Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(9):966-971

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Drug design, development and screening of pyrazolo pyridazine as potential agent for treatment of breast cancer

S. Hurmath Unnissa* and Shyaleen Rose[2]

[1]Department of Pharmaceutical Chemistry, K M C H College of Pharmacy, Coimbatore
[2]Department of Pharmacology, K M C H College of Pharmacy, Coimbatore

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ABSTRACT

Pyrazolo pyridazine are obtained by diazotization of substituted anilines followed by coupling to form corresponding hydrazones which on Intramolecular cyclisation forms 3-acetyl- substituted-cinnolin-4-ones. Further, treatment with hydrazine hydrate yields the expected 3'-methyl-substituted-pyrazolo [4, 3-C] Cinnoline derivatives. The compounds were characterized by analytical techniques like TLC, UV, IR, NMR Spectral studies. All the synthesised compounds were checked for drug likeliness using QIKPROP software and found to be efficacious and Screening for anti-tumor activity against breast cancer cell lines. The compound 1C was found to be safe and potent drug moderately incomparison to standard drug.

Keywords: Breast cancer, Pyrazolo pyridazine , Cinnoline, MCF-7 cell line, qikprop, Breast cancer

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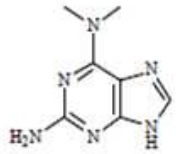
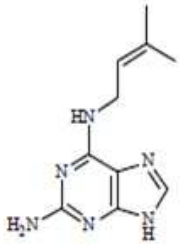
INTRODUCTION

Cancers involve a wide group of diseases, which are characterized by the unregulated cell growth in different parts of the body, affecting many tissues and organs. There are more than 200 different types of cancer, but four particular tumour types: breast, lung, colorectal and prostate constitute over half of all new cases diagnosed. Cancers are presently responsible for about 25% of deaths in developed countries and for 15% of all deaths worldwide, being therefore recognized as one of the foremost health problems, with about 1.45 million new cancer cases being expected yearly. A recent study showed that approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers), and 7.6 million people died of cancer worldwide.

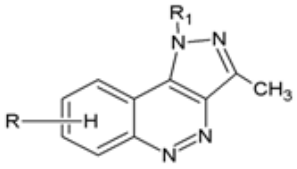
Globally, breast cancer is one of the leading causes of death due to cancer. The incidence of breast cancer is rapidly rising, amounting to a significant percentage of all cancers in women. One in nine women in the UK and USA will develop the disease in their lifetimes. It is more common in the Western countries than Africa, South America or Asia, and several aetiological factors have been implicated in its pathogenesis.[1-3] Pyrazoles are versatile lead molecule showing analgesic, anti-inflammatory, anti-cancer, anti-pyretic, antiarrhythmic, tranquilizing, muscle relaxing, MOA inhibiting, anti-diabetic and anti-bacterial activities[4,5]. Literature review shows the Pyrazole ring when fused with pyridine, pyrimidine and pyridazine show potent anti-cancer activity against various tumors by inhibition of various enzymes involved in various stages of cell cycle[6,7,8.9].Therefore efforts were taken to design and develop Pyrazolo pyridazine derivatives whose basic nucleus will be a structural analog and isosteric with nucleosides involved in cell cycle and to study its effect against breast cancer cells to get a potent anti-tumor agents.



Dimethylaminopurine



Isopentenyladenine



Pyrazolo benzpyridazine derivatives

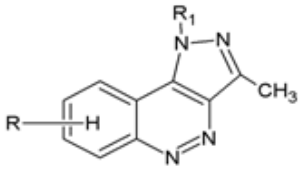
In the present investigation it has been tried to design and synthesized such novel compounds which include both the advantage of pyrazole and Benzo Pyridazine[Cinnoline] nucleus in the single molecule. All the title compounds (1c–6c) were subjected to molecular properties prediction by QikProp v3.1[GLIDE] software in order to filter the drugs for synthesis and biological screening and to reduce enormous wastage of expensive chemicals and precious time.

EXPERIMENTAL SECTION

MOLECULAR PROPERTIES PREDICTION

QikProp predicts physically significant descriptors and pharmaceutically relevant properties for organic structures. In addition to predicting molecular properties, QikProp provides ranges for comparing a particular molecule's properties with those of 95% of known drugs. QikProp produces the following descriptors and properties like molecule name, Number of property or descriptor values that fall outside the 95% range of similar values for known drugs. Number of non-conjugated amine groups. , Number of nitro groups, Number of carboxylic acid groups, Number of non-conjugated amide groups, Number of non-trivial, non-hindered ,rotatable bonds, Number of reactive functional groups, Predicted central nervous system activity on a -2 (inactive) to +2 (active) scale, Molecular weight of the molecule, Computed dipole moment of the molecule, Total solvent accessible surface area (SASA) , FOSA Hydrophobic component of SASA , FISA Hydrophilic component of the SASA ,PISAcarbon and attached hydrogen) component of the SASA,WPSA Weakly polar component of the SASA (halogens, P, and S),volume ,donorHB Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, accptHB -Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution, dip^2/V† Square of the dipole moment divided by the molecular volume,ACxDN^.5/SA- Index of cohesive interaction in solids,glob Globularity descriptor, QPpolrz- Predicted polarizability in cubic ångströms,QPlogPC16- Free energy of solvation in hexadecane, QPlogPoct- Free energy of solvation in octanol, QPlogPw- Free energy of solvation in water. QPlogPo/w- Predicted octanol/water partition coefficient. QPlogS Predicted aqueous solubility, log S. BIPCaco- Predicted apparent Caco-2 cell permeability in nm/sec usingthe Boehringer-Ingelheim scale, AffyPCaco- Predicted apparent Caco-2 cell permeability in nm/sec usingthe Affymax scale, QPlogBB-Predicted brain/blood partition coefficient, AffyPMDCK- Predicted apparent MDCK cell permeability in nm/sec using the Affymax scale, QPlogKp- Predicted skin permeability , IP(ev)† PM3-calculated ionization potential, EA(eV)† PM3- calculated electron affinity., #metabol- The number of likely metabolic reactions, QPLog Khsa- Prediction of binding to human serum albumin.

All the title compounds (1c–6c) were subjected to molecular properties prediction to check drug likeliness by QikProp v3.1[GLIDE] software in order to filter the drugs for biological screening.



Pyrazolo benzpyridazine derivatives

TABLE 1: MOLECULAR PROPERTIES PREDICTION USIJG QIKPROP

(Please find attachments qikprop table)

SEPARATE ATTACHMENT- Qikprop tables

SYNTHESIS OF PYRAZOLO PYRIDAZINE DERIVATIVES (1C-6C)

The Pyrazolo pyridazine derivatives have been prepared by the Intramolecular cyclisation of the corresponding phenyl hydrazones obtained from diazotization of substituted Anilines followed by coupling with ethyl aceto acetate in aqueous ethanolic solution containing sodium acetate and by reaction with hydrazine according to the reported procedure[10]. The physico chemical parameters and spectral data of all synthesised were analysed. Melting points were recorded on SMP1 Stuart apparatus and are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker DPX-300 spectrometer in CDCl3 with TMS as an internal standard. Mass spectra were acquired using Bruker APEX-4 instrument.

SCHEME

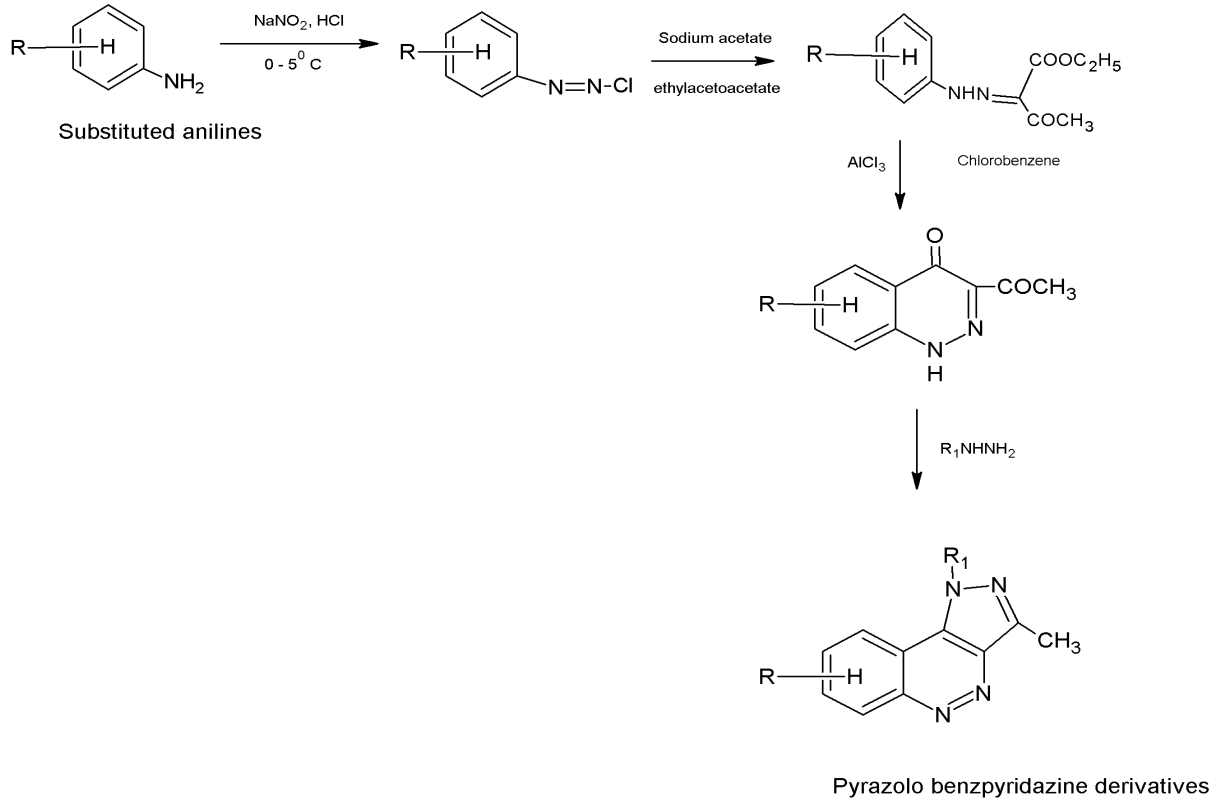


TABLE:2 Physicochemical properties of Pyrazolo Pyridazine derivatives

SL.NO	COMP CODE	R	R_1	MOLECULAR FORMULAE	M.WT (gm/mol)	% YIELD	COLOUR	SOLUBILITY	M.P ° C	[x] R_f VALUE
1	**1 C**	Cl	H	**$C_{10}H_7ClN_4$**	**218.64**	**74.5**	**Orange**	**DMSO**	**232-240**	**0.76**
2	**2 C**	H	H	**$C_{10}H_8N_4$**	**184.19**	**67.8**	**Orange**	**DMSO**	**218-220**	**0.83**
3	**3 C**	COOH	H	**$C_{11}H_8N_4O_2$**	**228.20**	**91.2**	**Yellowish brown**	**DMSO**	**300-310**	**0.54**
4	**4C**	COOH	C_6H_5	**C17H12N4O2**	**304.3**	**77.9**	**Orange**	**DMSO**	**290-300**	**0.67**
5	**5 C**	NO_2	H	**C10H7N5O2**	**229.19**	**68.4**	**Yellow**	**DMSO**	**224-230**	**0.86**
6	**6 C**	F	H	**C10H7FN4**	**202.18**	**61.5**	**Yellowish brown**	**DMSO**	**210-220**	**0.73**

[x] *Mobile phase - Chloroform: methanol - 0.2: 9.8.*

TABLE : 3 SPECTRAL VALUES OF PYRAZOLO PYRIDAZINE DERIVATIVES

Comp code	IR	^{1}H NMR	^{13}C NMR	MASS M+
1 C	2911(CH stretching)	7-8.2(Aromatic H)	109 -116c[benzene]	**219**

	3162.69(NH stretching) 1588.59(N=N stretching) 1667(C=N) 1045.71(C-N)	2.1(H in CH_3 group)	12.6 -CH3-methyl 145 –C-pyrazole	
2 C	900-675 (Aromatic group) 1661.86(C-C) 3177.63(NH stretching) 1456.96(C=N Stretching) 1236.15(C-N Stretching)	7-8(Aromatic H) 2.72(H in CH_3 group)		**185**
3 C	2954.08(CH stretching) 3107.43(NH stretching) 1581.68(N=N stretching) 1428.30(C=N) 1682.95(COOH Group)	7-8.5(Aromatic H) 2.166(H in CH_3 group) 11.6 (H in COOH)		**229**
4c	2954.08(CH stretching) 3420.62(NH stretching) 1541.33(N=N stretching) 1558.68(C=N) 1716.34(COOH Group)	7-8.5(Aromatic H) 2.18(H in CH_3 group)		**305**
5 C	2926.93(CH stretching) 3420.62(NH stretching) 1557.72(N=N stretching) 1456.93(C=N)	7-8.5(Aromatic H) 2.106(H in CH_3 group)		**230**
6 C	2911(CH stretching) 3162.69(NH stretching) 1588.59(N=N stretching) 1667(C=N)	7-8.5(Aromatic H) 2.209(H in CH_3 group)		**203**

ANTI-TUMOR ACTIVITY

CELL LINES AND CELL CULTURE

MCF-7 breast cancer cells were obtained from ATCC and were cultured in DMEM. All media were supplemented with 2 mM glutamine and 10% Fetal Bovine Serum (FBS, Gibco Life Technologies) and cells were maintained under standard cell culture conditions at 37 °C in a water-saturated atmosphere of 5% CO2 in air.

CELL PROLIFERATION ASSAY [10]

MCF-7 cells were seeded at a density of 1 × 104 and 4 × 104 per well in 96-well plates in appropriate medium. For anti-MCF-7 screening, the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations and doxorubicin as positive control.

Cell viability was assessed, after 3 days of treatment, with tetrazolium dye 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyltetrazolium bromide (MTT), obtained from Sigma (Dorset, UK). The % cell inhibition was determined using the following formula.

% Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using GraphPad Prism software.

TABLE : 4 *IN VITRO* CYTOTOXICITY AGAINST BREAST CANCER- MCF-7 CELL LINES

COMPOUND	CONCENTRATION (µM)	% GROWTH INHIBITION	IC_{50} (µM)
1 C	0.25 2.5 25 50 100	3.43 25.89 66.87 88.87 99.76	5.376
2 C	0.25 2.5	3.14 11.15	21.892

	25 50 100	69.20 87.09 98.9	
3 C	0.25 2.5 25 50 100	1.52 12.29 59.02 72.83 99.56	16.89
4C	0.25 2.5 25 50 100	3.24 6.38 69.06 80.07 98.63	12.332
5 C	0.25 2.5 25 50 100	2.28 21.9 68.12 79.28 99.98	6.34
6C	0.25 2.5 25 50 100	2.95 7.91 44.29 74.98 99.45	25.687

FIG:1 % cell inhibition of 1c

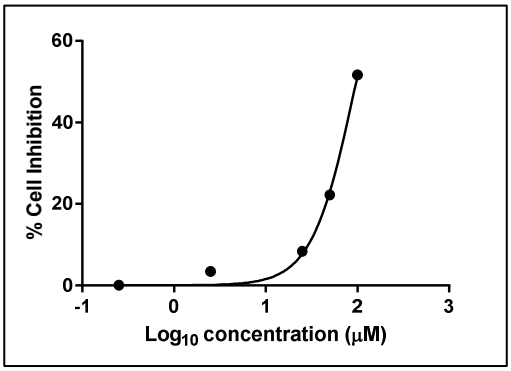
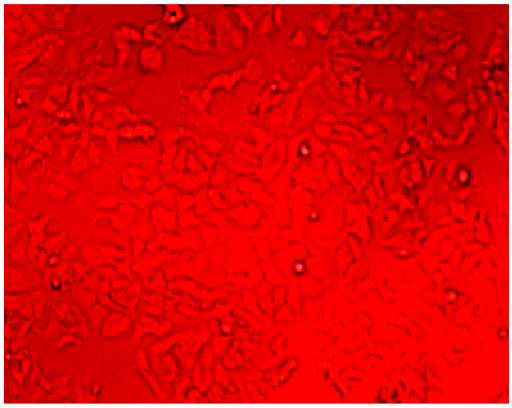


FIG:2 cell inhibiton at 25 μM



RESULTS

The Pyrazolo pyridazine derivatives have been prepared by the Intramolecular cyclisation of the corresponding phenyl hydrazones by reported methods and the physico chemical parameters and spectral data are given in Table 2&3.

All the title compounds (1c–6c) were subjected to molecular properties prediction to check drug likeliness by QikProp v3.1 [GLIDE] software in order to filter the drugs for biological screening and the prediction scores are given in Table 1.

The antitumor activity of compounds (1c–6c) was characterized by conducting cell inhibition against of MCF-7 breast cancer cells using the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) the results are shown in Table 4. Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC50 was determined using Graph Pad Prism software and graph of compound 1C is given in fig. 1

DISCUSSION

Some novel Pyrazolo pyridazine and its substituted derivatives were synthesized. The melting points were found and were uncorrected. The purity of the synthesized compounds, checked by thin layer chromatography was found to be pure. The structures of the compounds were elucidated by UV, IR and NMR spectral studies and was found to be in specified ranges.

From the Qikprop predictive values, none of the compounds violated drug likeliness parameters, making them potentially promising agents for anti-tumor therapy.All the synthesised compounds shown moderate MCF-7 cell inhibition, among which Compounds **1C**, 4C, and **6C** showed potential anti-MCF-7 activity and were able to reduce the viability. the determined IC50 values are given in the Table :4

Acknowledgement
The authors are thankful to KMCH college of Pharmacy, Coimbatore for providing all facilities for this research. We acknowledge CUSAT, STIC, COCHIN for spectral studies.

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