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Cytotoxic Activity of Some Spirooxindole-4*H*-pyran Derivatives

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Authors' contributions

This work was carried out in collaboration among all authors. Author ZF prepared the cytotoxicity tests. Author ZF analyses the cytotoxic results. Author MD managed the literature searches. Author SK wrote the manuscript and submitted it to the journal site. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Isatin is a honored scaffold and one of the most favorable class of heterocyclic systems that possesses many interesting biological activities and well-tolerated in humans. Here a series of fifteen spirooxindole-4*H*-pyran derivatives containing both isatin and pyran moieties ***(IC_a-IC_o)*** will be examine for their anti-cancer activity.
Study Design: Cytotoxic evaluation of some spirooxindole-4*H*-pyran derivatives in two cancerous cell lines.
Place and Duration of Study: Pharmaceutical Science Research Center and Shiraz Institute for Cancer Research, Medical School in Shiraz University of Medical Sciences, Shiraz, Iran, between June 2018 and July 2019.
Methodology: MTT assay was used to evaluate the cytotoxic activities of these compounds. The anticancer properties of the tested compounds were determined using A549 and MCF-7 cell lines.
Results: Among the tested compounds ***IC_c***, ***IC_d*** and ***IC_f*** showed the best cytotoxic activities against both cancerous cell lines. Compounds ***IC_h*** and ***IC_i*** showed desirable cytotoxic

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activities against A549 cell line. Compound IC_b showed desirable cytotoxic activities against MCF-7 cell line.
Conclusion: We conclude that the isatin-linked pyran analog can serve as a prototype molecule for further development of a new class of anticancer agents.

Keywords: Spirooxindole-4H-pyran; cytotoxic activity; MTT.

1. INTRODUCTION

Molecular hybridization is a new idea in drug design based on the combination of pharmacophoric moieties of unlike bioactive substances to produce a new hybrid compound with developed affinity and efficacy [1,2]. Nowadays cancer is a major problem of disease worldwide and in many countries, cancer ranks the second most common cause of death following cardiovascular diseases [3,4]. Although policy of the new chemotherapy agents is growing speedily, effective chemotherapy agent has not been discovered against the advanced stage of cancer. The cancer cell resistance against the anticancer agents can be happened by different mechanisms [5-7]. Cancers cells have the ability to develop chemotherapy resistance which is a major problem fronting current cancer treatment [6,8,9]. Therefore, there is an urgent need to improve new classes of chemotherapeutic agents to treat cancer. Isatin (1*H*-indole-2,3-dione) and its products represent a main class of heterocyclic compounds that can be used as precursors for drug synthesis [10-13]. Recently it has reached excessive attention in medicinal chemistry due to its numerous biological actions. The firmness of its indole ring has interested medicinal chemists to present many pharmacophore moieties to obtain novel bioactive compounds [14]. On the other hand pyran is an oxygen-containing heterocyclic moiety, which shows a range of pharmacological properties. Pyran is also one of the important structural subunits found widely in natural products, e.g. coumarins, benzopyrans, sugars, flavonoids, xanthones, etc [15-17]. There are also some publications have reported that the combination of isatin and pyran nucleus could be a worthy choice for anticancer activity against various human tumor cell lines [18-20]. The hybrid approach can also be used to optimize certain biological properties like affinity and selectivity, but also to gain novel biological activities distinct from the ones of the components. It will be shown that hybrids can be more than the sum of their components, but in many cases should be considered as pharmacological entities in their own respect [14, 21]. So due to the great potential of isatin and pyran moieties, we synthesized fifteen new analogous of spirooxindole-4*H*-pyran compounds in our previous work. We prepared these compounds via a three step reactions including alkylation, schiff bases and cyclocondensation [22]. In all our compounds isatin and pyran tings are linked together via a spiro carbon atom (Table 1). Our compounds are differing in the alkyl chain and also in the substitution on the pyran ring. Here we are going to evaluate these compounds against two tumor cell lines A549 and MCF-7.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals and solvents were provided from Merck Company (Germany). Selected synthesized compounds, as illustrated in Table 1, were provided from our previous study (22). Cisplatin was used as positive control.

2.2 Cell Lines and Cell Culture

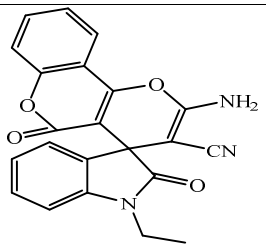
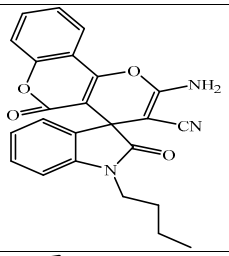
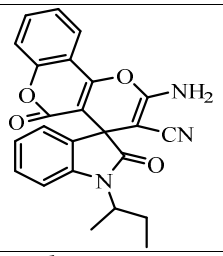
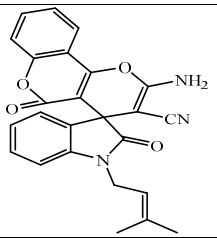
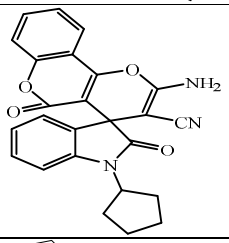
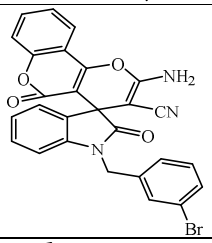
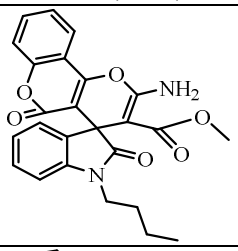
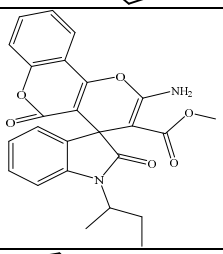
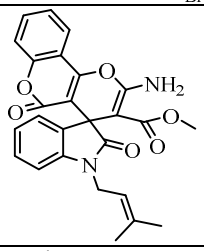
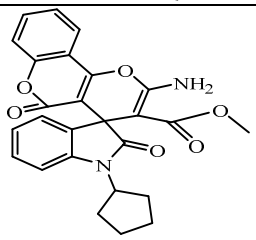
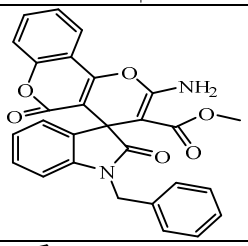
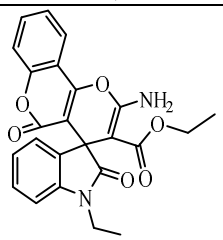
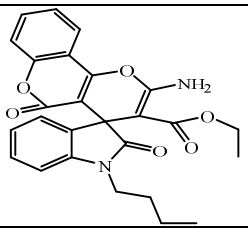
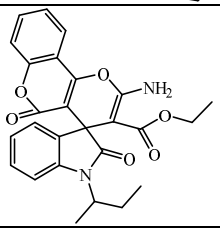
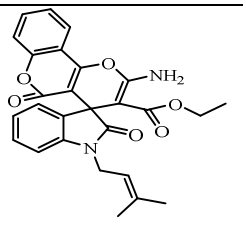
Two human cancer cell lines, A549 (lung carcinoma) and MCF-7 (breast carcinoma) were purchased from the National Cell Bank of Pasteur Institute of Iran. Using aseptic techniques, the cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Biosera, France), containing 10% fetal bovine serum (FBS) (Biosera, France), 100 unit penicillin and 100 µg/ml streptomycin (Biosera, France), and incubated at 37°C in a humidified atmosphere with 5% CO_2. Following enough confluence, the cells were treated with 25% trypsin–EDTA (Biosera, France) and sub-cultured. The cells were then washed, counted and prepared for cytotoxic MTT assay as previously described [23, 24].

2.3 MTT Assay

The cytotoxic effects of spirooxindole-4*H*-pyran compounds were investigated using a standard MTT assay. Briefly the cells were plated at the

8×10^3 cells concentration for A549 and 10×10^3 cells concentration for MCF-7 in 100 µl complete culture media per well. Then, the cells were incubated for 24 h to reattach. After attachment the cells were treated with different concentrations of each compound (1–500 µM). Three wells were left without treatment as cell-based negative controls, and three wells containing cell culture medium alone were considered as blanks. After 72 h incubation, the culture media were completely removed and 100 µl of MTT solution with 0.5 mg/ml concentration were added to the wells including controls. The plate was incubated for 3–4 h at 37°C and checked periodically for the appearance of purple precipitate. Then, after complete removing of MTT solution, 150 µl of DMSO was added to the wells and leaved in the 37˚C incubator for more 30 min. The absorbance of all wells including the blanks, were measured at 492 nm. Each experiment was separately repeated at least three times.

Table 1. Chemical structures of the spirooxindole-4*H*-pyran derivatives

Entry	Structure	Entry	Structure	Entry	Structure
IC_a		IC_b		IC_c	
IC_d		IC_e		IC_f	
IC_g		IC_h		IC_i	
IC_j		IC_k		IC_l	
IC_m		IC_n		IC_o	

2.4 Data Analysis

Excel 2013 software package was used for calculation. The average values from triplicate readings were determined and subtracted the average value for the blank. The inhibitory concentration (IC) of each compound was calculated and reported using following formula:

$$IC = 100 - [(OD_{test} - OD_{blank}) / (OD_{negative}) \times 100]$$

For each chemical a plot of the IC versus concentration was depicted using Curve Expert 1.4 software and an Inhibition Concentration 50 (IC_{50}), indicating the 50% growth inhibition of the cells was obtained for each compound. P values less than 0.05 (two-tailed) were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Cytotoxic Activities

The MTT colorimetric test is a customary method of determining viable cell number in proliferation and cytotoxicity studies. As the MTT assay is rapid, appropriate, and cost-effective, it has become a very prevalent technique for quantification of viable cells in culture [25]. The cytotoxic effects of fifteen spirooxindole-4*H*-pyran analogues ***(IC_a-IC_o)*** were determines using a colorimetric MTT assay on A549 and MCF7 cancer cell lines as in-vitro models. In 6 compounds ***(IC_a-IC_f)*** there is a cyano group on the pyran ring, in 5 other compounds ***(IC_g-IC_k)*** there is a methyl ester and in the 4 compounds ***(IC_l-IC_o)*** there is a ethyl ester on the pyran ring. The IC_{50} for each compound is displayed in Table 2. As shown in this table, compounds ***IC_c, IC_d*** and ***IC_f*** showed cytotoxic activity against both investigated cell lines in lower concentration. Compound ***IC_f*** was the most active compound with IC_{50} between 16-29 µM/ml against both cell lines. It seems that the cyano group is more effective for cytotoxic activities. Compounds ***IC_g*** and ***IC_k*** were also the second and third active compounds against A549 and MCF7 with IC_{50} between 247-436 µM/ml. In the point of substitution on the isatin ring it appears that aromatic substitution such as benzyl and bromobenzyl were more active. Our results indicated that among the other analogues, compound ***IC_n*** also showed desirable cytotoxic activities against both cell lines. Compounds ***IC_h*** and ***IC_j*** were active against A549 with IC_{50} between 332-516 µM/ml. Compounds ***IC_b*** and ***IC_i*** were active compounds against MCF7 with IC_{50} between 179-881 µM/ml. Of the alkyl substitutions on the isatin ring the branched alkyls such as 2-butene or isopentene showed more cytotoxic activities.

Table 2. In vitro cytotoxicity effects of spirooxindole-4H-pyran on different cancer cell lines

Compoumds	A549	MCF-7
IC_a	>1000	>1000
IC_b	>1000	179.42 ± 0.58
IC_c	34.14 ± 1.91	37.58 ± 1.18
IC_d	94.49 ± 1.96	65.13 ± 1.49
IC_e	>1000	>1000
IC_f	29.33 ± 2.22	16.18 ± 0.53
IC_g	272.30 ± 40.79	247.99 ± 17.86
IC_h	332.54 ± 19.97	>1000
IC_i	>1000	881.59 ± 106.88
IC_j	516.04 ± 29.98	>1000
IC_k	371.54 ± 25.92	436.04 ± 98.18
IC_l	>1000	>1000
IC_m	>1000	>1000
IC_n	411.17 ± 12.26	502.04 ± 57.08
IC_o	>1000	>1000

4. CONCLUSION

Singh et al. synthesized some hybrid of isatin and coumarin analogues which showed desirable cytotoxic ability [26]. Thota et al. also synthesized some coumarin thiazole derivatives enclosing indole ring with high cytotoxic potency [27]. We synthesized some novel spiro compounds of isatin covering pyran ring as well in our previous work [22]. Our compounds contain different substitutions in nitrogen atoms of the indole and also pyran rings. Compound ***IC_c, IC_d*** and ***IC_f*** with 2-butene, isopentene and bromo-benzyl substitutions on the indole ring respectively and cyano group in pyran ring were the most active compounds against both tested cell lines. Compounds ***IC_g*** and ***IC_k*** with butane and benzyl substitutions on the indole ring respectively and methyl carboxylate group in pyran ring showed moderate cytotoxic activities. Compounds ***IC_h*** and ***IC_j*** with 2-butane and cyclopentyl substitutions on the indole ring respectively and methyl carboxylate group in pyran ring showed moderate cytotoxic activities against A549. Compound ***IC_b*** with butane substitution on the indole ring and cyano group in pyran ring showed enough cytotoxic activities against MCF7. We conclude that the isatin-linked pyran analog can serve as a prototype molecule

for further development of a new class of anti-breast cancer agents.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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