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Research Article

EXPLORATION OF ANTICANCER POTENTIAL OF SPIROPYRANOPYRAZOLE DERIVATIVES AS CDK7 INHIBITORS

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ABSTRACT :

Purpose : The Spiropyranopyrazole derivatives are used as cytotoxic agents and nitrogen containing heterocyclic analogs targeting CDK7 inhibition shows better cytotoxic activity. Methodology: A new series of compounds were synthesized using various substituted isatin derivatives and then characterized and analyzed for biological activity by in-silico and using MTT assay targeting CDK7. All the synthesized compounds were analyzed for their biological activity for this purpose breast cancer cell lines (MCF7) were used and analyzed by MTT assay. Docking studies into ATP binding site of CDK7 were performed to predict their binding affinity scores and possible interactions with receptor to evaluate bioactivity in-silico using VLife MDS 4.3.

Findings : A novel series of Spiropyranopyrazole derivatives were successfully synthesized via Multicomponent reaction (MCR). From experimental data indicated that compounds 2c and 3c showed most promising results as their inhibitory activity with 23.20% and 26.50% respectively at 10μM and these were selected for further preclinical studies.

Social Implications : If the present findings of spiropyranopyrazole derivatives passes preclinical studies and we develop drug like candidate then it is a massive achievement in anticancer therapy that could save many lives.

Original : Successfully develop a novel series of spiropyranopyrazole having CDK7 inhibitor activity. This could be helpful for development of a drug like candidate having significant cytotoxic activity.

Key words : *Cell cycle, CDK7, Isatin, Spiropyranopyrazole derivatives, Anticancer, VLife MDS 4.3.*

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1. INTRODUCTION

Living habits, cancer and environment changes have become the largest cause of death. Cancer is reflecting a multi-step process, reflecting from acquired defects in genes involved in the mutation or mis-regulation of cell cycle controlling genes and proteins to guide an abnormal control of cell proliferation[1]. Cancer is the second largest cause of mortality in the last decade. Many of the genes involved in cell cycle progression are frequently mutated, leading to uncontrolled cell division. Progression of a cell through the cell cycle is dependent on one family of serine/threonine kinases, called CDKs[2-6]. The CDK7 is a major constituent of both CDK-activating kinase (CAK) and transcription factor TFIIH, involved in upstream of cell cycle regulatory CDKs and it directly affects on transcription[7]. The CDK7 is highly expressed in many types of cancers especially

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breast and colon cancers[8]. Hence CDK7 have been actively considered as active targets for anticancer therapy[9]. This knowledge provides information about recent rationale advances for considering the cell cycle and its complexes involved in regulation system as potential targets for design and development of new selective drugs in cancer therapeutics[10]. Targeting of CDK7 receptor is a new approach for the design and development of novel anticancer drugs[11]. Molecular docking studies of all the synthesized compounds 1a-3c into ATP binding site of CDK7 was performed to investigate the ability of these novel derivatives to inhibit these tumorogenic agents.

2. EXPERIMENTAL

2.1 Materials and Methods :

All the used reagents were purchased from Sigma-Aldrich, HiMedia Laboratories Ltd. and Loba-Chemie Pvt. Ltd. and were used without further purification. Shimadzu BL220H analytical balance was used for the weighing purposes. The progress of the reactions were monitored by TLC using ether/chloroform as a mobile phase on pre-coated Merck TLC plates in iodine chamber. All melting points were recorded on Stuart melting point apparatus (SMP-30) with open glass capillary tube and were uncorrected. IR spectra of compounds were recorded with KBr on a Bruker FT-IR spectrophotometer. ^{1}H NMR spectra were obtained in DMSO on 400 MHz Bruker Advance II NMR spectrometer using TMS (d=0) as an internal standard.

2.2 Synthesis :

Molecules for the present study were synthesised via procedures reported by Pore et al.[12], 2013 and Yu et al.[13], 2013 showed in Fig. 1 and table No. 1.

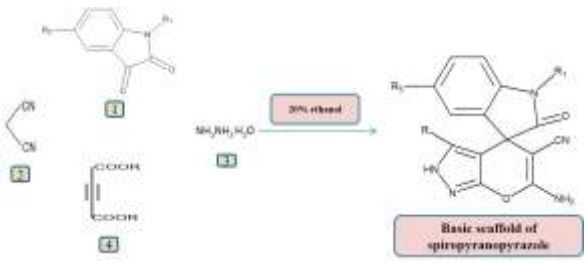
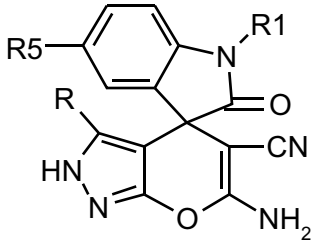


Figure 1: Synthetic scheme

Table 1: Showing spiropyranopyrazole derivatives synthesized and taken for study.



General Structure			
Compounds	**R1**	**R5**	**R**
1a	H	H	COOC2H5
1b	H	Cl	COOC2H5
1c	COCH3	H	COOC2H5
1d	CH2CH3	H	COOC2H5
2a	H	H	COOCH3
2b	H	Cl	COOCH3
2c	COCH3	H	COOCH3
3a	H	H	CH3
3b	H	Cl	CH3
3c	COCH3	H	CH3

2.2.1 General procedure for the synthesis of pyrano[2,3-c]pyrazole derivatives :

A mixture of DEAD or DMAD (1 mmol) and hydrazinehydrate (1 mmol) was stirred for 4-5 min at room temperature. Then 20% ethanol (5 mL), malononitrile (1 mmol) and substituted isatin (1 mmol) were added and stirring mixture at reflux temperature for 10 min. Finally, the precipitated product was filtered and washed with distilled water and dried to get pyrano [2, 3-c] pyrazole derivatives[12].

2.2.2 General procedure for the synthesis of spiro[indoline-3,4'-pyrano [2,3-c] pyrazole] derivatives :

A 50 mL flask charged with hydrazine (1.2 mmol), β-keto ester (1 mmol), substituted isatin (1 mmol), malononitrile (1 mmol) and L-proline (0.1 mmol) was stirred at 80 °C in water (5 ml. The completion of the reaction was monitored by TLC; the reaction mixture was cooled to room temperature. Then the precipitated product was filtered and washed with water and cold ethanol to afford the pure product as a solid in good to excellent yields[13].

2.3 MOLECULAR DOCKING

Docking studies of all designed compounds were performed by downloading 3-dimensional crystal structure of CDK7, retrieved from Protein Data Bank (PDB ID: 1UA2) and further refined for protein preparation by removing water molecules and then hydrogen was added and pocket modelling was carried out using proviz module of VLife MDS 4.3 software. The designed spiropyranopyarazole derivatives were drawn in 2D and then converted into 3D. Finally energy was minimized using Merck Molecular Force Field (MMFF) and residues at surrounding of the active pocket at a distance less than 5 Å from reference ligand surface were marked. The selected pocket was used for optimization of structural requirements of designed compounds. We docked all designed compounds into the active site of the receptor and then recorded H-bonding, Pi-stacking, hydrophobic and van der Waal interactions with the designed compounds. The spiropyrano-pyarazole moiety considers as a basic scaffold and was used as template to built compounds in dataset of module builder of VLife MDS 4.3 software[14].

2.4 BIOLOGICAL EVALUATION

2.4.1 Cell culture and treatments :

Cell lines (Breast cancer cell lines MCF7) were procured from National cell repository situated at National Centre For Cell Science (NCCS), Pune. Breast cancer cell lines were grown in DMEM media supplemented with 10% fetal bovine serum (FBS) and antibiotic solution (1×Penstrip). Cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) media with 10% FBS, 50 U/mL penicillin G, 50 µg/mL streptomycin sulfate and 1.25 µg/mL amophotericin B. The cells were incubated at 37^{0}C with 5% CO_2 and 95% humidity conditions. For experiments, all cells were seeded in equal numbers after trypan blue cell counting (5,000 cells per well of 96-well plates). Then cells were washed once with sterile 1× PBS and cultured with serum free media for 8 h for synchronization. The test compounds were dissolved in cell culture grade DMSO upto concentration of 100 mM and further dilutions were done in serum free DMEM media. The total amount of media per well (200 µL per well of 96 well plate) was kept constant[15,16].

2.4.2 MTT [3-(4, 5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay :

Evaluation of anticancer activity of synthesized compounds was carried out using MTT assay (Breast MCF7 cell lines). MTT assay was carried out using 96-well plates (total volume of media was 200 µL/well). After the treatments cells were washed with 1× PBS and were mixed with 100µL/mL well of MTT (5 mg in 10 mL of 1×PBS) and finally incubated at room temperature for 24hrs to allow formation of formazan crystals. Each well was then mixed with 100µL of DMSO to dissolve the crystals followed by ELISA readings at 570 nm[15,16]. Anticancer activity was evaluated as a function of percent inhibition with respect to 5-FU as a standard and was calculated by the following formula:

$$\% \text{ inhibition} = (1-[A_{570\text{treated}}/A_{570\text{control}}]) \times 100\%$$

3. RESULT AND DISCUSSION

3.1 Chemistry :

Multicomponent reactions used in the synthesis of the target compounds are illustrated in Fig.1. A new series of compounds pyrano [2, 3-c] pyrazole and spiro [indoline-3, 4'-pyrano [2, 3-c] pyrazole] derivatives were synthesized by following the reported procedures. Moreover, all synthesized compounds were achieved in good yield.

3.2 Anticancer activity :

All synthesized compounds 1a-3c were evaluated for their anticancer activity against breast MCF7 cancer cell lines in comparison with marketed 5-FU as standard drug. The anticancer activities (µM) of two series of compounds pyrano[2,3-c]pyrazole and spiro[indoline-3,4'-pyrano [2,3-c]pyrazole] derivatives are shown in Table 1. The percent inhibition values of compound demonstrated that all compounds showed significant inhibition against CDK7 at concentrations 10 µM, 20 µM and 50 µM except compounds 3a and 3c. The most active compounds 1c, 2c and 3b with percent inhibition values 15.67%, 23.20% and 26.50%, respectively as shown in table 2.

Table 2: Percent inhibition of compounds (1a-3c) against breast cancer MCF7 cell lines

Table 1: Showing spiropyranopyrazole derivatives synthesized and taken for study.

Compound No.	% inhibition 10 µM	20 M	50 µM
1a	4.91	6.24	8.25
1b	4.60	7.50	22.64
1c	15.67	21.33	27.73
1d	12.69	13.72	20.55
2a	13.45	17.85	25.55
2b	9.75	12.14	13.86
2c	23.20	28.78	27.50
3a	ND	ND	ND
3b	26.50	29.95	34.73
3c	ND	ND	ND
5-FU[a]	17.27	29.11	40.12

ND: not determined
5-FU[a] was used as a reference drug.

3.3 Docking studies :

We find out the binding mode for all the synthesized compounds, docking studies were conducted using VLife MDS 4.3 (figure 2). From the docking procedures we were satisfied with the all designed compounds, the target compounds were subjected to V Life MDS 4.3 as described in the experimental section. Compounds 1c, 2c and 3b shows good interactions with CDK7 and also shows better bioactivity hence designed leads are superior. Compound 1b virtually showed good interactions with the CDK 7 via formation of hydrogen bond interaction ASN414, LEU 114 and MET 125. Compound 1b also showed significant aromatic interaction with PHE 91. Compound 2c showed hydrogen bond interaction with LYS114 and LEU 144. Compound 3c showed three hydrogen bond interactions with ASP155, ASN 141 and ASP 97 (Figure 3, 4 and 5). Compound 1b not produced promising activity because there are two main reasons taken, concentration not sufficient for produce toxicity or there is no direct correlation between binding interactions and its biological activity.

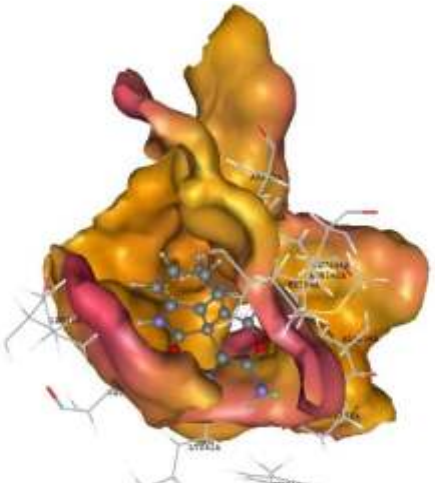


Figure 2: The best pose of the compounds 2c obtained from docking study in the active site of CDK7.

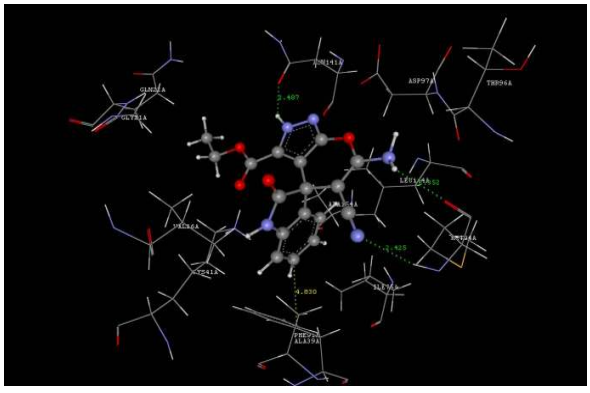


Figure 3 : A representative models for interaction of compound 1a the CDK7. The H bonding and p-p interactions are illustrated as green and yellow dashed line, respectively.

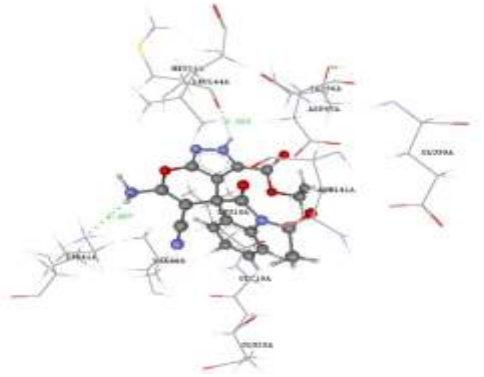


Figure 4 : Representatives model for interaction of compound 2c the CDK7. The H bonding interactions are illustrated as green dashed line.

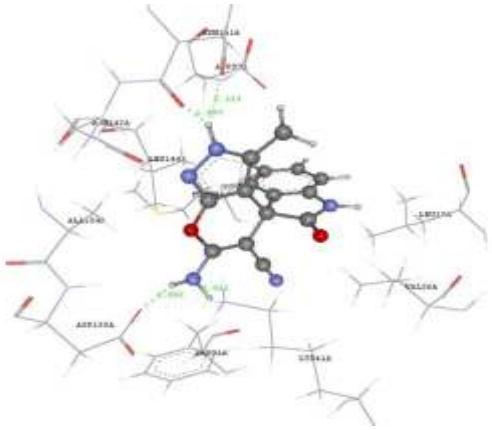


Figure 5 : Representatives models for interaction of compound 3b the CDK7. The H bonding interactions are illustrated as green dashed line.

3.4 Pharmacokinetic study :

Predicted percent oral absorption of all synthesized compounds was calculated by considering total polar surface area (TPSA). Calculated predicted percent oral absorption by using following formula Predicted percent oral absorption = 109 – (TPSA × 0.345) × 100. All derivatives showed acceptable Lipinski parameters and oral absorption within 61% and 75% as shown table no. 3[17,18,19].

Table 3: The Lipinski's filter for designed molecules

Compound	MW	HDC	HAC	RBC	Log P	% ABS
1a	351.3215	3	5	6	1.695	61.587
1b	337.2946	3	5	5	1.282	72.652
1c	371.7394	3	5	5	1.901	61.785
1d	385.7662	3	5	6	2.314	68.247
1e	477.2212	3	5	6	2.793	65.574
2a	393.3588	2	6	8	1.763	68.333
2b	379.3319	2	6	7	1.350	66.142
2c	379.3752	2	5	8	2.247	75.534
2d	365.3484	2	5	7	1.834	65.267
3a	293.2848	3	3	3	1.654	71.348
3b	327.7296	3	3	3	2.273	69.415
3c	335.3220	2	4	5	1.722	66.142

**HAC: H-Acceptor Count*
HDC: H-Donor Count
RBC: Rotatable Bond Count
%ABS: % Oral Absorption

4. CONCLUSION

A novel series of spiropyrano[2,3-c]pyrazole and spiro[indoline-3,4'-pyrano [2,3-c]pyrazole] derivatives have been successfully synthesized via Multicomponent reaction (MCR). Evaluation of their anticancer activity against breast cancer MCF7 cell lines and molecular docking were studied with CDK7. It was observed that all synthesized compounds were active toward the tested breast MCF7 cell lines, in which compound 2c and 3c were shown to be most potent activity with 23.20% and 26.50%, respectively compared with standard 5-FU without affecting on normal cell line. There were good correlations between virtual screening and observed biological activities of lead molecules which suggest that rationale used to optimize structural requirements of ligand with respect to selected targets was appropriate. These studies have confirmed identification of novel lead compounds 2c and 3b for further investigation which may produce therapeutic agents for treatment of breast and ovary cancer.

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