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RESEARCH ARTICLE

Molecular docking studies of novel s-triazine derivatives incorporating amino methoxy chalcone for EGFR inhibitor in breast cancer

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Abstract

Background: The Epidermal growth factor receptor (EGFR) receptor is involved in apoptosis and angiogenesis. An upregulation of EGFR activity can potentially expedite the proliferation of malignant cells. Anticancer activity is among the many pharmacological activities of s-triazine derivative compounds. The potential of amino chalcone derivatives as anticancer agents has been documented. The anticipated outcome is that the derivatives of these two compounds will enhance their efficacy as antiproliferative agents. **Objective:** Through molecular docking, this study aimed to assess the potential of eight novel trichlorotriazin compounds derived from amino chalcone as EGFR inhibitors in breast cancer. **Method:** The molecular docking process was carried out with the assistance of the software package identified as the molecular docking environment (MOE) 2023.0901. **Results:** Compounds two and four, which have a similar binding orientation to the positive control, have the potential to inhibit EGFR, according to molecular docking results. For developing new s-triazine derivatives that are potent agents against breast cancer, the molecular docking outcomes of compounds two and four may require additional consideration. **Conclusion:** Compounds two and four have surfaced as noteworthy contenders.

Introduction

Compounds derived from S-triazine (1,3,5-triazine) are a major area of interest in medicinal chemistry, as their fundamental structure provides a basis for developing new drugs (Shah *et al.,* 2014). S-triazine scaffolds confer a variety of biological activities to these compounds, which include anticancer, antimicrobial, anti-inflammatory, anti-HIV, and antituberculosis properties (Patel *et al.,* 2012; Sharma *et al.,* 2017; Barakat *et al*., 2019; Alhameed *et al*., 2020; Faham *et al*., 2020). To generate novel drug molecules, chemical modifications of these derivatives are performed to improve their pharmacological activities. Due to the chlorine atoms in the main ring, s-triazine derivatives can form a wide variety of compounds that may have therapeutic applications (Zacharie *et al.,* 2018).

Cancer is a prominent contributor to mortality on a global scale, with female breast cancer being the most frequently diagnosed subtype (Sung *et al*., 2020; Bray *et al*., 2021). Epidermal growth factor receptor (EGFR), a tyrosine kinase implicated in cellular proliferation and signalling, has been identified as a crucial target in cancer treatment, specifically breast cancer (Mokhtar *et al*., 2020). The aberrant cellular behaviours induced by EGFR overexpression in cancer cells, such as uncontrolled proliferation and invasion, underscore its significance as a target for therapeutic interventions. The clinical application of numerous EGFR-targeting drugs to treat lung and breast cancers demonstrates the receptor's importance in cancer pathogenesis (Giordano & Petrelli, 2008).

It has been reported that amino-methoxy chalcone derivatives exhibit antiproliferative properties; the maintenance of this activity is dependent on particular structural groups (Anwar *et al.,* 2018; Lu *et al.,* 2020). By combining s-triazine structures with additional anticancer fragments, novel anticancer agents can be designed strategically. As potential EGFR inhibitors, this study employs molecular docking, an in silico technique, to forecast the bioactivity of s-triazine derivatives based on amino methoxy chalcone (Frimayanti *et al.,* 2021). By revealing the potential interactions, conformations, and binding affinities between these s-triazine derivatives and the EGFR, molecular docking provides a predictive model for their therapeutic efficacy against cancer.

Notwithstanding the encouraging characteristics of s-triazine derivatives, a substantial knowledge deficit persists regarding the comprehension and enhancement of their interaction with EGFR in female breast cancer. Prior research has established a fundamental understanding; however, a dearth of exhaustive data exists concerning s-triazine derivatives that have undergone amino methoxy chalcone modifications. By providing detailed molecular insights into the interaction mechanisms of these derivatives with EGFR, this study hopes to close this knowledge gap and guide the development of more effective and targeted cancer therapies.

The main aims of this study were to determine how s-triazine derivatives bind to amino methoxy chalcone on EGFR, assess their viability as inhibitors of breast cancer, and comprehend the structural characteristics that contribute to their biological activity. Identifying potent s-triazine-based EGFR inhibitors, establishing a molecular basis for their activity, and establishing a foundation for subsequent development and clinical evaluation of these compounds as anticancer agents are all anticipated results of this research. By conducting this research, the authors anticipate advancing the understanding of cancer therapeutics and providing breast cancer patients with optimism for more efficacious interventions.

Methods

Protein preparation

The protein's crystallographic structure (PDB-ID code: 1XKK) was downloaded in PDB format from the website www.rcsb.org. This protein is made up of one chain (Chain A). BIOVIA Discovery Studio Visualiser (DSV) and Molecular Operating Environment (MOE) software were used to create protein crystal structures. The BIOVIA discovery studio visualiser is used for the first protein preparation (DSV). Water molecules, unused chains, and ligands attached to the protein that are not required are removed during preparation and saved in "*pdb*" format. The first protein preparation results were opened in the MOE 2023.0901 software for the second preparation process. The existing ligands are removed during this process, and the protein structure is optimised by setting the minimise energy, force field of CHARMM27, fix hydrogens, fix charges, and coordinates X, Y, and Z of 19.173, 42.442, and 36.9445, respectively. RMS Gradient 0.001 is chosen from the QuickPrep menu and saved in "*pdb*" format.

Ligand preparation

Chemdraw Professional 15.0 was utilised to generate the "*cdx*" file containing the framework of s-triazine derivative compounds utilising amino methoxy chalcones as the ligand and Lapatinib as the positive control (Figure I). Fixed hydrogen, fixed charges, and an RMS Gradient of 0.0001 (kcal/mol/A) were selected in the Molecular Operating Environment (MOE) 2023.0901 (Chemical Computing Group) to generate the ligand structures, which were then saved as a ligand database in'mdb' format.

Determination of the active site on the protein, Lapatinib redocking and validation of the docking process

The protein's active site was annotated as a receptor using the'site finder' function in MOE. Assign the value of site one to the role of a dummy atom. When the RMSD value is less than 2.0, and the re-docked ligand and the co-crystal ligand exhibit high similarity in their interactions with amino acid residues located in the protein's active site, the docking protocol is considered valid. Superimposition was performed to verify that the ligand and co-crystal ligand share the same binding orientation.

Molecular docking

Docking simulations are conducted by incorporating a pre-existing ligand database into the protein that has been formulated. The active side is then identified using the "*site finder*" menu. Correspondingly, configure the 'placement and refinement poses' parameters for sites 1 to 50 and 10. After that, click "*run*". The docking outcomes will be displayed in the database table labelled "*docking results*". After that, choose "*ligand interactions*". RMSD values less than 2.0 indicate the optimal binding. Following this, the docking results are visualised and interpreted.

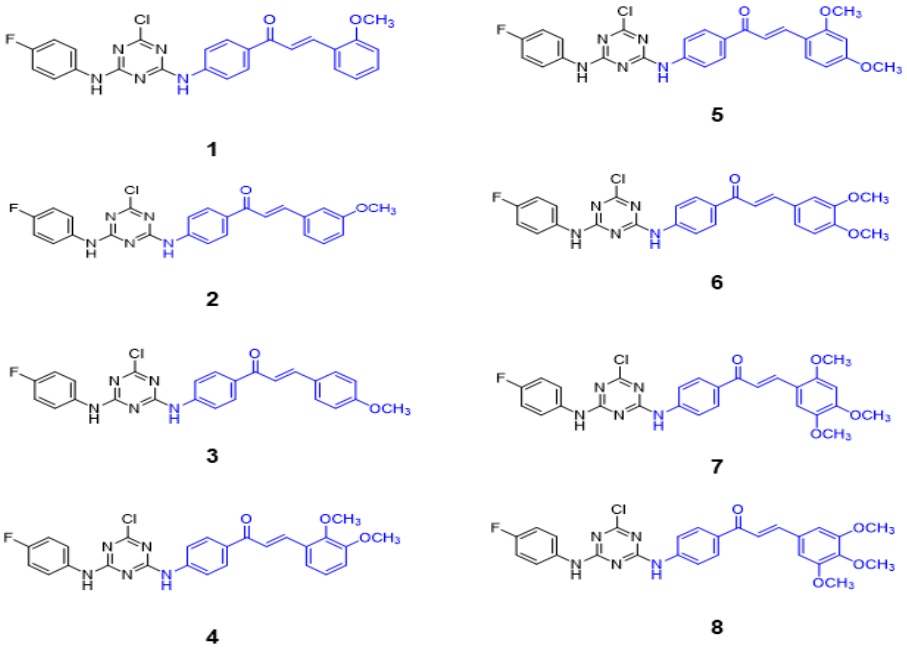

Figure I: Molecular structures of a novel s-triazine derivatives compounds based on amino methoxy chalcone

Results

To determine the potential of eight s-triazine-derived compounds designed with amino methoxy chalcone as EGFR inhibitors, the authors examined their binding interactions with the protein receptor (PDB ID: 1XKK) in this exhaustive study. The results for each of the ligands obtained through the molecular docking procedure are systematically listed in Table I.

Compounds	S (kcal/mol)	RMSD	Hydrogen bond	Hydrophobic bond	Van der Waals	Other interaction	Binding factor
Positive control (Lapatinib)	-13.1180	1.6584	Leu718	Lys745, Arg776, Arg841, Met793	Asp855	Gly719, Ser720, Gly721, Ala722, Val726, Ala743, Ile744, Met766, Cys775, Leu777, Leu788, Thr790, Gln791, Leu792, Cys797, Gly796, Asn842, Leu844, Thr854, Phe856, Met1002	23
1	-10.9923	1.1569	Val726 Arg841 Asp855	Lys745 Arg776	Asp800	Ser720, Gly721, Ala722, Ala743, Met766, Cys775, Leu777, Leu788, Thr790, Gly796, Cys797, Asn842, Leu844, Thr854, Phe856, Leu858	21
2	-10.6605	1.2672	Lys745 Asp855	**Arg776** Arg841	Asp800	Leu718, Gly719, Ser720, Val726, Ala743, Ile744, Met766, Val769, Cys775, Leu777, Leu788, Thr790, Phe795, Gly796, Cys797, Asn842, Thr854, Phe856, Leu1001, Met1002	**22**
3	-10.8989	1.1955	Lys745 Asp855	**Arg776** Arg841	Asp800	Leu718, Val726, Ala743, Ile744, Met766, Val769, Cys775, Leu777, Leu788, Thr790, Gly796, Cys797, Asn842, Thr854, Phe856, Leu1001, Met1002	20

Compounds	S (kcal/mol)	RMSD	Hydrogen bond	Hydrophobic bond	Van der Waals	Other interaction	Binding factor
4	-10.8172	1.3798	**Leu718** Val726	**Lys745** **Arg776**	Asp800 Glu804 **Asp855**	Gly719, Ala743, Ile744, Met766, Cys775, Leu777, Leu788, Thr790, Leu792, Phe795, Gly796, Cys797, Leu844, Thr854, Phe856, Leu858, Tyr998, Leu1001, Met1002	**19**
5	-11.0309	0.9912	Lys745 Cys797	Arg776	Asp800 Glu804 Asp855	Leu718, Val726, Ala743, Met766, Cys775, Leu777, Leu788, Ile789, Thr790, Phe795, Gly796, Leu844, Thr854, Phe856, Leu858, Phe997, Tyr998, Leu1001	17
6	-10.4518	1.3752	Cys797 Lys745 Leu844	Arg776 Arg841	Asp800 Glu804 Asp855	Leu718, Val726, Met766, Cys775, Leu777, Thr790, Phe785, Gly796, Tyr801, Asn842, Thr854, Phe856, Leu858, Phe997, Tyr998, Leu1001	17
7	-11.7282	1.2649	-	Lys745 Arg776	Asp800 Asp855	Leu718, Val726, Ala743, Ile744, Met766, Cys775, Leu777, Thr790, Met793, Gly796, Cys797, Leu844, Thr854, Phe856, Phe997, Tyr998, Met1002	19
8	-10.3832	1.4994	-	Lys745	Asp800 Glu804 Asp855	Leu718, Gly719, Val726, Ile744, Ala743, Met766, Cys775, Leu777, Leu788, Thr790, Leu792, Met793, Phe795, Gly796, Cys797, Tyr801, Leu844, Thr854, Phe856, Tyr998, Leu1001, Met1002	22

A comprehensive strategy was implemented to ascertain the most effective ligand-protein interactions. The selection process for optimal interaction outcomes incorporated the criterion of minimising binding free energy values, which signified the most advantageous binding affinity. Furthermore, the authors took measures to guarantee that the root mean square deviation (RMSD) remained below 2.0, indicating the docking poses' consistency and dependability. To determine the similarity of the binding patterns, the authors also compared the number of amino acids shared by the positive control, lapatinib. In addition, a superimposition analysis was performed to validate the ligand's alignment and orientation within the binding site compared to the positive control.

Figure II illustrates the spatial configuration of lapatinib, which serves as the positive control concerning the protein receptor. This visual representation functions as a standard against which the binding efficiency and orientation of the s-triazine derivatives can be evaluated. The visual depiction facilitates comprehension of the molecular interactions in operation, offering a more distinct viewpoint on how these innovative compounds emulate or deviate from the binding characteristics of established EGFR inhibitors.

The comprehensive molecular docking analysis demonstrates that the s-triazine derivatives differ concerning their affinity for binding, alignment, and interactions with pivotal amino acids within the binding site. Comprehending the structure-activity relationship and directing future modifications to improve these compounds' therapeutic potential depends on these variations. The findings unveiled in this research article not only enhance our comprehension of s-triazine derivatives as prospective EGFR inhibitors but also establish a foundation for subsequent refinement and advancement in creating anticancer agents that are more efficacious and selective.

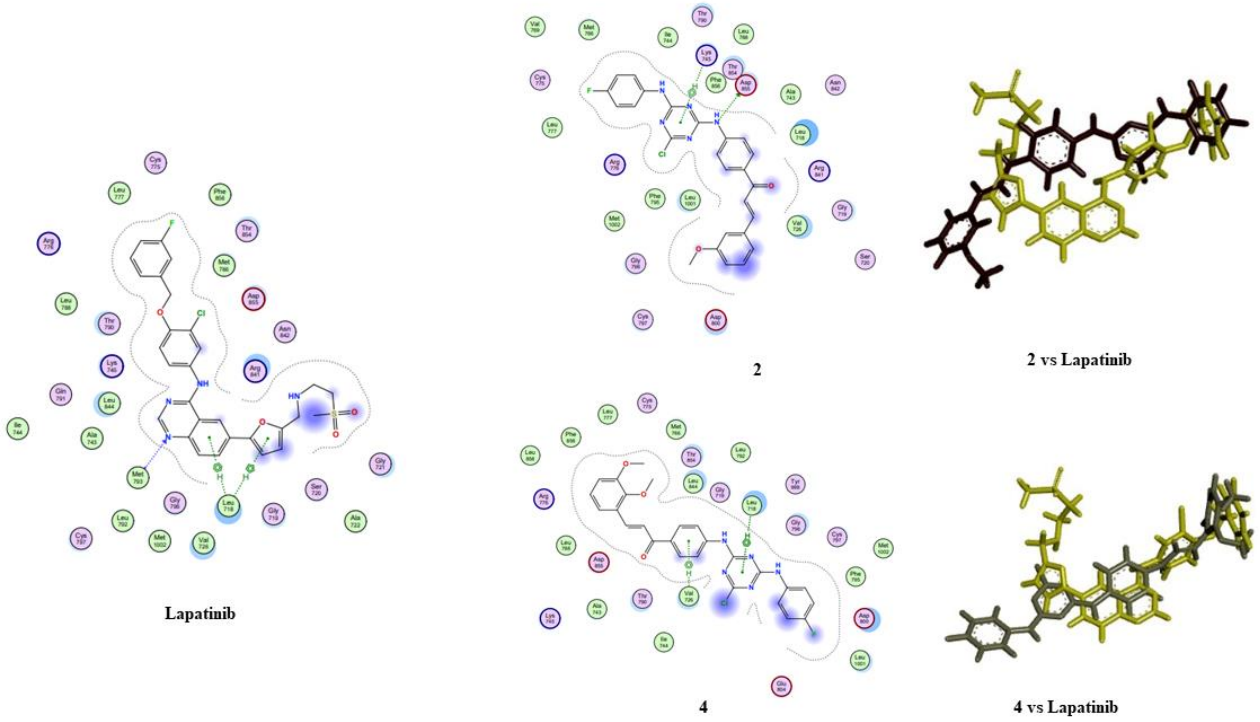

Figure 2: Spatial arrangement of the binding site (1XKK) for Lapatinib as the positive control. Visualisation of spatial arrangement of compound two and four; superimposition of the two compounds (dark brown) and lapatinib (yellow), visualised using MOE 2023.0901 software.

Discussion

The rapid advancement of computational methodologies, specifically molecular docking, has substantially accelerated the process of discovering new drugs. The present investigation utilised molecular docking to forecast interaction patterns between molecule ligands and protein receptors, thereby facilitating the estimation of the bioactivity of compounds (Lin *et al.,* 2020). Oriented docking, a technique that improves the docking procedure's precision and specificity by identifying the receptor's active site, was implemented (Syahputra *et al.,* 2014). An in-depth comprehension of the interactions between s-triazine derivative compounds and the EGFR receptor is essential for developing new cancer therapeutics, as demonstrated by our results employing this technique.

Lapatinib, our positive control, exhibited minimal binding free energy and maintained a stable interaction with the receptor by forming critical hydrogen bonds with 23 amino acid residues (Figure II). By conducting this benchmarking, a comparative foundation was created to evaluate the effectiveness of the s-triazine derivatives. Compounds one, two, and eight were particularly promising, which exhibited a comparable or greater number of amino acid interactions than the remaining compounds in the investigation. It is worth mentioning that compounds one, five, and seven displayed the highest binding free energy values, indicating their potential efficacy as inhibitors.

In contrast, prior investigations have frequently concentrated on ligands' structural arrangement and binding affinity toward the EGFR receptor (Lin *et al.,* 2020). Our research contributes to the existing literature by illustrating that effective inhibition requires consideration of specific interactions with amino acids, overall ligand orientation, and binding affinity. Particularly for compound four, the superimposition highlights the significance of ligand orientation when attempting to imitate the binding pattern of known effective inhibitors.

However, there are limitations to this study that should be addressed in future research. Although the authors have presented a thorough examination of the binding interactions exhibited by these s-triazine derivatives, their therapeutic potential must be confirmed through in vitro and in vivo validation. Furthermore, conducting additional research on the dynamic components of ligand-receptor interactions may yield additional

knowledge regarding the stability and effectiveness of these substances in natural environments.

Further investigation in this area should concern the alteration of these compounds to improve their specificity and mitigate possible adverse effects. The clinical application of these findings might be substantially altered by creating derivatives with enhanced pharmacokinetic properties and reduced off-target effects. Furthermore, conducting comparative analyses with alternative EGFR inhibitors could provide additional insight into the standing of s-triazine derivatives as cancer therapeutics in the broader spectrum.

Furthermore, in light of the intricate characteristics of cancer and the wide array of genetic and molecular variations it harbours, further research may wish to investigate the potential synergistic impacts of these s-triazine derivatives in conjunction with additional therapeutic agents. Potentially more effective at overcoming resistance mechanisms and producing a synergistic therapeutic effect, this method may be implemented.

In conclusion, this research has contributed significantly to the knowledge regarding how s-triazine derivatives interact with the EGFR receptor, thereby illuminating their potential as therapeutic agents for cancer. By expanding upon these discoveries and attending to the suggested avenues for future investigation, further scholarly inquiry can facilitate the advancement of cancer therapies that are more efficacious, precise, and risk-free. With the ongoing development of computational drug discovery, incorporating increasingly advanced models and experimental validation will undeniably bolster the effectiveness and dependability of these computational prognostications in the practical context of pharmaceutical advancement.

Conclusion

Molecular docking analysis has generated insights into the potential of eight s-triazine derivative compounds based on amino methoxy chalcones as EGFR inhibitors. In particular, compounds two and four have surfaced as noteworthy contenders. Compound two exhibits considerable similarity to the positive control, as evidenced by its interactions with 22 amino acids. This suggests that compound two may possess the capability to replicate the inhibitory effects observed in established EGFR inhibitors. Although compound four interacts with 19 fewer amino acids than the positive control, the characteristics and arrangement of its interactions—including hydrophobic, van der Waals, and hydrogen bonding—are strikingly similar to those observed in the positive control. The observed similarity in amino acid sequences and interaction types implies that compound four, similar to compound two, may potentially inhibit the EGFR receptor in a therapeutically beneficial manner. The aforementioned results emphasise the potential of these s-triazine derivatives in advancing novel cancer therapeutics and bolster the significance of molecular docking as a valuable instrument in drug discovery. Additional investigation and validation are required to validate these findings and comprehensively establish the therapeutic capabilities of compounds two and four as inhibitors of EGFR.

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References

Alhameed, R., Almarhoon, Z., N. Sholkamy, E., Ali Khan, S., Ul-Haq, Z., Sharma, A., ... & El-Faham, A. (2020). Novel 4, 6-disubstituted s-triazin-2-yl amino acid derivatives as promising antifungal agents. *Journal of Fungi*, **6**(4), 237. https://doi.org/10.3390/jof6040237

Anwar, C., Prasetyo, Y. D., Matsjeh, S., Haryadi, W., Sholikhah, E. N., & Nendrowati, N. (2018). Synthesis of chalcone derivatives and their in vitro anticancer test against breast (T47D) and colon (WiDr) cancer cell line. *Indonesian Journal of Chemistry*, **18**(1), 102–107. https://doi.org/10.22146/ijc.26864

Asadi, P., Alvani, M., Hajhashemi, V., Rostami, M., & Khodarahmi, G. (2021). "Design, synthesis, biological evaluation, and molecular docking study on triazine based derivatives as anti-inflammatory agents". *Journal of Molecular Structure*, 1243(2021), 130760. https://doi.org/10.1016/j.molstruc.2021.130760

Barakat, A., El-Senduny, F. F., Almarhoon, Z., Al-Rasheed, H. H., Badria, F. A., Al-Majid, A. M., ... & El-Faham, A. (2019). Synthesis, X-ray crystal structures, and preliminary antiproliferative activities of new s-triazine-hydroxybenzylidene hydrazone derivatives. *Journal of Chemistry*, **2019**. https://doi.org/10.1155/2019/9403908

Bray, F., Laversanne, M., Weiderpass, E., & Soerjomataram, I. (2021). The ever-increasing importance of cancer as a leading cause of premature death

worldwide. *Cancer*, **127**(16), 302–3030. https://doi.org/10.1002/cncr.33587

El-Faham, A., Farooq, M., Almarhoon, A., Alhameed, R.A., Wadaan, M.A.M., de la Torre, G., & Albericio, F. (2020). Di- and tri-substituted s-triazine derivatives: Synthesis, characterization, anticancer activity in human breast cancer cell lines, and developmental toxicity in zebrafish embryos. *Bioorganic chemistry*. https://doi.org/10.1016/j.bioorg.2019.103397

Frimayanti, N., Marzieh, Y., Ihsan, I., Dhea, R.W.P., Hamid, N., & Fatemeh, S.B. (2021). Insight on the in silico study and biological activity assay of chalcone-based 1, 5-benzothiazepines as potential inhibitor for breast cancer MCF7. *CMUJ*, **20**(1), e2021019.

Giordano, S., & Petrelli, A. (2008). From single-to multi-target drugs in cancer therapy: When aspecificity becomes an advantage. *Current medicinal chemistry*, **15**(5), 422–432. https://doi.org/10.2174/092986708783503212v

Hashem, H.E., E.Amr, A.E., Nossier, E.S., Anwar, M.M., & Azmy, E.M. (2022). New Benzimidazole-, 1,2,4-Triazole-, and 1,3,5-Triazine-based derivatives as potential EGFRWT and EGFRT790M inhibitors: Microwave-assisted synthesis, anticancer evaluation, and molecular docking study. *ACS Omega*, **7**, 7155–7171. https://doi.org/10.1021%2Facsomega.1c06836

Lin, X., Li, X., & Lin, X. (2020). A review on applications of computational methods in drug screening and design. *Molecules*, **25**(6), 1375. https://doi.org/10.3390/molecules25061375

Lu, C. F., Wang, S. H., Pang, X. J., Zhu, T., Li, H. L., Li, Q. R., ... & Zhang, S. Y. (2020). Synthesis and biological evaluation of amino chalcone derivatives as antiproliferative agents. *Molecule*s, **25**(23), 5530. https://doi.org/10.3390/molecules25235530

Marín-Ocampo, L., Veloza, L. A., Abonia, R., & Sepúlveda-Arias, J. C. (2019). Anti-inflammatory activity of triazine derivatives: A systematic review. *European Journal of Medicinal Chemistry*, **162**, 435–447. https://doi.org/10.1016/j.ejmech.2018.11.027

Mokhtar, A. M., El-Messery, S. M., Ghaly, M. A., & Hassan, G. S. (2020). Targeting EGFR tyrosine kinase: Synthesis, in vitro antitumor evaluation, and molecular modeling studies of benzothiazole-based derivatives. *Bioorganic chemistry*, **104**, 104259. https://doi.org/10.1016/j.bioorg.2020.104259

Patel, R. V., Kumari, P., Rajani, D. P., Pannecouque, C., De Clercq, E., & Chikhalia, K. H. (2012). Antimicrobial, anti-TB, anticancer and anti-HIV evaluation of new s-triazine-based heterocycles. *Future Medicinal Chemistry*, **4**(9), 1053–1065. https://doi.org/10.4155/fmc.12.57v

Shah, D.R., Modh, R.P., & Chikhalia, K.H. (2014). Privileged s-triazines: structure and pharmacological applications", *Future Medicinal Chemistry*, **6**(4), 463–477. https://doi.org/10.4155/fmc.13.212

Sharma, A., Ghabbour, H., Khan, S.T., de la Torre, B.G., Albericio, F., & El-Faham, A. (2017). Novel pyrazolyl-s-triazine derivatives, molecular structure and antimicrobial activity. *Journal of Molecular Structure*, **1145**, 244–253. https://doi.org/10.1016/j.molstruc.2017.05.040

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, **71**(3), 209–249. https://doi.org/10.3322/caac.21660

Syahputra, G., Ambarsari, L., & Sumaryada, T. (2014). Simulasi docking kurkumin enol, bisdemetoksikurkumin dan analognya sebagai inhibitor enzim12-lipoksigenase. *Jurnal Biofisika*, **10**(1), 55–67.

Zacharie, B., Abbott, S. D., Duceppe, J. S., Gagnon, L., Grouix, B., Geerts, L., Gervais, L., Sarra-Bournet, F., Perron, V., Wilb, N., Penney, C. L., & Laurin, P. (2018). Design and synthesis of new 1,3,5-trisubstituted triazines for the treatment of cancer and inflammation. *Chem PubSoc Europe*. https://doi.org/10.1002%2Fopen.201800136