Characterization of cell lines and culture media

Subculturing of cell lines (DMEM, FBS)

Drugs (Doxorubicin)

Initial cell viability, density and population doubling time

Preparation of plate for cytotoxic activity (Drug Addition)

After 4hrs, 12hrs, 24hrs cell viability calculation (XTT Assay)

Cell lines and Culture Medium ^{4, 5}

VERO, NCI, MDA-MB-453 and MDA-MB-468 cell cultures used in the experiments were derived from National Centre for Cell Science, Pune. Stock cells of these cell lines were cultured

in DMEM, supplemented with 10% FBS (fetal bovine serum). Along with media cells were also supplemented with penicillin, streptomycin and amphotericin-B, in a humidified atmosphere with 5% CO₂ at 37°C until confluence reached. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in phosphate buffer saline solution. The stock cultures were grown initially in 25 cm² tissue culture flasks, then in 75 cm² and finally in 150 cm² tissue culture flasks and all cytotoxicity experiments were carried out in 96 well microtitre plates.

Experiment Design

Cell lines in exponential growth phase were washed, trypsinized and re-suspended in complete culture media. Cells were plated in 96 well micro titer plate and incubated for 24 hours during which a partial monolayer form. The cells were then exposed to various concentrations of the test compounds and standard doxorubicin. Control wells were received only maintenance medium. The plates were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 24 hours. Morphological changes of drug treated cells were examined using an inverted microscope and compared with the cells serving as control. At the end of, cellular viability was determined by use of XTT assay.

Screening of Test Compounds by XTT Assay

Preparation of solutions ³⁻¹⁵

XTT labeling reagent and activation reagent was put at room temperature. Each vial was mixed thoroughly to obtain a clear solution.

XTT labeling mixture

A cell proliferation assay (XTT) with one micro-plate (96 wells) was performed by mixing 5 ml XTT labeling reagent with 0.1 ml activation reagent.

Protocol

Cells were preincubated at a concentration of 1×10^6 cells/ml in culture medium for 3 hours at 37°C and 5% CO_2 . Cells were seeded at a concentration of 5×10^4 cells/well in 100 μl culture medium and various amounts of compound (final concentration,

e.g. $100\mu\text{M}$ - $0.005\mu\text{M}$) into microplates (tissue culture grade, 96 wells, flat bottom). Cell cultures were incubated into microplates for 24 hours at 37°C and 5% CO_2 . 50 μl XTT labeling mixture was added and incubated for 18 hours at 37°C and 6.5% CO_2 . The spectrophotometrical absorbance of the samples was measured using a microplates (ELISA) reader. The wavelength to measure absorbance of the formazan product was 450 nm. (The reference wavelength was not more than 650 nm.) Percentage cell growth inhibition or percentage cytotoxicity was calculated by following formula,

$$\% \text{ viability} = (A_T - A_B) / (A_C - A_B) \times 100$$

Compounds Structure ^{16, 17}

Comp. No.	-R
1
2
3
5
6
7
8
9
10
11

RESULTS AND DISCUSSION

Anticancer activity on NCI Cell line

Figure 1: anticancer activity on NCI cell line (log conc. Vs %inhibition)

Table 1: % inhibition on NCI cell line

LOG CONC.	1	2	3	5	6	7	8	9	10	11	Std.*
2	73.113	78.349	63.207	66.037	73.113	79.905	63.632	67.169	63.207	70.012	85.283
1.5228	70.283	77.500	61.933	71.839	72.688	74.811	70.990	78.349	62.532	66.352	84.292
1.0457	66.320	72.405	61.226	70.990	72.405	53.018	58.537	67.020	60.254	64.252	76.933
0.5686	64.764	65.613	60.518	70.424	71.550	51.037	57.264	65.470	58.215	62.254	74.245
0.0915	49.481	59.245	53.726	53.160	59.386	46.509	57.547	47.641	56.21	60.592	63.632
-0.385	43.679	53.018	50.754	51.603	53.160	45.518	57.405	44.103	51.215	58.265	58.820
-0.862	29.386	47.924	49.905	48.915	51.320	44.952	54.433	34.339	50.214	53.256	56.415
-1.339	27.971	29.528	47.924	40.283	44.103	44.520	52.311	29.811	48.654	50.245	55.990
-1.816	23.443	28.537	47.783	39.009	39.433	44.386	51.179	26.839	47.256	48.654	55.000
-2.294	21.462	24.858	47.075	37.452	28.820	43.820	41.839	20.188	42.235	42.251	54.433

*Doxorubicin

Anticancer activity on MDA-MB-453 Cell line

Figure 2: anticancer activity on MDA-MB-453 cell line (log conc. Vs inhibition)

Table 2: % inhibition on MDA-MB-453 cell line

LOG CONC.	1	2	3	5	6	7	8	9	10	11	Std.*
2	76.918	78.481	67.398	70.097	75.923	78.481	63.988	71.234	58.258	67.582	86.580
1.5228	69.245	70.524	56.315	62.282	71.802	69.529	55.462	60.719	57.265	65.254	84.164
1.0457	51.484	56.457	51.910	45.089	61.140	59.725	49.068	50.205	55.254	64.258	78.054
0.5686	49.921	51.199	42.390	45.374	53.615	55.320	48.358	63.990	52.325	62.548	74.076
0.0915	49.481	44.663	45.658	41.537	34.291	52.620	41.537	73.508	51.235	58.325	63.703
-0.385	40.969	40.969	43.384	35.001	33.864	36.990	41.111	54.752	50.254	55.258	58.304
-0.862	33.012	35.427	28.890	31.449	28.465	31.165	35.001	29.744	48.254	54.268	55.320
-1.339	22.497	31.165	18.661	27.186	35.427	31.165	46.226	28.465	46.985	53.258	55.170
-1.816	21.218	24.628	18.377	22.781	28.465	29.744	39.832	18.519	45.845	49.845	54.752
-2.294	20.080	21.218	19.655	21.500	27.754	26.760	44.095	38.411	40.587	45.258	52.762

*Doxorubicin

Anticancer activity on MDA-MB-468 Cell line

Figure 3: anticancer activity on MDA-MB-468 cell line (log conc. Vs %inhibition)

Table 3: % inhibition on MDA-MB-468 cell line

LOG CONC.	1	2	3	5	6	7	8	9	10	11	Std.*
2	71.029	72.575	66.358	65.266	66.352	73.840	64.001	63.579	71.450	62.450	83.960
1.5228	69.623	71.029	65.685	56.973	65.35	69.483	59.360	53.599	69.623	55.427	80.868
1.0457	62.595	63.579	64.358	49.945	63.352	64.001	46.009	48.399	61.049	50.080	76.651
0.5686	55.427	55.286	63.589	45.447	62.21	56.551	41.230	45.447	56.129	51.210	73.418
0.0915	46.990	45.588	60.358	45.447	59.645	49.664	39.262	43.900	48.539	41.230	59.784
-0.385	41.230	41.652	58.648	41.371	58.358	37.710	40.527	44.322	41.230	35.889	54.864
-0.862	32.797	32.797	57.354	32.937	56.358	33.210	34.343	36.029	32.375	28.580	55.005
-1.339	28.861	27.174	56.358	29.564	55.351	26.893	32.375	29.283	29.283	27.455	54.443
-1.816	24.640	26.190	55.325	34.905	54.365	27.174	23.379	24.925	34.062	23.098	54.443
-2.294	18.881	24.785	50.255	17.195	49.652	25.909	20.287	21.833	27.030	18.741	52.756

*Doxorubicin

Anticancer activity on VERO Cell line

Figure 4: anticancer activity on VERO cell line (log conc. Vs %inhibition)

Table 4: % inhibition on VERO cell line

LOG CONC.	1	2	3	5	6	7	8	9	10	11	Std.*
2	10.022	22.922	14.800	15.090	14.517	15.854	11.235	14.517	20.534	22.731	12.989
1.5228	9.325	19.484	20.343	13.180	14.040	14.326	9.325	13.940	17.191	17.478	12.034
1.0457	9.235	12.989	13.180	12.034	13.276	14.517	8.268	13.276	15.090	15.854	10.888
0.5686	8.564	11.938	13.940	12.034	13.940	12.989	6.356	13.180	12.034	12.702	10.697
0.0915	7.265	14.040	15.854	11.079	11.747	10.124	5.235	11.079	8.695	7.640	6.399
-0.385	6.3254	4.393	2.674	3.342	2.865	3.151	4.259	4.393	3.304	2.483	2.473
-0.862	2.356	5.253	4.393	1.814	2.578	4.393	2.358	2.196	2.483	4.966	1.623
-1.339	1.25	4.393	3.915	2.196	4.680	3.438	1.365	1.910	2.578	3.438	1.442
-1.816	0.215	2.196	2.865	2.86	2.196	1.719	0.236	2.196	3.056	3.438	0.668
-2.294	0.111	2.960	1.337	0.191	0.382	0.191	0.135	3.056	3.056	2.674	0.191

*Doxorubicin

From the results tables and graphs we can discuss that in the cell line NCI, MDA-MB-453 and MDA-MB-468 compound no. 2 and 7 gives prominent anticancer activity with comparison of standard drug doxorubicin, whereas in the cell line VERO compound no. 2 and 11 gives prominent anticancer activity with comparison of standard drug doxorubicin.

CONCLUSION

From the discussion, we conclude that compound which contain o-nitrobenzene as attachment gives better anticancer activity in all cell lines, whereas compound contain p-fluorobenzene as attachment gives better anticancer activity in cell line like NCI, MDA-MB-453 and MDA-MB-468. The compound contains m-nitrobenzene gives positive results in VERO cell line. Both the nitro and fluorine groups are highly electronegative so it may give better activity on different cell line, hence give prominent antitumor activity.

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