SYNTHESIS, In-vitro AND In-silico ANTICANCER ACTIVITY STUDIES OF METHOXY SUBSTITUTED TETRALONE-BASED CHALCONE DERIVATIVES

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ABSTRACT
Chalcones are organic compounds with numerous biotic activities such as anticancer, antibacterial, and antifungal. Using the Claisen-Schmidt condensation reaction, three methoxy substituted chalcone derivatives were synthesized and crystallized using the slow evaporation method. Cytotoxicity evaluation showed that all three compounds were almost inoffensive to VERO cell lines and exhibited excellent anticancer activity on breast cancer (MCF-7) cell lines. Molecular docking analysis confirmed that all three molecules fit well at the active site of the target protein with PDB ID: 1M17. In silico pharmacokinetic analysis reveals that the compounds satisfied Lipinski’s rule of five and can be recommended as oral drug candidates. ADMET properties of the organic synthesized compounds revealed the efficacy of the compounds to be considered as drug candidates for treating breast cancer after in-vivo experiments and clinical authentication.

Keywords: Chalcones, Anticancer Activity, Molecular Docking, Pharmacokinetic Analysis, In Silico Analysis.

INTRODUCTION
In general, chalcones are natural open-chain flavonoids in which two arene rings are held together by three carbons with a carbonyl group and double bond.¹ They are polyphenolic compounds mostly present in fruits, vegetables, flowers, roots, tea, and barks.² Chalcones and their derivatives, either natural or synthetic, illustrate a ample variety of pharmacological activities such as antidiabetic, antispasmodic, antileishmanial, and especially more significant due to their anticancer activity.³⁻⁴ The production of effective and less-cytotoxic agents with low cost, high bioavailability, high selectivity, and drug absorption properties has become a challenge nowadays. Preclinical research has approximated that cancer can be prevented at very early stages by using chalcones and their derivatives as a composite in the drugs.⁵⁻⁷ Breast cancer has been diagnosed as the lead cause of the death toll in women.⁸ Based on the literature support for methoxy substituted at Para, Meta, and Ortho positions based chalcone derivatives as prominent anticancer materials, mainly due to relapse and reoccurrence of the tumor. Attempts have been made to a synthesize such class of compounds using the Claisen-Schmidt condensation reaction method.¹³

EXPERIMENTAL
Materials and Methods
The chemicals utilized for the synthesis of the title compounds were obtained from the chemical distributors, in Chennai, Tamilnadu. 3,4 dimethoxy benzaldehyde (1a), 3,4,5 trimethoxy benzaldehyde(1b), and 3- hydroxy 4- methoxy benzaldehyde (1c) were combined with alpha tetralone in the presence of 10% NaOH solution and absolute alcohol to derive the final products (2a-2c) (Fig.-1 and Table-1).¹⁴

Synthesis of (2E)-2-[(3,4-dimethoxyphenyl)methylidene]-3,4-dihydronaphthalen-1(2H)-one(PMMD)
The title compound PMMD was synthesized with 3, 4 dimethoxybenzaldehyde and alpha tetralone (3-Dihydro-1(2H)-naphthalenone) in the presence of a base (NaOH). The resultant compound was solved for its molecular structure and crystallographic information was reported.¹⁵
Synthesis Procedure of (2E)-2-[(3,4,5-trimethoxyphenyl)methylidene]-3,4-dihydronaphthalen-1(2H)-one (TMMD)

An equimolar amount of 3, 4-Dihydro-1(2H)-naphthalene (2mL, 0.015mol) and 3,4,5-dimethoxy benzaldehyde (3g, 0.015mol) were dissolved in 100mL of ethanol and stirred. Adding 10% NaOH solution, the mixture was stirred again for 24h. To the resultant precipitate, ice-cold water was added and filtered. The dried filtrate was crystallized twice in a mixture of acetone and ethyl methyl ketone in the ratio of 1:1, and yellow blocks of crystals of size, 0.20 x 0.20 x 0.15 mm were harvested after seven days with a 90% yield (MP:115°C using melting point apparatus).

Synthesis Procedure of (2E)-2-[(3-hydroxy-4-methoxyphenyl)methylidene]-3,4-dihydronaphthalen-1(2H)-one (HMMD)

A mixture of 3, 4-Dihydro-1(2H)-naphthalenone (2mL, 0.015mol) and 3- hydroxy 4- methoxy benzaldehyde (3g, 0.015mol) was stirred after dissolving in ethanol (100 mL). The mixture was added with 10% NaOH solution and stirred for 3h. The prepared solution was kept undisturbed overnight, to which 36% hydrochloric acid (50 mL) was added and again kept at rest for one day at 7°C. The precipitate obtained was filtered and dried. The product obtained was recrystallized thrice in acetone. After 5 days colorless block crystals of size, 0.02 x 0.20 x 0.15 mm were obtained with 86% yield (MP: 120°C using melting point apparatus).

Evaluation of Biological Activities

The Micro culture Tetrazolium Assay (MTT) was utilized to evaluate the biological activities of the as-synthesized compounds. The Vero (normal) and MCF-7 (Michigan Cancer Foundation-7) cell lines were procured from National Centre for Cell Sciences, Pune (NCCS). The MTT assay was performed as reported in D. Angeline Shirmila et al.

In-silico Molecular Docking Analysis

Molecular docking was carried out using AutoDock 4.2.6 software package, and PyMOL software to prepare the protein target and ligand. The ligand and target interactions were visualized and interpreted. The protein structure (PDB ID: 1M17) was procured from RCSB Protein Data Bank. The protein was saved in PDBQT format after computing the Kollman and Gasteiger charges and was then used as the target. The conversion of crystallographic information file (ligand) to program database file (PDB) format was done using Open Bable software. The Lamarckian Genetic Algorithm (LGA) was administered on the target protein with a grid spacing of 1Å and grid size of 96 (x-axis) x 80 (y-axis) x102 (z-axis) Å.

In-silico Molecular Properties

The molecular properties of the as-synthesized compounds were studied and calculated using the molinspiration online toolkit (http://www.molinspiration.com) to check whether the compounds satisfy Lipinski’s requirements. The molecular weight, the number of hydrogen-bond donors and hydrogen
bond acceptors, the total count of rotatable bonds, the value of logP, molecular refractivity, and the total polar surface area were evaluated according to Lipinski’s rule of five and Veber’s rule. In pharmacology, the bioavailability of any compound is evaluated depending on the absence of violations of Lipinski’s rule of five.

Swiss ADME Pharmacokinetic Prediction

Chalcone derivatives have been proven to display high anticancer activity in various cancer cell lines without affecting the tissues or cells in the surrounding environment by employing their efficient cytotoxic effect. Swiss ADME software package is exploited to determine the absorption capacity, distribution efficacy, rate of metabolism, and excretion of the waste or unused materials from the living organism. These studies assist in the estimation of the favorable results obtained in the process of discovery of a suitable drug candidate for the treatment of various diseases.

RESULTS AND DISCUSSION

Evaluation of Cytotoxicity of PMMD, TMMD, HMMD on Vero Cell Lines

The 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was executed to assess the cell survival rate of three compounds (PMMD, TMMD, and HMMD). The cells obtained from NCCS were carefully placed on a plate with 24-wells and treated with different concentrations of all three synthesized compounds. Their corresponding absorbance and cell viability (%) were recorded. The change in response with their corresponding dose was plotted, and the IC<sub>50</sub> value of each compound was obtained by extrapolating the graph (Fig.-3). The morphological changes of treated cells (Fig.-2) were compared to untreated cells, and from the results, it is noticed that as the concentration of the sample increases, the cell viability decreases. These results can be visualized from the change of color of the solutions from purple to yellow. The IC<sub>50</sub> values of the synthesized chalcone derivatives clearly show that they are nontoxic on Vero (normal) cell lines since even at 1000µg/mL of the sample concentration, all three compounds showed a high percentage of cell viability.

![Fig.-2: Image Showing the Cytotoxic Effect of PMMD, TMMD, and HMMD](image_url)

Evaluation of Anticancer Activity of PMMD, TMMD, HMMD on MCF-7 Cell Lines

MTT assay was perpetrated to evaluate the efficacy of PMMD, TMMD, and HMMD against the multiplication of breast cancer cells. The as-synthesized compounds show a preferable benignant effect on MCF-7 cell lines even at an optimum concentration of 62.5 µg/mL of PMMD with 48.20% of cell viability, 15.6 µg/mL of TMMD with 52.33% of cell viability and 125 µg/mL of TMMD with 50.46% of cell viability. Comparatively, TMMD exhibits an excellent anticancer effect than PMMD and HMMD. The morphology variations due to the effect of the synthesized compound on MCF-7 cell lines at different concentrations and their corresponding graphs with IC<sub>50</sub> curves are represented pictorially (Fig.-4 and Fig.-5). The low toxicity of the as-synthesized compounds has been exemplified by the IC<sub>50</sub> values reflecting the percentage of cell survival and the prospective anticancer activity of the compounds. The biomolecules PMMD, TMMD, and HMMD have the potential to be nominated as antiproliferative drugs to inhibit breast cancer after proper clinical testing.
Molecular Docking Studies
The PyMOL image of the interaction between co-crystal (Erlotinib) and target protein (PDB ID: 1M17) is shown in Fig.-6a and the binding domain interactions between the ligands (small molecules) and the target protein are illustrated in Fig.6.-b-d. The finest match of interactions of all the three as-synthesized compounds, PMMD, HMMD, and TMMD along with D-H...A Values corresponding to their lowest binding energy with their respective runs were estimated using AutoDock Tools 1.5.6, and presented in Table-2. The inhibition constant (Ki) for the ligands (PMMD, TMMD, and HMMD) with 1M17 which indicates the measure of the ease with which the ligand binds with the target protein is also tabulated (Table-2). The Ki value is comparable to the quantity of dose mandatory to hinder the proliferative activity. The stability of the compound is precisely analogous to the bond distance between the protein target and the ligands fitted in its active site which in turn is directly proportional to the least value of binding energy. The amino acid residues engaged in the binding site interaction of all three compounds (Table-2) are present in the active site of the commercially available drug for breast cancer, Erlotinib, which is used as the co-crystal in the current research work. All three compounds were found to have N-H…O binding interaction with the amino acid residue Met’769 and the same type of interaction can be visualized between the co-crystal, Erlotinib, and the target protein, 1M17. This result strongly supports the recommendation of the as-synthesized compounds, PMMD, TMMD, and HMMD, to be eligible drug candidates for breast cancer treatment.
Drug-likeness and the Bioavailability Investigation of the Chalcone Derivatives

The synthesized compounds PMMD, TMMD, and HMMD, obeys and satisfy Lipinski’s and Veber’s rules which are the basic criteria to be fulfilled by any compound to possess the property of drug-likeness and bioavailability. A Molinspiration server was availed to screen all three synthesized compounds for their ADMET properties, to evaluate their toxicity factor and total drug-likeness. All three compounds exhibited no violation with absolute non-toxicity. These results confirm that the title compounds have a high binding affinity with the receptors. The synthesized compounds were found to have good penetrability through the cell membranes, which can be confirmed by their LogP values being less than five. The molecular weights of all three compounds were found to be less than 500 Dalton, proving to be convenient for easy transportation, can be absorbed readily, and also diffuse easily. The number of hydrogen bond acceptors (should be less than 5) and hydrogen bond donors (should be less than 10) were well within the range. All these values are in the optimum range and anticipate the drug-likeness of the compounds (Table-3 and 4).

Table-2: Scoring Functions Obtained by Molecular Docking Simulation of the Ligands with Target Protein 1M17

| Ligand | Run number | Binding site interaction | D-H…A | Binding energy kcal/mol | Inhibition constant(Ki)nM |
|--------|------------|--------------------------|--------|------------------------|-------------------------|
| PMMD   | 7          | [Met-769] N-H…O [Lys-721]N-H…O | 2.0    | -10.57                 | 17.72                   |
| TMMD   | 4          | [Met-769] N-H…O [Lys-721]N-H…O | 2.0    | -10.96                 | 9.28                    |
| HMMD   | 3          | [Met-769] N-H…O [Lys-721]N-H…O [Asp-831]O-H…O | 2.2    | -10.11                 | 39.09                   |
Fig.-6: PyMOL Plot Representing the Interactions Between (a) Co-crystal (Erlotinib) and the Protein (1M17), (b) the Ligand (PMMD) and the Protein (1M17), (c) the Ligand (TMMD) and the Protein (1M17), (d) the Ligand (HMMD) and the Protein (1M17)

Table-3: Drug-likeness Score

| Compound | miLogP | TPSA  | nAtoms | nON  | nOHNH | n violations | rotb | volume | MW   |
|----------|--------|-------|--------|------|-------|--------------|------|--------|------|
| PMMD     | 3.91   | 35.54 | 22     | 3    | 0     | 0            | 3    | 275.71 | 294.34|
| TMMD     | 3.89   | 44.77 | 24     | 4    | 0     | 0            | 4    | 301.25 | 324.37|
| HMMD     | 3.60   | 46.53 | 21     | 3    | 1     | 0            | 2    | 258.18 | 280.32|

Table-4: Bioactivity Score

| Compound | GPCR Ligand | Ion Channel Modulator | Kinase Inhibitor | Nuclear Receptor Ligand | Protease Inhibitor | Enzyme Inhibitor |
|----------|-------------|-----------------------|------------------|-------------------------|--------------------|------------------|
| PMMD     | -0.17       | -0.33                 | -0.38            | -0.14                   | -0.41              | -0.01            |
| TMMD     | -0.14       | -0.31                 | -0.33            | -0.17                   | -0.35              | -0.01            |
| HMMD     | -0.16       | -0.30                 | -0.38            | -0.09                   | -0.46              | 0.06             |

Physicochemical and Pharmacokinetics Properties

One of the requirements for an orally active drug is the ability to be absorbed by the gastrointestinal tract of the living organism, all the three synthesized compounds PMMD, TMMD, and HMMD exhibit high gastrointestinal absorption. All three compounds exhibit good bioavailability scores and were found to possess high permeability towards the blood-brain barrier (BBB) signifying negligible toxicity. The ease with which a compound can be synthesized commercially in bulk quantity is assessed by the synthetic accessibility scores (0 to 10 – easy to hard) and the scores of all the as-synthesized compounds are well within the range i.e. 2.92 (PMMD), 3.10 (TMMD), and 2.80 (HMMD). The affinity to bind with the receptor, cell ingestion, and bioavailability are governed by the assimilability factors like molar refractivity
and lipophilicity. The prescribed range of molar refractivity values must lie between 40 and 130. The molar refractivity of the compounds and the lipophilicity values of all three molecules were well within the reported range (Table-5). The above results explicitly reveal that the compounds are well underway to be considered as oral drug candidates.

Table-5: Physicochemical and Pharmacokinetics Properties

| Physiochemical properties | PMMD | TMMD | HMMD |
|---------------------------|------|------|------|
| Compounds                | C₁₉H₁₈O₃ | C₂₀H₂₀O₄ | C₁₈H₁₈O₃ |
| Molecular formula         | 86.89 | 93.38 | 82.42 |
| Molar refractivity        | 3.69  | 3.66  | 3.35  |
| Lipophilicity (consensus) | Moderately soluble | Moderately soluble | Moderately soluble |
| Pharmacokinetics          | Gastrointestinal absorption | High | High | High |
| Blood-brain barrier permeation | Yes | Yes | Yes |
| P-glycoprotein substrate  | No   | No   | No   |
| Cytochrome P450 1A2 inhibitor | Yes | Yes | Yes |
| Cytochrome P450 2C19 inhibitor | Yes | Yes | Yes |
| Cytochrome P450 2C9 inhibitor | Yes | Yes | No |
| Cytochrome P450 2D6 inhibitor | Yes | Yes | Yes |
| Cytochrome P450 3A4 inhibitor | Yes | Yes | Yes |
| Skin permeation           | -5.23 cm/s | -5.44 cm/s | -5.38 cm/s |

CONCLUSION

Three new methoxy substituted tetralone-based chalcone derivatives were successfully synthesized and screened for anticancer activity against MCF-7 cell lines. Results of the biological studies revealed that all three compounds showed good anticancer activity. The compound TMMD was more sensitive to the MCF-7 cell line than the other two. It showed a 52.33% of cell survival rate at a concentration of 15.6µg/mL. The other two compounds (PMMD and HMMD) also showed good anticancer activity reaching the IC₅₀ values with moderate concentrations. From the cytotoxicity study, it is observed from the IC₅₀ values that the synthesized compounds are nontoxic on the Vero cell line even beyond 1000 μg/mL (sample concentration). The molecular docking simulations reveal the good binding ability of the ligands (synthesized compounds) at the active site of the target protein 1M17 forming hydrogen bonds with the amino acid residues Met 769 and Lys 721 with a shorter bond distance than the commercially available Erlotinib drug proving to be highly reactive at the active site of the protein. The in-silico pharmacokinetic analysis of the three synthesized compounds resulted in no violations of Lipinski’s and Veber’s rules. All the compounds show a good pharmacokinetic profile and hence, the studied compounds, specifically, PMMD, TMMD, and HMMD could be promising lead molecules for the development of more potent and safer anticancer drugs after further preclinical testing.

ACKNOWLEDGEMENT

We the authors sincerely thank the Central Instrumentation Facility (DST–FIST), Queen Mary's College (A), Chennai-4 for the computing facility.

REFERENCES

1. S.H. Mah, Chalcones in Diets. In: J. Xiao, S.D. Sarker and Y. Asakawa (Eds.), Handbook of Dietary Phytochemicals, Springer, Singapore, 273(2021), https://doi.org/10.1007/978-981-13-1745-3_10-1
2. C. Awinash and S. Prafulla, The Pharma Innovation Journal, 9(1), 39(2020)
3. R. Pemmereddy, K.S. Chandrashekar, K.P.S. Ranganath, V. Pai, A. Mathew and B.V. Kamath, Rasayan Journal of Chemistry, 15(1), 1(2022), http://dx.doi.org/10.31788/RJC.2022.1516421
4. A. Sumathy, R. Suresh and N.L. Gowrishankar, Rasayan Journal of Chemistry, 15(1), 497(2022), http://dx.doi.org/10.31788/RJC.2022.1516738
5. T. Enoki, H. Ohnogi, K. Nagamine, Y. Kudo, K. Sugiyama, M. Tanabe, E. Kobayashi, H. Sagawa and I. Kato, Journal of Agricultural and Food Chemistry, 55(15), 6013(2007), https://doi.org/10.1021/jf070720q
6. A. Escobar-Ramos, C. Lobato-Garcia, A. Zamilpa, A. Gómez-Rivera, J. Tortoriello and M. González-Cortazar, *Molecules*, 1405, 22(9), 1(2017), https://doi.org/10.3390/molecules22091405

7. N. Tajuddeen, M.B. Isah, M.A. Suleiman, F.R. van Heerden and M.A. Ibrahim. *International Journal of Antimicrobial Agents*, 51(3), 311(2018), https://doi.org/10.1016/j.ijantimicag.2017.06.010

8. D.K. Mahapatra, S.K. Bharti and V. Asati, *European Journal of Medicinal Chemistry*, 98, 69(2015), https://doi.org/10.1016/j.ejmech.2015.05.004

9. H. L. Qin, Z.P. Shang, I. Jantan, O.U. Tan, M.A. Hussain, M. Sher and S.N.A. Bukhari, *Royal Society of Chemistry Advances*, 5(57), 46330(2015), https://doi.org/10.1039/c5ra02995c

10. Y.L. Hsu, P.L. Kuo, W.S. Tzeng and C.C. Lin, *Food and Chemical Toxicology*, 44, 704(2006), https://doi.org/10.1016/j.fct.2005.10.003

11. D. Elkhalifa, F. Alali, A.A. Moustafa and A. Khalil, *Journal of Drug Targeting*, 27(8), 830(2018), https://doi.org/10.1080/1061186X.2018.1561889

12. V. Gote, A.R. Nookala, P.K. Bolla and D. Pal, *International Journal of Molecular Sciences*, 22(9), 4673(2021), https://doi.org/10.3390/ijms22094673

13. K.R. Ethiraj, J. Mathew and F.R.N. Khan, *Medicinal Chemistry Research*, 22, 5408(2013), https://doi.org/10.1007/s00044-013-0520-9

14. F. Dong, C. Jian, F. Zhenghao, G. Kai and L. Zuliang, *Catalysis Communications*, 9, 1924 (2008), https://doi.org/10.1016/j.catcom.2008.03.023

15. D. A. Shirmila, D.R. Jonathan, M. K. Priya, J. Hemalatha and G. Usha, *IUCr Data Reports*, x210309, 6, 1(2021), https://doi.org/10.1107/S2414314621003096

16. T. Mosmann, *Journal of Immunological Methods*, 65, 55(1983), https://doi.org/10.1016/1016-1759(83)90303-4

17. G. Mahanthesha, T. Suresh and T.R.R. Naik, *Rasayan Journal of Chemistry*, 15(1), 155(2022), http://dx.doi.org/10.31788/RJC.2022.1516577

18. K. Assidqi, N.F. Sianipar and R. Tarian, *Rasayan Journal of Chemistry*, 15(1), 232(2022), http://dx.doi.org/10.31788/RJC.2022.1516468

19. J. Stamos, M.X. Sliwkowski, C. Eigenbrot, *Journal of Biological Chemistry*, 277(48), 46265(2002), https://doi.org/10.1074/jbc.M207135200

20. C. Shivanika, S. D. Kumar, V. Ragunathan, P. Tiwari, A. Sumitha and P. B. Devi, *Journal of Biomolecular Structure and Dynamics*, 40(2), 1(2020), https://doi.org/10.1080/07391102.2020.1815584

21. G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Huey, W.E. Hart, R.K. Belew and A.J. Olson, *Journal of Computational Chemistry*, 19(14), 1639(1998), https://doi.org/10.1002/(SICI)1096987X(19981115)19:14<1639::AIDJCC10>3.0.CO;2-B

22. The PyMOL Molecular Graphics System, LLC, Schrodinger, Version 1.5.0.4; (2009), https://doi.org/10.1002/SIC10196987X(19981115)19:14<1639::AIDJCC10>3.0.CO;2-B

23. G. Subramanian, F. Sherin, N. M. Joy, D.W. Ashish, G. Byran and A. Antony, *Rasayan Journal of Chemistry*, 15(1), 483(2022), http://dx.doi.org/10.31788/RJC.2022.1516628

24. N.M. O'Boyle, M. Banck, C.A. James, C. Morley, T. Vandermeersch and G.R. Hutchison. *Journal of Cheminformatics*, 3(1), 1(2011), https://doi.org/10.1186/1758-2946-3-33

25. R.A. Biswal, R. Venkataraman, V. Pazhamalai and S.I. Romain, *Journal of Applied Pharmaceutical Science*, 9(5), 21(2019), https://doi.org/10.7324/JAPS.2019.90503

26. G.S. Reddy, V.R. Anna, S. Balabadra and I.V.K. Viswanath, *Rasayan Journal of Chemistry*, 15(1), 726(2022), http://dx.doi.org/10.31788/RJC.2022.1516645
30. Molinspiration Cheminformatics, Novaulica, SK-90026, Slovak Republic.
31. C.A. Lipinski, F. Lombardo, B.W. Dominy and P.J. Feeney. *Advanced Drug Delivery Reviews*, 23, 3(1997), https://doi.org/10.1016/S0169-409X(96)00423-1
32. D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward and K.D. Kopple, *Journal of Medicinal Chemistry*, 45(12), 2615(2002), https://doi.org/10.1021/jm020017n
33. B.G. Vijayakumar, D. Ramesh, A. Joji, J.J. Prakasan and T. Kannan, *European Journal of Pharmacology*, 886, 173448, 1(2020), https://doi.org/10.1016/j.ejphar.2020.173448
34. A. Daina, O. Michielin and V. Zoete, *Scientific Reports*, 7, 42717(2017), https://doi.org/10.1038/srep42717
35. V.S.S.L.P. Talluri, S.S. Lanka, B. Mutaliyeva, A. Sharipova, A. Suigenbayeva and A. Tleuova, *Rasayan Journal of Chemistry*, 15(2), 786(2022), http://dx.doi.org/10.31788/RJC.2022.1526842
36. Y.H. Lim, C.W. Oo, R.Y. Koh, G.L. Voon, M.Y. Yew, M.F. Yam and Y.C. Loh, *Drug Development Research*, 81, 994(2020), https://doi.org/10.1002/ddr.21715
37. S. Sevvanthi, S. Muthu, M. Raja, *Journal of Molecular Structure*, 1173, 251(2018), https://doi.org/10.1016/j.molstruc.2018.07.001
38. N. Dege, A.S. Aydin, E. Ağar, S. Kansız, S. JoseKavitha, K. BalaSubramani, M. Hemamalini, and V. Rajakannan, *Chemical Data Collections*, 25, 100320 (2020), https://doi.org/10.1016/j.cdc.2019.100320
39. M.K. Priya, D.R. Jonathan, S. Muthu, D.A. Shirmila, J. Hemalatha and G. Usha. *Journal of Molecular Structure*, 1253, 132296(2022), https://doi.org/10.1016/j.molstruc.2021.132296
40. J. G. Wagner, *Pharmacology and Therapeutics*, 12, 537(1981), Pergamon Press Ltd 198L Printed in Great Britain, https://doi.org/10.1016/0163-7258(81)90097-8
41. A.K. Ghose, V.N. Viswanadhan, J.J. Wendoloski, *Journal of Combinatorial Chemistry*, 1, 55(1999), https://doi.org/10.1021/cc9800071
42. P. Prajapati, J. Pandey, R.S. Manishkumar, A. Srivastava, P. Tandon, S.P. Velaga and K. Sinha, *Journal of Molecular Structure*, 1125, 193(2016), https://doi.org/10.1016/j.molstruc.2016.06.070
43. P. Pacák and Z. Kodejš, 66(9), 2244(1988), https://doi.org/10.1139/v88-356
44. R.T. Sawale, T.M. Kalyankar, R. George and S.D. Deosarkar, *Journal of Applied Pharmaceutical Science*, 6(3), 120(2016), https://doi.org/10.7324/JAPS.2016.60321

[RJC-7030/2022]