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Discovery of a new tetramethylpyrazine based chalcone with α, β-Unsaturated ketone moiety as a potential anticancer agent

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ABSTRACT

In this study, a new ligustrazine-based chalcone molecule has been synthesized that contains an extra α, β-Unsaturated ketone moiety along with α, the β-Unsaturated carbonyl group of chalone. A new tetramethylpyrazine (TMP) based aldehyde was synthesized to make the TMP (ligustrazine) as part of chalcone and then it was reacted with newly synthesized ketone containing additional α, β-Unsaturated ketone moiety. After characterization, this new compound was evaluated for its effect on different types of cancer cell lines and very promising results were obtained. The growth of these cancer cells was inhibited by newly designed and synthesized compounds, especially for colon and pancreatic cancer cells with IC_{50} 0.04 - 0.05 μM.

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INTRODUCTION

The second most shared reason of death now a day is cancer and if we see recent statistics regarding the epidemiology of cancer, it shows that only in the United States, in 2019, approximately 11,060 children can be spotted and 1,190 can die by this disease. (Siegel *et al.*, 2019). This data is very alarming and encouraging the entire scientific world to do more and more efforts regarding the discovery of potent anticancer agents with minimal side effects.

In our previous studies (Seyed *et al.*, 2016; Qin *et al.*, 2016a, 2015; Bukhari *et al.*, 2014) it has been observed that natural compounds or synthetic derivatives of natural compounds can be very effective against cancer as they have potential to act as multitarget agents. Recently we have done the intensive investigations regarding the anticancer potential of natural compound ligustrazine chemically known as tetramethylpyrazine (TMP) (Zha *et al.*, 2017) by incorporating it in the chemical backbone of chalcones. On the other hand, we are also working from last decade on chalcone related α, β-unsaturated carbonyl based compounds (Bukhari *et al.*, 2012a,b; Qin *et al.*, 2015) and it has been proven that α, β-unsaturated carbonyl based moiety is also very effective for anticancer effects (Bukhari *et al.*, 2014; Zha *et al.*, 2017).

Other than our previously reported research, a number of other research groups has also proven that the α, β-unsaturated ketone functionality, a Michael acceptor is critical for the cancer cell growth inhibition (Rana *et al.*, 2016; Heller *et al.*, 2015). These examples and our previous research exhibited that uniting an α, β-unsaturated ketone in a new synthetic compound can increase the anticancer attributes of a natural product derivatives, and α, the β-unsaturated moiety can be observed as functionality structure to new drug design. So, in our recent study, we have designed and synthesized an agent to combine all three main functionalities, including TMP, chalcone and additional α, β-

unsaturated ketone moiety.

MATERIALS AND METHODS

Synthesis of TMP based aldehyde *(4)*

As we reported previously, modified aldehyde was synthesized by Boekelheide reaction and it was used for the synthesis of the new target compound. (Zha *et al.*, 2017)Scheme 1.

Synthesis of α, β-unsaturated ketone substituted 4-aminoacetophenone (5)

For the synthesis of the desired ketone, 4-aminoacetophenone (4) was reacted with acrylyl chloride to give the ketone (5) with required substituted α, β-unsaturated ketone.

Synthesis of TMP based chalcone with α, β-Unsaturated ketone moiety (6)

Tetramethylpyrazine based chalcone with α, β-Unsaturated ketone moiety (6) was synthesized using direct coupling method as we have reported previously for similar compounds Scheme 1 (Qin *et al.*, 2016b). Claisen-Schmidt condensation reaction was performed by using the base as catalyst for the reaction of ketone (5) with TMP-based aromatic aldehyde (3) at feed ratio 1:1 to get new compound (6). Reaction details are explained and reported previously.

N-{4-[3-(3,5,6-Trimethyl-pyrazin-2-yl)-acryloyl]-phenyl}-acrylamide (6)

Yield: 51%; Mp: 212-214 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 10.52 (s, 1H, NH), 8.29 (d, J=9.2 Hz, 1H), 7.92 (d, J=8.5 Hz, 2H), 7.69 (d, J=8.5 Hz, 2H), 6.94 (d, J=8.0 Hz, H), 6.55 (d, J=8.0 Hz, 2H), 6.12-5.92 (m, H), 2.59 (s, 3H) 2.51 (s, 3H) 2.40 (s, 3H) HRMS (ESI) m/z: 322.37 $[M+H]^+$, Microanalysis calculated for $C_{19}H_{19}N_3O_2$ (321.37), C: 71.01%, H: 5.96%, N: 13.08%. Found C: 71.04%, H: 5.98%, N: 13.01%.

MTT assay

In MTT assay, mammary epithelial cells (MCF-10A) were used to evaluate the effect of new compounds and assay was carried out exactly as reported by us in previous studies (Bukhari *et al.*, 2014). Percentage inhibition of cell proliferation was compared with controls containing 0.1% DMSO to present the data of MTT assay for the test compound.

Propidium iodide fluorescence assay

Propidium iodide fluorescence assay was done to explore the antiproliferative potential of the test compound (Bukhari *et al.*, 2014). Five different cell lines such as prostate cancer cell line (PC-3), breast cancer cell line (MCF-7), epithelial cancer cell line (A-549), colon cancer cell line (HT-29) and pancreatic carcinoma cell line (PaCa-2) were used to see the effects of the test compound on the cancer cell growth. Erlotinib, a well-known anticancer drug, was used as a positive control in the assay. Assay detailed procedure is well reported previously by us.

RESULTS AND DISCUSSION

Chemistry

As mentioned in methodology section equimolar quantities of chemically modified ligustrazine based aldehyde (3) and α, β-Unsaturated ketone moiety substituted acetophenone (5) were reacted in 1 mmol, 50% ethanolic NaOH solution to yield the novel chalcone derivative (6) by using Claisen-Schmidt condensation reaction Scheme 1 (Bukhari *et al.*, 2015). For the synthesis of modified aldehyde (3), 2-hydroxymethyl-3,5,6-trimethyl pyrazine (2) was synthesized first from ligustrazine (1), by using Boekelheide reaction and IBX oxidation of (2) produced an aldehyde functionality-containing intermediate (3) Scheme 1 (Zha *et al.*, 2017).

Cell viability assay

MCF-10A cells were used to accomplish *in vitro* cell viability assay. Synthesized compound N-{4-[3-(3,5,6-Trimethyl-pyrazin-2-yl)-acryloyl]-phenyl-acrylamide (6) was treated with MCF-10A cells for 96h and MTT assay was used to investigate cell viability. Tetramethylpyrazine based chalcone with α, β-unsaturated ketone moiety (6) exhibited a cell viability percentage of 94% and was found to be safe for further use. Activity, exposure and toxicity are included in the rule of three that is always a difficult task confronted by potential new drugs in the development stage. In drug discovery, absorption, distribution, metabolism and excretion (ADME) studies are regularly carried out to alter lead compounds into drugs which are both effective and safe (Nassar *et al.*, 2004).

Antiproliferative effects of N-{4-[3-(3,5,6-Trimethyl-pyrazin-2-yl)-acryloyl]-phenyl}-acrylamide (6)

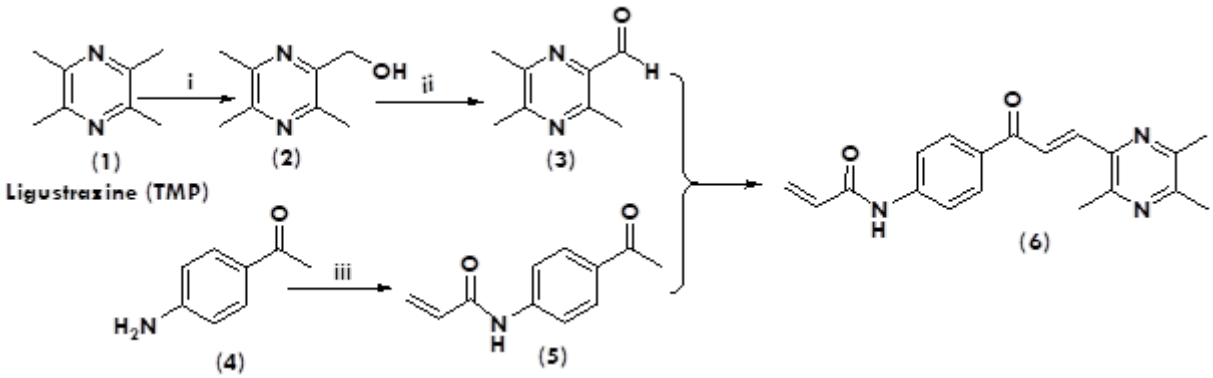
For evaluation of antiproliferative effects of a new compound (6), Propidium iodide (PI) fluorescence assay was performed. Synthetic compound (6) strongly inhibited all five cancer cell lines and results are shown in Table 1. Cytotoxicity of chalcone compound (6) to the HT-29 and PaCa-2 was highest, while for the other three cell lines, including A-549, PC-3 and MCF-7 were also strongly inhibited. Erlotinib, that is a well-known anticancer drug used in this study as a positive control.

Previously Zhu et al. designed and synthesized a

Table 1: Inhibition of cancer cell growth by synthetic compound(6)

Comp.	Inhibition of cancer cell growth IC50 ± SEM (μM)				
	A-549	PaCa-2	MCF-7	PC-3	HT-29
6	0.09±0.04	0.04±0.01	1.02±0.07	1.02±0.03	0.05±0.01

A-549 (epithelial cancer cell line): PaCa-2 (pancreaticcarcinoma cell line): PC-3(prostate cancer cell line): MCF-7 (breast cancer cell line): HT-29 (colon cancer cell line).



Scheme 1: Synthesis scheme of TMP-aldehyde (3)and new chalcone derivative (6). Reagents and conditions: (i-a) acetic acid, 30% H_2O_2, 70 °C, for 8 h.;(i-b) acetic anhydride, reflux, 2 h;(i-c) 20% NaOH; (ii) DMSO, IBX, room temperature, for 0.5 h; (iii) EtOH, NaOH, Room temperature.

series of similar chalcone analogs by incorporating α,β-Unsaturated ketone functionality and all new compounds were evaluated as potential anti-lung cancer agents by evoking ROS to induce pyroptosis and some compounds were found very effective, but that series was not including ligustrazine in main backbone of chalcone structure (Zhu *et al.*, 2018).

CONCLUSIONS

Chalcones are extensively reported previously for their diverse pharmacological propertives and chemically they are α, β-unsaturated carbonyl-based compounds so pharmacological importance of α, β-Unsaturated ketone moiety is noticeable. Here we tried to merge the chalcone with an additional α, β-Unsaturated ketone moiety and biologically very active compound ligustrazine was also incorporated in the chalcone backbone by using the modified aldehyde, so new compound with these three main biological active components was found strong inhibitor of cancer cell growth. Further studies can be performed to check the in vivo effects and bioavailability of this compound.

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