Synthesis and cytotoxic activities of some heterocyclic chalcones

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Abstract

Chalcone (1,3-diphenylprop-2-en-1-one) compounds are of natural or synthetic origins and possess a diversity in bioactivities. Recently, the inhibitory activities of heterocyclic chalcones against different cancer cell lines have raised much concern.

The study aimed at synthesizing some heterocyclic chalcones and determining their *in vitro* cytotoxicity on rhabdomyosarcoma (RD) cell line.

Heterocyclic chalcones in this study were developed on the basis of the idea that heteroaryl moieties either on ring A or ring B of chalcones might provide cytotoxicity on cancer cells. These compounds were prepared by Claisen-Schmidt condensation the *in vitro* cytotoxicity of the compounds on rhabdomyosarcoma (RD) cell line has been evaluated using the microculture tetrazolium (MTT) assay.

A total of synthesized heterocyclic chalcones has been synthesized and their structures were elucidated by spectrometric methods. Bioassay results revealed that 5 out of 20 compounds showed activities against RD cells with IC_{50} values less than 20μM. The compound **7** possessing two heterocyclic moieties (phenothiazine as A ring and thiophene as B ring) was considered as the most potential with IC_{50} at 12.51 μM (compare with taxol having IC_{50} at 10.88μM).

The results demonstrate that heterocyclic chalcones are promising compounds for developing anticancer drugs.

Keyword: Heterocyclic chalcones, cytotoxicity, rhabdomyosarcoma, MTT

1. INTRODUCTION

The devastating ravages of cancer have become a current major problem the global human population is facing. Statistics show that cancer is second only after cardiovascular disease as the leading cause of death in the U.S. and the leading cause of death in the UK.[1,2] Some of the dominant drawbacks of present anticancer drug therapy involve their lack of significant greater toxicity towards cancer cells in comparison with normal tissue and the rise of multi-drug resistance. Tumor-selective cytotoxic agents whose structures are sufficiently different from anticancer medication currently on the market are therefore highly sought.

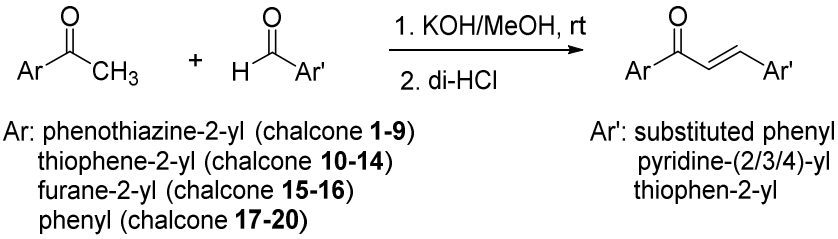
Chalcones, also known as α,β-unsaturated ketones, form a central scaffold of various

plant flavonoids and isoflavonoids. This family has been documented with diverse biological function, including antibacterial, antifungal, anti-inflammatory, anticancer, antiproliferative and antidiabetic activities and some of them have received approval for clinical application or tested in humans.[3,4] Notably, synthetic heterocyclic chalcones, whose either ring A or ring B of the original structure is replaced by heteroaryl moieties hold promise as potential agents for cytotoxic activities on various cancer cell lines.[5] Thus, the identification of new heterocyclic chalcones with anticancer activities is of great interest. Towards this objective, we have synthesized 20 heterocyclic chalcone compounds and evaluated for their cytotoxicity on rhabdomyosarcoma (RD–A) cells by MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) cell proliferation assay[7].

2. MATERIALS AND METHODS

2.1. Chemical synthesis

Chemical reagents were purchased from Acros, Merck and Sigma Aldrich used directly without further purification. Heterocyclic chalcones in this study were developed on the basis of the idea that heteroaryl moieties either on ring A or ring B of chalcones might trigger cytotoxicity on cancer cells. The structures of studied compounds were shown in Table 1.



Scheme 1. General key step for the synthesis of chalcones

General procedure: to solution of Acetyl heteroaryl ketone (2 mmol) and appropriated aromatic aldehyde (2 mmol) in methanol (15 mL) cooled to 5-10°C in an ice bath added a small portion of pulverized KOH (2.5 mmol). The reaction mixture was magnetically stirred for 60 minutes and then left overnight or longer, monitored by T.L.C. The reaction mixture was diluted with ice water and carefully neutralized using diluted HCl acid. The target chalcone which separated as yellow solid was further purified by crystallization from methanol. All the synthesized chalcones were taken their analytical spectroscopy and elucidated structure (data not showed).

2.2. Evaluation of cytotoxic activities

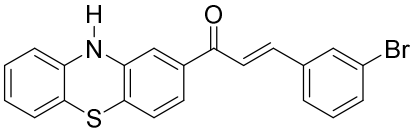
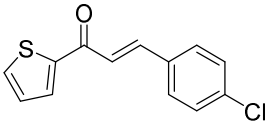
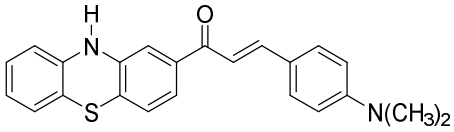
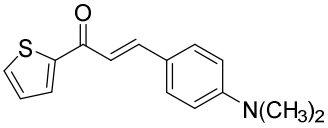
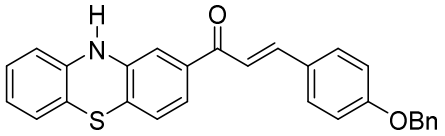
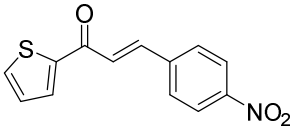
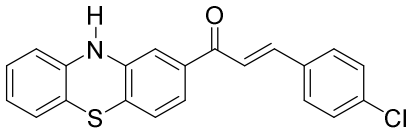
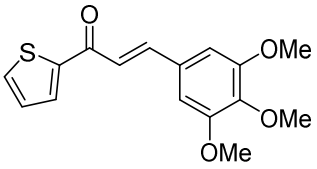
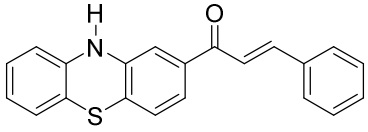
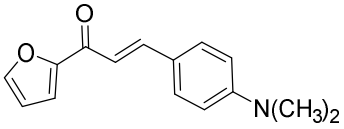
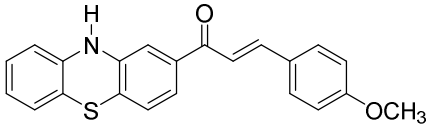
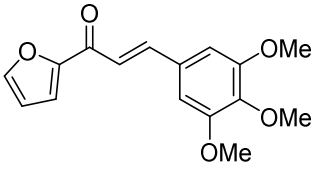
General procedure: RD-A cells suspension was harvested from the EMEM medium containing 10% FCS, 2 mM L–glutamine and 100 IU/ml penicillin. The samples were prepared in DMSO in the 5 concentrations of 100; 50; 25; 12.5 and 6.25 μM. The cells were incubated in 96–well plates and treated with samples at 5 different

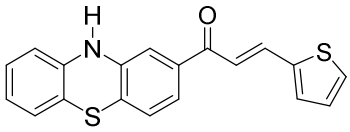
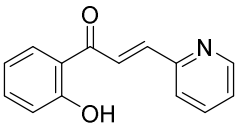
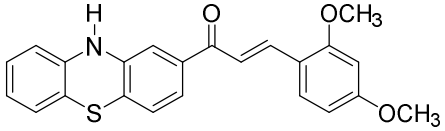
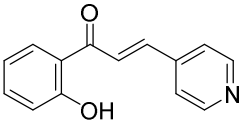
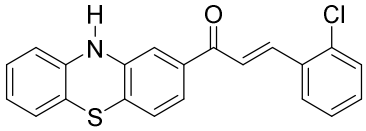
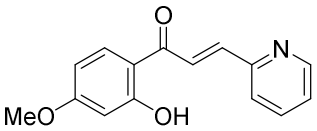
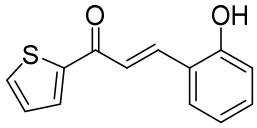
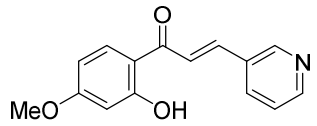
concentrations in 24 hours. After removal of the incubation medium, 0.05 mg/mL MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) was added and the wells was subjected to continued incubation in 3 hours with 5% CO_2 at 37°C. Absorbance values at the wavelength of 570 nm were recorded using microplate Multiskan™. The percentage of proliferation inhibition was calcutated as following: % Proliferation inhbition = 100 – ($A_{sample}/A_{control}$) × 100

Assay was performed in duplicate and IC_{50} was deduced by linear regression of the inhibitory percentage – log (concentration).

3. RESULTS AND DISCUSSION

Table 1. Heterocyclic chalcones' structures and their cytotoxicities

No	Structure and IC_{50} (µM)*	No	Structure and IC_{50} (µM)*
1	 **IC_{50}> 100 µM**	11	 **IC_{50}> 100 µM**
2	 **IC_{50}> 100 µM**	12	 **IC_{50}> 100 µM**
3	 **IC_{50}> 100 µM**	13	 **IC_{50}> 100 µM**
4	 **C_{50}> 100 µM**	14	 **IC_{50} = 24.75 µM**
5	 **IC_{50} > 100 µM**	15	 **IC_{50} = 77.34 µM**
6	 **IC_{50} > 100 µM**	16	 **IC_{50} = 18.04 µM**

7	 **IC_{50} = 12.51 µM**	17	 **IC_{50} = 91.12 µM**
8	 **IC_{50} > 100 µM**	18	 **IC_{50} = 92.85 µM**
9	 **IC_{50} = 18.04 µM**	19	 **IC_{50} = 20.77 µM**
10	 **IC_{50} = 49.55 µM**	20	 **IC_{50} = 26.62 µM**

**: taxol (paclitaxel) was used as reference with IC_{50} = 10.84 µM*

Overall, the studied heterocyclic chalcone compounds demonstrated low to strong cytotoxic properties on RD–A cells, in which compounds **7**, **8**, **9**, **19** and **20** exhibited promising potentials with predicted IC_{50} value of below 10 µM. These results are in consistent with reported initial structure-activity relationship of anticancer chalcones, i.e., that heteroaryl moieties either on ring A or ring B of chalcones provide cytotoxicity on cancer cells.[6] For phenothiazinyl chalcones, it is notable that an electronegative center at the position 2 of ring B enhance cytotoxicities greatly (**7** and **9**). In terms of chalcones with ring B replaced with pyridine moieties (typically **19** and **20** in comparison with **17** and **18**), a hydroxy group at position 2' and a methoxy group at position 4' on ring A also enhance the cytotoxic properties.

4. CONCLUSIONS

Several lines of investigation suggest that heterocyclic chalcone compounds may be effective anticancer agents. The study presented herein demonstrates that some representative heterocyclic chalcones, namely, phenothiazinyl chalcones and pyridinyl chalcones exhibit promising anticancer properties. These structures are simpler than those of anticancer drugs using for chemotherapy recently. As a result, these compounds may be promising leads for the development of effective anticancer agents.

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7. Tim Mosmann, Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays, Journal of lmmunological Methods. 1983; 65, 55-63