Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 13s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Insilico, Synthesis and Invitro Evaluation of Novel Thiadiazoles as Inhibitors Caspase 3 For Potential of Anticancer

Patibandla. Jahnavi[1], Gaurav Tiwari[2], A Rajendiran[3], Varsha Patwekar[4], Ramdas Baburao Rode[5], Jamdhade Ashwini Ashok[6], Sindhu Priya E S[7], Praveencumar R[*8]

[1]Department of pharmaceutics, KVSR siddhartha college of pharmaceutical sciences, Vijayawada, Andhra Pradesh.

[2]PSIT-Pranveer Singh Institute of Technology (Pharmacy), Kalpi road, Bhauti, Kanpur-209305.

[3]School of Pharmaceutical Sciences, CSJM University, Kanpur- 208024, (U.P), India.

[4]Department of Pharmacology, Dr. Vedprakash Patil College of pharmacy Chhatrapati Sambhaji Nagar, Maharashtra.

[5]KVN Naik SPS's Institute of Pharmaceutical Education and Research, Nashik.

[6]Department of Pharmaceutics, Sanjivani College of Pharmaceutical Education and Research, Kopargaon, Maharashtra, India.

[7]Department of Pharmacology, Yenepoya Pharmacy College and Research Centre, Yenepoya (Deemed to be University), Naringana, Deralakatte, Mangalore 575023.

[*8]Paavai group of institutions, Paavi group of institutions, Paavai nagar, Paachal, NH#44, Namakkal – 637018.

***Corresponding Author:**

Dr. Praveencumar R,

Head and Professor, Paavai group of institutions, Paavi group of institutions, Paavai nagar, Paachal, NH44, Namakkal - 637018

Email ID: cumar.pravin@gmail.com

Cite this paper as: Patibandla. Jahnavi, Gaurav Tiwari, A Rajendiran, Varsha Patwekar, Ramdas Baburao Rode, Jamdhade Ashwini Ashok, Sindhu Priya E S, Praveencumar R, (2025) Insilico, Synthesis and Invitro Evaluation of Novel Thiadiazoles as Inhibitors Caspase 3 For Potential of Anticancer. *Journal of Neonatal Surgery*, 14 (13s), 252-260.

ABSTRACT

This paper explains how to create different new compounds that include thiadiazole and imidazole with a cyclopropyl group. The diverse biological activities and therapeutic applications of imidazothiadiazoles render them intriguing. We predominantly perform docking experiments using the reference structure PDB ID 3KJ7. To focus on the cysteine proteases in the caspase family, we have created a new group of diaryl compounds that have imidazothiadiazoles attached. To monitor the reaction, we employed thin-layer chromatography with an appropriate mobile phase. By comparing the Rf values, the melting point of the produced compounds was ascertained. To further characterize and validate these derivatives, we used mass spectrometry analyses, one-dimensional nuclear magnetic resonance, carbon-13 nuclear magnetic resonance, and infrared spectroscopy. All cancer cell lines were effectively inhibited by compounds 1 and 3. The manufactured medications were assessed using a colorimetric assay using a caspase 3 inhibitor kit.

Keywords: *Cancer, caspase 3, thiadiazole, imidazole, synthesis.*

1. INTRODUCTION

We must continuously seek new treatment medicines for cancer, as it remains one of the world's top causes of death [1]. Inducing apoptosis, a method of programmed cell death that destroys malignant or damaged cells, is one of the most promising approaches to cancer treatment [2]. A key executioner protease in the apoptotic cascade, caspase-3, is essential for breaking down cellular constituents and ultimately causing cell death. As a result, focusing on caspase-3 activation has become a useful strategy for creating anticancer medications [3].

Heterocyclic compounds, especially thiadiazoles, have attracted a lot of attention in medicinal chemistry due to their many pharmacological characteristics, such as antibacterial, anti-inflammatory, and anticancer effects [4]. Thiadiazoles have outstanding bioavailability, metabolic stability, and target selectivity. They are distinguished by a five-membered ring that contains nitrogen and sulfur atoms [5]. Because of their structural adaptability, new derivatives that can specifically alter biological processes, such as apoptosis, can be created and synthesized [6].

Because of their capacity to alter the pathways leading to cell death, caspase-3 inhibitors have become promising therapeutic agents in oncology [7]. Besides potentially making cancer cells more responsive to other forms of cell death, like necroptosis, these inhibitors can also help protect healthy cells from harm during chemotherapy and radiation therapy [8]. Also, blocking caspase-3 might prevent too much inflammation and immune system weakening caused by apoptosis, which can otherwise help cancer spread [9].
Recent studies have shown that thiadiazole derivatives can block caspase-3, which may lead to cancer cells dying through a process called apoptosis [10]. These substances can cause cell death in cancers while reducing toxicity to healthy cells by specifically increasing or altering caspase-3 activity [11]. A promising way to find new cancer-fighting drugs is to develop new caspase-3 inhibitors that are based on heterocyclic thiadiazole [12].

The goal of this research is to study how thiadiazole derivatives are built and how they work, specifically as blockers of caspase-3, and to explore their potential use in treating cancer. We will cover recent developments in their synthesis and pharmacological assessment to further emphasize these compounds' effectiveness and potential as anticancer medicines in the future [13, 14].

The first cues to encourage tumor repopulation come from dying cells inside the tumor bulk. Dying cells, in particular, release growth-promoting signals to stimulate the division of living cells [15]. To facilitate the quick repopulation of tumors from a limited number of living tumor cells, caspases are essential for intratumoral death cells. Also, caspase 3, which is a type of enzyme involved in the final stage of cell death, is an important controller of the growth signals that come from dying cells [16]. In cytotoxic cancer treatment, the caspase-mediated tumor repopulation pathway plays a crucial role [17].

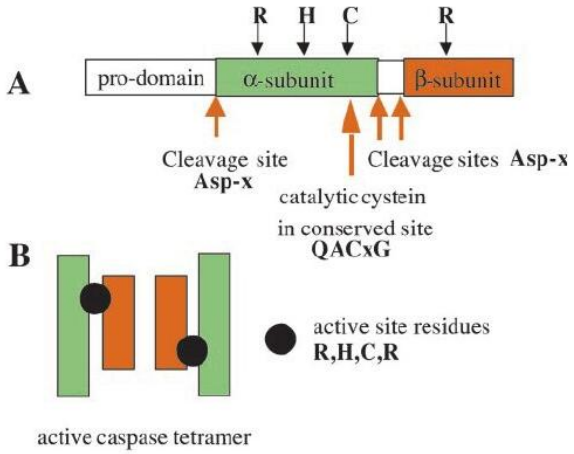


Figure 1: Mammalian caspases' structural characteristics are shown schematically.

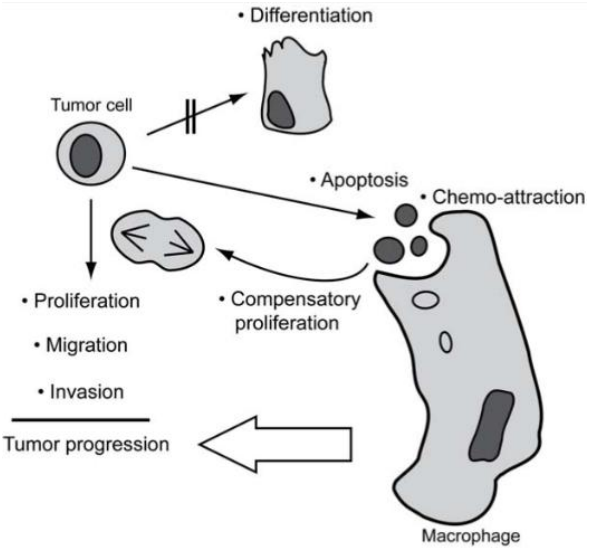


Figure 2: Role of caspase in cancer

A series of fused heterocyclic compounds termed imidazo[2,1-b][1,3,4]thiadiazoles has garnered significant interest due to its diverse pharmacological and chemical properties. These compounds are rigid bicyclic complexes with unique electrical and structural characteristics, created by the fusion of an imidazo ring to a 1,3,4-thiadiazole core. Their distinctive reactivity and biological activity are ascribed to the heteroatoms of sulfur and nitrogen inside the framework [18-20].

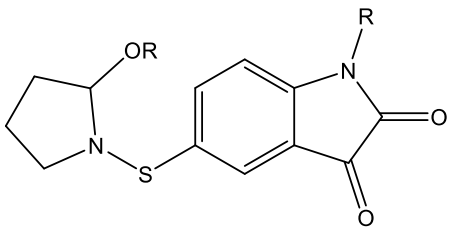


Figure 3: Basic Nucleus

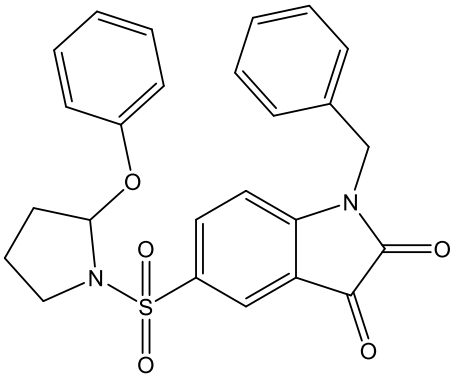
Imidazo[2,1-b][1,3,4]thiadiazoles have been used in a variety of sectors, such as pharmaceutical chemistry, materials science, and catalysis, because of their distinctive structural characteristics [21]. They are useful scaffolds in drug development because of their biological activity, which include antibacterial, anticancer, anti-inflammatory, and antiviral qualities. In summary, imidazo [2, 1-b] [1, 3, 4] thiadiazoles are interesting types of compounds that can be used in many scientific areas, have special chemical reactions, and can be made in different ways. Further research into changing and improving them could lead to new uses in advanced materials and medicines. Additional investigation into their functionalization and alterations may open up new possibilities for their application in innovative materials and pharmaceuticals [22].

2. MATERIALS AND METHODS

Molecular Docking:

Using a computational approach, we discovered strong and specific inhibitors of Caspase-3 in this investigation. Using the well-established set of Caspase-3 inhibitors, three-dimensional (3D) pharmacophore models were created to outline the molecular characteristics necessary for its action [23]. The best pharmacophore is verified via docking and structure-based pharmacophore studies. By using these models to quickly screen compounds from a database, a number of new and extremely powerful Caspase-3 inhibitors were found. Docking scores, expected binding locations, and drug-like characteristics obtained from virtual screening with a pharmacophore as a query were used to choose compounds for synthesis and in vitro screening [24].

To determine that each synthesized derivative has a different chemical nature from its original component, the following process was used to identify and define the substance [25].



1-benzyl-5-((2-phenoxypyrrolidin-1-yl)sulfonyl)indoline-2,3-dione

imidazo[2,1-*b*][1,3,4]thiadiazole imidazo[5,1-*b*][1,3,4]thiadiazole

Figure 4: showing imidazole ring system

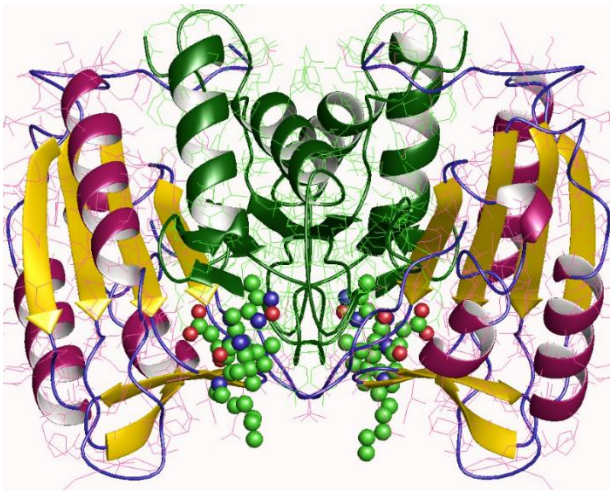
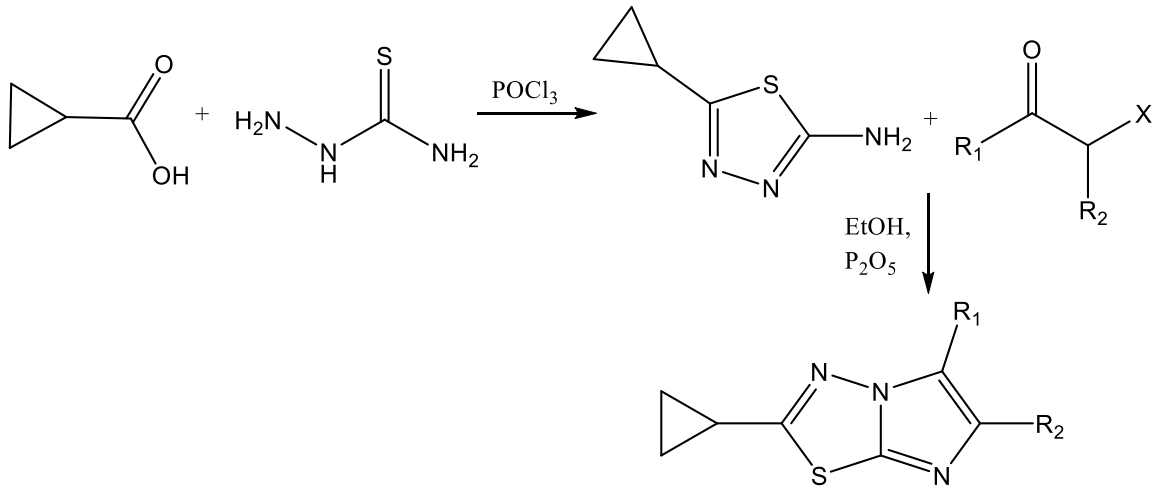


Figure 5: Caspases 3 inhibitor docking structure (PDB ID: 3KJ7)

Spectrochem contributed the additional substances, such as cyclopropanecarboxylic acid, bromine, various acetophenones, DMF, and $POCl_3$, while Sigma-Aldrich provided the chemicals required for the synthesis, such as thiosemicarbazide and trifluoroaceticanhydride. All of the solvents were employed after distillation. Following distillation, every solvent was used. LR-grade chemicals and solvents were used most frequently. Utilizing pre-made TLC plates, benzene and acetone mixes in various ratios, T:E:F, and chloroform:methanol combinations, thin layer chromatography was utilized to assess the compounds' purity [26-28]. The dots were illuminated with a UV lamp. Open capillary tubes with one end submerged in a liquid paraffin bath were used to measure uncorrected melting points. A Bruker Model Advance II 400 (400 MHz, 1H NMR) device and a Perkin Elmer IR 4000-400 (ν max in cm^{-1}) spectrophotometer with KBr pellets were used to measure the compounds' infrared (IR) and 1H nuclear magnetic resonance (1H NMR) readings, respectively. For reporting chemical shifts, tetramethylsilane (TMS), expressed in δ parts per million (ppm), is the reference standard [29].

Synthesis of 5-cyclopropyl-1, 3, 4-thiadiazol-2-amine



A combination of 13 milliliters of $POCl_3$, 0.05 moles of cyclopropanecarboxylic acid, and 0.05 moles of thiosemicarbazide was subjected to heating at 75 °C for 0.75 hours. Water was introduced once it reached room temperature. The reaction mixture was subjected to reflux for four hours. After cooling, we incrementally added a 50% NaOH solution while stirring to adjust the mixture's pH to 7. The ethanol precipitate was consolidated after filtration [30].

Synthesis of 2-bromo-1, 2-(substituted-aryl)ethanone

At 25ºC, trifluoroacetic anhydride (29.5 mmol) was quickly added while stirring hard into a mix of phenyl acetic acid/p-substituted phenyl acetic acid (7.3 mmol), substituted aromatic hydrocarbon (8.8 mmol), and 88–93% orthophosphoric acid (8.8 mmol). The mixture experienced a vigorous exothermic reaction, resulting in a dark-hued solution. The reaction mixture was introduced into 50 milliliters of ice-cold water while being stirred for one minute at the same temperature. We then used 10 mL of cold hexane to wash it until it solidified [31].

Synthesis of 2-cyclopropyl-5, 6-diarylsubstituted imidazo- thiadiazole

Combine a-bromo-1-(4''-substituted) and 2-amino-5-substituted-1,3,4-thiadiazole (10 mmol). 3-(4'-substituted) Phenyl-2-phenyl-1-ethanone (10 mmol) in 150 mL of anhydrous ethanol was subjected to reflux in a water bath for 6–8 hours. Subsequently, phosphorus pentoxide (3 mmol) was introduced, and the refluxing procedure was extended for an additional 4 to 6 hours. The reaction mixture was permitted to cool to ambient temperatures overnight. After taking out the extra solvent using low pressure, the solid hydrobromide was filtered, washed with cold ethanol, and allowed to dry. The free bases were made by mixing hydrobromide salts with a cold water solution of Na_2CO_3 to neutralize them, and then they were further purified by dissolving and re-crystallizing from dry ethanol. The chemicals were cleaned up using column chromatography with fine silica gel, and they were washed out with either a mix of ethyl acetate and hexane or a mix of chloroform and hexane [32].

Caspase colorimetric kit

The p-nitroaniline (pNA) molecule is released when acetyl-Asp-Glu-Val-Asp p-nitroanilide is broken down by caspase 3, which is the basis for the Caspase 3 Colorimetric Assay Kit. At 405 nm, p-nitroaniline has an extinction coefficient of 10.5 $mM^{-1}cm^{-1}$. The absorbance readings at 405 nm or a calibration curve made from pNA standards (provided with the kit) are used to determine how much pNA has been released from the substrate. You can use a spectrophotometer to test the sample in a 1 mL amount, or an ELISA reader to check the sample in a 100 µL amount in a 96-well plate [33,34].

3. RESULT AND DISCUSSION

We have worked to create different versions of imidazo [2, 1, b] thiadiazole using cyclopropane carboxylic acid as the starting point. We monitored the Reaction Scheme to facilitate the synthesis. Reacting 2-amino 5-substituted 1, 3, 4-thiadiazole of general formula 3 with substituted a-haloaryl/heteroaryl ketones, as outlined in the scheme, yields compounds 1–9, including a substituted aryl group at positions 5 and 6. Thin-layer chromatography was used to check the reaction with different mixtures of solvents, such as n-hexane: ethyl acetate: formic acid (5:4:1), benzene: acetone (7:3), and chloroform: methanol (9.5:0.5). Comparison revealed that the Rf values differed from each other. The determination of the melting points of the derivatives was made.

Table 1: The molecular docking score with Hydrogen Bond Donor and Hydrogen bond Acceptor

Sr. No.	Molecule No	Docking Score	Hydrogen Bond Donor	Hydrogen bond Acceptor
1	1	-78.3	02	06
2	2	-22.8	03	07
3	3	-83.2	03	07
4	4	-48.7	02	06
5	5	-14.7	02	06
6	6	-70.3	03	07
7	7	-45.2	02	07
8	8	-20.3	03	06
9	9	-65.4	02	07
10	3KJ7	-89.40	03	07

Human Tumor Cell Line (NCI-60 DTP):

A single administration of 10µM is employed to evaluate all compounds across the 60 cell lines in the initial phase of the two-stage screening process. The comparison program may evaluate the outcomes from the single dose screen, shown as a mean graph. The 60-cell panel is employed to further evaluate substances exhibiting significant growth inhibition across five distinct concentration levels.

Table 2: List of design molecules

Molecule No	R1	R2
1	4-$(OCH_3)C6H_4$	C_6H_5
2	C_6H_5	C_6H_5
3	4-$(CH_3)C_6H_4$	C_6H_5
4	4-$(SCH_3)C_6H_4$	C_6H_5
5	4-ClC_6H_4	C_6H_5
6	4-BrC_6H_4	4-$OCH_3C_6H_4$
7	4-$(OCH_3)C_6H_4$	4-$OCH_3C_6H_4$
8	4-$(SCH_3)C_6H_4$	4-$OCH_3C_6H_4$
9	4-$(Cl)C_6H_4$	-

***Invitro* cancer activity:**

The human cancer cell lines used for cancer screening are grown in a special liquid called RPMI 1640, which has added L-glutamine and 5% fetal bovine serum. Depending on how quickly the isolated cell lines grow, cells are placed into 96-well microtiter plates with 100 µL of liquid, using between 5,000 and 40,000 cells in each well for a typical screening test. Before adding the experimental drugs, the microtiter plates are kept at 37°C for 24 hours with 5% CO_2, 95% air, and 100% humidity after the cells are added. Two plates of each cell line are fixed in situ with TCA after 24 hours to quantify the cell population at the time of drug administration (Tz). Before giving the drugs, the experimental pharmaceuticals are frozen and then mixed with dimethyl sulfoxide to make a solution that is 400 times stronger than the highest concentration that will be tested. A portion of frozen concentrate is thawed and diluted with complete medium containing 50 µg/ml gentamicin upon medication addition. This is executed at twice the desired ultimate maximum test concentration. Five drug concentrations, along with a control, are achieved through the preparation of four further ten-fold serial dilutions. The final drug concentration is reached by adding 100 µl samples of the different drug dilutions into the right microtiter wells, which already have 100 µl of medium in them. Following the administration of the drug, the plates are incubated for a further 48 hours at 37 °C, with 5% CO_2, 95% air, and 100% relative humidity. The introduction of cold TCA concludes the experiment for adherent cells. 50 µl of cold 50% (w/v) TCA (final concentration, 10% TCA) is meticulously applied to the cells for fixation, followed by a 60-minute incubation at 4 °C. The plates are rinsed five times with tap water and permitted to air dry, while the supernatant is discarded. Each well is allocated 100 µl of a sulforhodamine (SRB) solution at a concentration of 0.4% (w/v) in 1% acetic acid. The plates are subsequently permitted to rest at ambient temperatures for 10 minutes. After staining, we allow the plates to air dry and then remove the excess dye by washing them five times with 1% acetic acid. After the dye is dissolved using 10 mM Trizma base, the amount of light absorbed is measured at 515 nm with an automatic plate reader. The process is the same for suspension cells, but 50 µl of 80% TCA is gently added to the wells to fix the cells that have settled at the bottom of the test. Upon achieving the activity level, values are calculated for each parameter; if the effect is not achieved or surpassed, the value for that parameter is indicated as exceeding or falling short of the tested minimum or maximum concentration. Additional structures of the synthesized compounds were elucidated using spectral data. In their spectra, compounds 1–9 showed absorption bands starting at 1600 cm^{-1} for the C=N stretch, while compound 3 had peaks at cm^{-1} for NH_2. The creation of bridgehead nitrogen heterocycles 1–9 through cyclodehydration via 3 is shown by the loss of a single peak in the 1H NMR spectrum between δ-6.0 and 6.5 ppm. The presence of a methoxy group in compounds 1–7 was shown by single peaks around 3.8 ppm, while the cyclopropane, methoxy, and methyl groups in their structures were indicated by separate peaks at 1.16-2.39, 3.73, and 2.47 ppm. Aromatic protons were distinctly observable in compounds 1–9 at δ 7.09–7.8 ppm. The strongest signals in the C-NMR spectra were found at δ 123.961, 159.137, and 162.905 ppm for the imidazole's C-N and the thiadiazole carbon's C-S. Carbon signals of the phenyl group were detected at δ values ranging from 128.0 to 132 ppm. The M+1 peak of Compound 1 was seen at 348.0. The in vitro anticancer effectiveness of synthetic compounds was evaluated at NCI, USA. We examined the effects of several substituents at the 4' and 4" positions of the aromatic ring. Compounds 1 and 3 exhibited notable anticancer activity.

4. CONCLUSION

The synthesis of imidazo-thiadiazole derivatives is covered by the current work. As part of a new series of derivatives, imidazo-thiadiazole with a cyclopropyl moiety was created by refluxing several substituted 2-bromo-1,2-diarylethanones with substituted 2-amino-1,3,4-thiadiazole in anhydrous ethanol. The yield of the produced chemicals ranged from 70 to 80

percent. In contrast to substituted phenyl compounds, we were able to produce a favorable yield of phenyl derivatives. Each of the recently created substances was characterized using physical, spectroscopic, and analytical data. The usual compounds' mass, NMR, and infrared spectra were examined.

5. DECLARATIONS

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

All the authors approved the manuscript for publication.

Availability of data and material:

All required data is available.

Competing interests:

All authors declare no competing interests.

Funding:

Not applicable.

REFERENCES

[1] Tiwari, G., Gupta, M., Devhare, L. D., & Tiwari, R. (2024). Therapeutic and phytochemical properties of thymoquinone derived from Nigella sativa. Current Drug Research Reviews Formerly: Current Drug Abuse Reviews, 16(2), 145-156.

[2] Mostafa, M. S., Radini, I. A. M., El-Rahman, N. M. A., & Khidre, R. E. (2024). Synthetic Methods and Pharmacological Potentials of Triazolothiadiazines: A Review. Molecules, 29(6), 1326.

[3] Tiwari, R., Khatri, C., Tyagi, L. K., & Tiwari, G. (2024). Expanded Therapeutic Applications of Holarrhena Antidysenterica: A Review. Combinatorial Chemistry & High Throughput Screening, 27(9), 1257-1275.

[4] Dincel, E. D., & Güzeldemirci, N. U. (2019). Discovery, Synthesis and Activity Evaluation of Novel Compounds Bearing 1, 2, 4-triazolo [3, 4-b][1, 3, 4] thiadiazine Moiety: A Review. Sağlık Bilimlerinde İleri Araştırmalar Dergisi, 2(2), 60-70.

[5] Tiwari, G., Tiwari, R., & Kaur, A. (2023). Pharmaceutical Considerations of Translabial Formulations for Treatment of Parkinson's Disease: A Concept of Drug Delivery for Unconscious Patients. Current Drug Delivery, 20(8), 1163-1175.

[6] Tiwari, R., Tiwari, G., & Parashar, P. (2023). Theranostics Applications of Functionalized Magnetic Nanoparticles. In Multifunctional And Targeted Theranostic Nanomedicines: Formulation, Design And Applications (pp. 361-382). Singapore: Springer Nature Singapore.

[7] Tiwari, R., Tiwari, G., Mishra, S., & Ramachandran, V. (2023). Preventive and therapeutic aspects of migraine for patient care: An insight. Current Molecular Pharmacology, 16(2), 147-160.

[8] Boraei, A. T., Ghabbour, H. A., Gomaa, M. S., El Ashry, E. S. H., & Barakat, A. (2019). Synthesis and anti-proliferative assessment of triazolo-thiadiazepine and triazolo-thiadiazine scaffolds. Molecules, 24(24), 4471.

[9] Tiwari, R., & Pathak, K. (2023). Local drug delivery strategies towards wound healing. Pharmaceutics, 15(2), 634.

[10] Tiwari, R., Tiwari, G., Sharma, S., & Ramachandran, V. (2023). An Exploration of herbal extracts loaded phyto-phospholipid complexes (Phytosomes) against polycystic ovarian syndrome: Formulation considerations. Pharmaceutical Nanotechnology, 11(1), 44-55.

[11] Tiwari, G., Chauhan, A., Sharma, P., & Tiwari, R. (2022). Nutritional Values and Therapeutic Uses of Capra hircus Milk. International Journal of Pharmaceutical Investigation, 12(4).

[12] Kaushik, D., Sardana, S., & Mishra, D. N. (2009). 5-fluorouracil loaded guar gum microspheres for colon delivery: preparation, characterization and in vitro release. Yao xue xue bao= Acta pharmaceutica Sinica, 44(11), 1278-1284.

[13] Deep, A., Kaur Bhatia, R., Kaur, R., Kumar, S., Kumar Jain, U., Singh, H., ... & Kishore Deb, P. (2017). Imidazo [1, 2-a] pyridine scaffold as prospective therapeutic agents. Current topics in medicinal chemistry, 17(2), 238-250.

[14] Dincel, E. D., Akdağ, Ç., Kayra, T., Coşar, E. D., Aksoy, M. O., Akalın-Çiftçi, G., & Ulusoy-Güzeldemirci, N. (2022). Design, synthesis, characterization, molecular docking studies and anticancer activity evaluation of novel hydrazinecarbothioamide, 1, 2, 4-triazole-3-thione, 4-thiazolidinone and 1, 3, 4-oxadiazole derivatives. Journal of Molecular Structure, 1268, 133710.

[15] Jyoti, K., Pandey, R. S., Kush, P., Kaushik, D., Jain, U. K., & Madan, J. (2017). Inhalable bioresponsive chitosan microspheres of doxorubicin and soluble curcumin augmented drug delivery in lung cancer cells. International journal of biological macromolecules, 98, 50-58.

[16] Kaushik, D., Sardana, S., & Mishra, D. N. (2009). In vitro cytotoxicity analysis of 5-fluorouracil loaded guar gum microspheres on HT-29 colon cancer cell line. Int J Pharm Sci Drug Res, 1(2), 83-4.

[17] Kaushik, D., Sharma, K., & Sardana, S. (2016). Colon targeting guar gum microspheres of 5-aminosalicylic acid: evaluation of various process variables, characterization and in-vitro drug release. Cell, 91, 130-2221072.

[18] Bléhoué IC, Koné M, Esmell EA, Fofana I,KéïtaM, MegnassanE. Molecular modelling and virtual screening application to the computer-aided design of anticancer inhibitors with a favorable pharmacokinetic profile againstE6 papillomavirus type 16. Universal Journal of Pharmaceutical Research 2024; 9(5): 51-67.http://doi.org/10.22270/ujpr.v9i5.1198

[19] El-Behairy MF, Fayed MAA, Ahmed RM, Abdallah IA.Computational drug reproposing to Identify SARS-CoV-2 Mpro Inhibitors: Molecular Docking, ADMET analysis, and in-silico/in-vitrotoxicity study. Universal Journal ofPharmaceutical Research 2022; 7(5):23-31.https://doi.org/10.22270/ujpr.v7i5.837

[20] Awofisayo O. Insilico identification of target palindromic genes as potential drug targets in breast cancer therapy. Universal Journal of Pharmaceutical Research 2020; 5(5):1-3.https://doi.org/10.22270/ujpr.v5i5.47

[21] Kouman KC, Kouassi AF, Fagnidi YKH, Fofana I, Allangba KNPG, KéitaM, MegnassanE. Virtual design of novel of orally bioavailable piperazine inhibitors of enoyl-acyl carrier protein reductase of Mycobacterium tuberculosiswith favorable pharmacokinetic profiles. Universal Journal of Pharmaceutical Research 2024; 9(5): 91-104.http://doi.org/10.22270/ujpr.v9i5.121

[22] AWofisayo O. In silicoantimalarial target selection conserved in four Plasmodiumspecies.Universal Journal of Pharmaceutical Research 2020; 5(4):27-31.https://doi.org/10.22270/ujpr.v5i5.48

[23] Ajmal S, Khan S. In silicoligand-based 2D pharmacophore generation for H+/K+ATPase inhibitors. Universal Journal of Pharmaceutical Research.2017; 2(4): 29-35.http://doi.org/10.22270/ujpr.v2i4.R7

[24] Pippalla, S., Kumar, V., Nekkalapudi, A.R.(2024).A Novel, Stability-Indicating RP-HPLC Method for Simultaneous Estimation of Assay and Organic Impurities of Pyridostigmine Bromide and Assay of Sodium Benzoate in Liquid Oral Formulation. Pharm Chem J 58, 1339–1347.

[25] Sreenivas Pippalla, Arjuna Rao Nekkalapudi, Suresh Babu Jillellamudi .(2022).Stability Indicating RP-UPLC Method for Quantification of Glycopyrrolate, Methylparaben and Propylparaben Assay in Liquid Oral Formulation American Journal of Analytical Chemistry 13(12).

[26] Pippalla S, Nekkalapudi AR, Jillellamudi SB, Reddy MP, Kumar CV.(2023) A stability-indicating, reversed-phase HPLC method for quantification of assay and organic impurities in doxycycline hyclate bulk and parenteral dosage forms. Biomed Chromatogr. 2023 Mar 17: e5626.

[27] Sreenivas Pippalla, Arjuna Rao Nekkalapudi, Venugopal Reddy Komreddy.(2024).A validated stability-indicating reversed-phase-UPLC method for simultaneous estimation of promethazine hydrochloride, methylparaben, propylparaben and sodium benzoate assay of cough suppressant and antihistamine liquid oral dosage forms. Biomed Chromatogr. 2024 July14: e5944.

[28] Sreenivas Pippalla, Srinivasulu Kasa, Dipak Goyal, Venugopal Komreddy, Poluri Venkata Reddy.(2024).A Novel Reversed Phase HPLC Assay Method for Simultaneous Estimation of Glucose, Sodium Citrate and Chlorides in Pharmaceutical Formulations and Drug Solution for Oral Rehydration. Journal of Pharmaceutical, Research & Reports. SRC/JPRSR-180.

[29] Gaurav Tiwari, Santosh Karajgi, Vattakkalvalasu Ramathan Ravikkumar, Ram Kumar Choudhary, Jegannathan Kannan Shyamala, Vinod Kumar, Sreenivas Pippalla.(2024).An In-depth Review of Exploring the Potential of Colloidosomes in Drug Delivery.International Journal of Pharmaceutical Investigation 14 (4).

[30] Dr. Dinesh Kaushik, Manisha, Dr. Saroj Jain(2024), Fast dissolving tablets of Nano-Steroids Anti-Inflammatory drug: A review in IJRTI, volume 9, Issue 3, pages 706-720.

[31] Gourav, Dinesh Kaushik, Saroj Jain(2022), SNEDDS: A vital role in Drug Delivery true or myth in WJPER, volume 11, Issue 13, pages 1965-1991.

[32] Arman Dalal, Dinesh Kaushik, Saroj jain (2022), Invasomes : A novel deformable vasicluar nanocarrier for

enhanced transdermal drug delivery in BJPMR, volume 7, Issue 5, pages 4044-4059.

[33] Balekundri, A., Ahire, E.D. and Keservani, R.K., (2024). Plant Metabolites and Vegetables for Diabetes Prevention and Treatment. In *Plant Metabolites and Vegetables as Nutraceuticals* (pp. 333-360). Apple Academic Press.

[34] Ahire, E.D., Surana, K.R., Keservani, R.K., Gupta, A.K., Yadav, A., Bharti, S.K., Jaiswal, M. and Singh, B.K., (2021). Current overview of the nutraceutical nanoparticulate delivery technology with special emphasis on herbal formulation.