Discovery of a new tetramethylpyrazine based chalcone with $\alpha$, $\beta$-Unsaturated ketone moiety as a potential anticancer agent

Syed Nasir Abbas Bukhari

Department of Pharmaceutical Chemistry, College of Pharmacy, Jouf University, Aljouf, Sakaka 2014, Saudi Arabia

Article History:
Received on: 15.09.2019
Revised on: 10.12.2019
Accepted on: 15.12.2019

Keywords:
Chalcone, Ligustrazine, Cancer, Cell line, Natural compound

ABSTRACT

In this study, a new ligustrazine-based chalcone molecule has been synthesized that contains an extra $\alpha$, $\beta$-Unsaturated ketone moiety along with $\alpha$, the $\beta$-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 $\alpha$, $\beta$-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 $\mu$M.

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 $\alpha$, $\beta$-unsaturated carbonyl based compounds (Bukhari et al., 2012a,b; Qin et al., 2015) and it has been proven that $\alpha$, $\beta$-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 $\alpha$, $\beta$-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 $\alpha$, $\beta$-unsaturated ketone in a new synthetic compound can increase the anti-cancer attributes of a natural product derivatives, and $\alpha$, the $\beta$-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 $\alpha$, $\beta$-
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 \( \alpha, \beta \)-unsaturated ketone substituted 4-aminoacetophenone (5)

For the synthesis of the desired ketone, 4-aminoacetophenone (4) was reacted with acryl chloride to give the ketone (5) with required substituted \( \alpha, \beta \)-unsaturated ketone.

Synthesis of TMP based chalcone with \( \alpha, \beta \)-Unsaturated ketone moiety (6)

Tetramethylpyrazine based chalcone with \( \alpha, \beta \)-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, CDCl₃) δ: 10.52 (s, 1H, NH), 8.29 (d, J=9.2 Hz, 1H), 7.92 (d, =8.5 Hz, 2H), 7.69 (d, =8.5 Hz, 2H), 6.94 (d, =8.0 Hz, H), 6.55 (d, =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 C₁₉H₁₈N₂O₂ (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 \( \alpha, \beta \)-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 \( \alpha, \beta \)-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 chalone 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 (pancreatic carcinoma 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% H2O2, 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 properties 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.

ACKNOWLEDGEMENT

Special thanks to Jouf University for the financial support provided through research project No. (39/399) by the deanship of scientific research.

REFERENCES

Bukhari, A., Jasamai, S., Jantan, M., ., I. 2012a. Synthesis and Biological Evaluation of Chalcone Derivatives (Mini Review). Mini-Reviews in Medicinal Chemistry, 12(13):1394–1403.

Bukhari, S. N. A., Jantan, I., Jasamai, M. 2012b.

Bukhari, S. N. A., Jantan, I., Tan, O., Sher, M., Naeem-Ul-Hassan, M., Qin, H. L. 2014. Biological Activity and Molecular Docking Studies of Curcumin-Related α,β-Unsaturated Carbonyl-Based Synthetic Compounds as Anticancer Agents and Mushroom Tyrosinase Inhibitors. Journal of Agricultural and Food Chemistry, 62(24):5538–5547.

Bukhari, S. N. A., Zhang, X., Jantan, I., Zhu, H. L., Amjad, M. W., Masand, V. H. 2015. Synthesis, Molecular Modeling, and Biological Evaluation of Novel 1, 3-Diphenyl-2-propen-1-one Based Pyrazolines as Anti-inflammatory Agents. Chemical Biology & Drug Design, 85(6):729–742.

Heller, L., Schwarz, S., Perl, V., Köwitsch, A., Siewert, B., Csuk, R. 2015. Incorporation of a Michael acceptor enhances the antitumor activity of triterpenic acids. European Journal of Medicinal Chemistry, 101:391–399.

Nassar, A.-E. F., Kamel, A. M., Clarimont, C. 2004. Improving the decision-making process in structural modification of drug candidates: reducing toxicity. Drug Discovery Today, 9(24):1055–1064.
Qin, H. L., Leng, J., Zhang, C. P., Jantan, I., Amjad, M. W., Sher, M., Bukhari, S. N. A. 2016a. Synthesis of \( \alpha,\beta \)-Unsaturated Carbonyl-Based Compounds, Oxime and Oxime Ether Analogs as Potential Anticancer Agents for Overcoming Cancer Multidrug Resistance by Modulation of Efflux Pumps in Tumor Cells. Journal of Medicinal Chemistry, 59(7):3549–3561.

Qin, H. L., Leng, J., Zhang, C. P., Jantan, I., Amjad, M. W., Sher, M., Bukhari, S. N. A. 2016b. Synthesis of \( \alpha,\beta \)-Unsaturated Carbonyl-Based Compounds, Oxime and Oxime Ether Analogs as Potential Anticancer Agents for Overcoming Cancer Multidrug Resistance by Modulation of Efflux Pumps in Tumor Cells. Journal of Medicinal Chemistry, 59(7):3549–3561.

Qin, H. L., Shang, Z. P., Jantan, I., Tan, O. U., Hussain, M. A., Sher, M., Bukhari, S. N. A. 2015.

Rana, S., Blowers, E. C., Tebbe, C., Contreras, J. I., Radhakrishnan, P., Kizhake, S., Natarajan, A. 2016. Isatin Derived Spiro cyclic Analogues with \( \alpha \)-Methylene-\( \gamma \)-butyrolactone as Anticancer Agents: A Structure-Activity Relationship Study. Journal of Medicinal Chemistry, 59(10):5121–5127.

Seyed, M. A., Jantan, I., Bukhari, S. N. A., Vijayaraghavan, K. 2016. A Comprehensive Review on the Chemotherapeutic Potential of Piceatannol for Cancer Treatment, with Mechanistic Insights. Journal of Agricultural and Food Chemistry, 64(4):725–737.

Siegel, R. L., Miller, K. D., Jemal, A. 2019. Cancer statistics. Cancer Journal for Clinicians, 69(1):7–34. CA: A.

Zha, G. F., Qin, H. L., Youssif, B. G. M., Amjad, M. W., Raja, M. A. G., Abdelazeem, A. H., Bukhari, S. N. A. 2017. Discovery of potential anticancer multitargeted ligustrazine based cyclohexanone and oxime analogs overcoming the cancer multidrug resistance. European Journal of Medicinal Chemistry, 135:34–48.

Zhu, M., Wang, J., Xie, J., Chen, L., Wei, X., Jiang, X., Wu, J. 2018. Design, synthesis, and evaluation of chalcone analogues incorporate \( \alpha,\beta \)-Unsaturated ketone functionality as anti-lung cancer agents via evoking ROS to induce pyroptosis. European Journal of Medicinal Chemistry, 157:1395–1405.