Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 14, Issue 3, 2025, 106
https://doi.org/10.33263/LIANBS143.106

Designing, Screening, Synthesis, and Biological Activities of *S*-Linked Tetrazoles Derived from Fmoc/Boc/Cbz Amino Acids

Lalithamba HaralurShankaraiah [1,*], Ramya Mariswamy [2], Kumari Dalli [2], Nagendra Govindappa [2,*]

[1] Department of Chemistry, Siddaganga Institute of Technology, Tumkur – 572103, Karnataka, India; hsl@sit.ac.in;
[2] Department of Chemistry, School of Applied Sciences, REVA University, Rukmini Knowledge Park, Kattigenahalli, Yelahanka, Bangalore - 560 064, Karnataka, India; ramyaanu62@gmail.com (R.M.); kumari.aums@gmail.com (K.D.); gnagendrachem@gmail.com (N.G.);
* Correspondence: hsl@sit.ac.in (L.H.S); gnagendrachem@gmail.com (N.G);

Received: 14.12.2023; Accepted: 5.02.2024; Published: 4.09.2025

Abstract: The synthesis and development of peptides containing heterocycles, such as tetrazoles, have gained significant attention due to their biological significance and pharmacophores. In this study, *S*-linked tetrazoles derived from Fmoc/Boc/Cbz-amino acids were synthesized. Seven target proteins were selected, including actibindTRnase protein from *Aspergillus niger* (PDB ID: 3D3Z), anti-inflammatory proteins like COX1, COX2, LOX5 (PDB ID: 1CQE, 1CX2, 3V99, respectively), and breast cancer-causing protein thioredoxin kinase (PDB ID: 1H6V, 1T46, 2J5F). The 3D structures of these proteins were obtained from the protein data bank, and the active sites were predicted for docking studies. Based on the docking scores, five lead amino acid-linked tetrazole compounds were chosen, which had the most hydrogen bonds. These compounds were tested for their stability using dynamic tests performed with Discovery Studio 3.5. The lead compounds were prepared using a mixed anhydride reaction and Hantzsch's protocol from protected amino acids. The keto thiocyanates were synthesized from the respective bromomethyl ketones and subjected to a cycloaddition reaction with azide ion with Lewis acid catalyst to obtain the keto *S*-linked tetrazoles. HRMS and NMR were used to characterize the compounds. Finally, the synthesized compounds were evaluated for their anticancer activity on MCF7 cell lines for breast cancer.

Keywords: peptidomimetics; molecular docking; Hantzsch protocol; molecular dynamics.

1. Introduction

Synthetic organic compounds are many, which are sulphanilamide, thiazolidinones, benzothiazines, thiazoles, and tetrazoles [1], etc., which are Schiff's bases [2]. Among them, tetrazoles are widely used in medical [3], pharmacological, and biological activities such as antibacterial [4-6], antifungal [7,8], analgesics, anti-inflammatory [9], antihypertensive [10], antiallergic, antibiotic [11], and anticonvulsant agents [12]. The fundamental core of tetrazoles [13,14] contains heterocycles of nitrogen, and this demonstrates biological and pharmacological properties [15] with structures similar to natural and synthetic compounds having biological activity [16-18]. Tetrazoles, other than pharmacological and biological activities, have applications in the photographic industry, organic chemistry, agriculture [19], coordination chemistry [20], and explosives [21]. Tetrazole compounds, also known as tetrazolic acids, are versatile molecules that exist in neutral, cationic, or anionic forms [22]. Tetrazoles can be synthesized by various methods, such as by using sodium azide [23], nitriles [24], and an excess of triethylamine hydrochloride. In addition, *tert*-butyl alcohol can be used along with the reaction of nitriles with sodium azide/acetic acid. Among these methods, cyclization processes using sodium azide [25] have been proven to be more effective.

Peptidomimetics is a technique where a peptide structure in 3D space is mimicked naturally by retaining its biological activity [26]. Peptides are made up of amino acids [27] since amino acids act as neurotransmitter transport agents[28,29] and biosynthesis, and some act as antibiotics [30]. Peptidomimetics of amino acids enhance their biological activity [31-33].

We are interested in mimicking tetrazoles with amino acids since they exhibit potent pharmacological and biological activities. We designed tetrazoles with Fmoc, Boc, and Cbz protecting groups. Target proteins such as actibindTRnase protein, COX1, COX2, LOX5, and Breast cancer-causing protein thioredoxin kinase. Molecular dockings were performed for the active region of proteins and designed structures [34,35]. Stable compounds were chosen based on the docking scores obtained, and their synthesis was performed using Hantzsch's procedure and a mixed anhydride reaction [36]. The intermediates like diazomethyl ketone, bromomethyl ketone, and keto thiocyanates were synthesized as per the Scheme 1 outlined below, and cycloaddition of these keto thiocyanates with azide ion is performed with Lewis acid catalyst to obtain the keto *S*-linked tetrazoles [37]. Dynamics studies have been executed for the lead compounds with proteins of interest to evaluate their stability in the organism using Discovery Studio 3.5 [38].

2. Materials and Methods

2.1. In silico approach.

The target protein 3-dimensional structure of *Aspergillus niger*, and anti-inflammatory targets like COX1, and COX2, were selected for in silico approach, and the structure (PDB id: 3D3Z, 1CQE, 1CX2) was downloaded from the PDB website and cleaned using Discovery Studio 3.5 (DS). A simulation box was set up for 20 nanoseconds, and the simulation was run to minimize protein [39]. The active site present on the protein molecules was predicted by using DS 3.5. The molecules of the ligand were sketched using ChemDraw software, where the amine group was protected by protecting groups such as Fmoc, Boc, and Cbz. The amino acid chain was extended along the carboxyl side and saved in a mol file format. Then, ligand minimization was carried out to get a stable ligand molecule by using Discovery Studio [40]. The ligand molecule was checked for Lipinski's rule of 5 and ADMET properties. The designed ligand is shown in Figure 1. Further, Ligand-Protein docking was conducted using Lead ITFlexX molecular docking, and Molecular Dynamics studies were performed using Discovery Studio 3.5 [41].

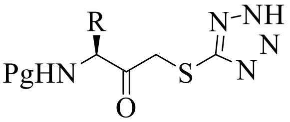
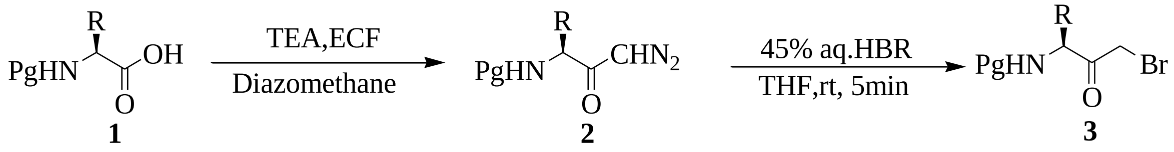


Figure 1. Designed ligand compound.

2.2. Synthesis.

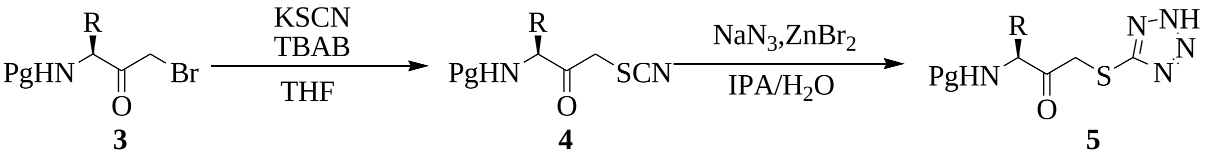
The *N*-protected amino acid was reacted with triethyl amine (TEA), ethyl chloroformate (ECF), and NMU (*N*-nitroso *N*-methyl urea) using the mixed anhydride method to form a diazomethyl ketone. The resulting intermediate was dissolved in THF, and to the solution, 45% aqueous HBr was added. The reaction progress was monitored using TLC. Finally, the bromomethyl ketone was obtained by diluting the reaction mixture with water, as shown in Scheme 1.



Pg = Protecting group
TEA = Triethyl amine
ECF = Ethyl chloroformate

Scheme 1. Synthesis of *N*-protected bromomethyl ketone.

Bromomethyl ketone acts as the precursor for the formation of keto thiocyanates. Bromomethyl ketone was dissolved in tetrahydrofuran (THF), made to react with potassium thiocyanate (KSCN) and TBAB (tetra *N*-butyl ammonium bromide), and kept for reflux for 2 hours. The obtained compound was observed through TLC. Ketothiocyanates obtained were reacted with a calculated amount of sodium azide and zinc bromide in water/isopropanol (1:1) for 16 hours of reflux, as shown in Scheme 2. A simple workup involving acidification to obtain tetrazoles in pure form, and checked by TLC.



Pg = Fmoc/Boc/Cbz

Scheme 2. Synthesis of *N*-protected ketomethylene *S*-linked tetrazoles.

3. Results and Discussion

3.1. Anticancer activity.

3.1.1. MTT assay.

The cell line was cultured in 25 cm^2 flasks and harvested using trypsin. A hemocytometer was used to count the number of cells. Next, each well of 96-well plates was added with 1 × 10^4 cells/100 µL medium and incubated for 24 hours. After that, the cell lines were treated with varying concentrations of a sample that was dissolved in a medium, and the incubation continued for a further 48 hours. Then, 20 µL of MTT (5 mg/mL) in phosphate-buffered saline (PBS) was added to each well, and the plate was incubated at 37°C for 4 hours. The medium was then removed, and each well was filled with 100 µL of dimethyl sulfoxide. The plate was incubated again for 10 minutes at 37°C, and finally, the plate was read at 570 nm using a microplate reader. The percentage of cell viability was calculated using the formula:

$$([AB - AA]/AB) \text{ x } 100 \qquad (1)$$

AB represents the absorption of the blank sample, whereas the absorption of the test sample is represented by AA.

3.1.2. *In silico* results.

Ligand preparation: In this study, we drew the structures using ChemDraw software to protect the amine end and extend the carboxyl end using *S*-linked ketomethylene tetrazoles. Around 80 structures were drawn for the study, 60 structures were protected (Fmoc, Boc, and Cbz, respectively), and 20 structures, 60 of which were protected (Fmoc, Boc, and Cbz, respectively) and 20 of which were without protection. These 80 structures were minimized and screened using Discovery Studio v3.5. The 78 screened compounds were again screened by the Lipinski rule of 5 and ADMET property using Discovery Studio v 3.5. The screened ligands were saved as library files in PDB format for docking studies. Lipinski rule and ADMET properties were assessed for five selected compounds, with the results summarized in Tables 1 and 2. The structures of the screened compounds are illustrated in Figure 2.

Table 1. Lipinski rule of 5 of compounds[#].

Compound	**H- Donors**	**H- Acceptors**	**A logP**	**Molecular weight**
Cbz-Ser-*ψ*[CO-CH_2-S-Tet] (**5a**)	5	9	0.313	337.35
Cbz-Arg-*ψ*[CO-CH_2-S-Tet] (**5b**)	5	9	3.39	407.47
Fmoc-Ala-*ψ*[CO-CH_2-S-Tet] (**5c**)	5	9	-0.339	409.46
Cbz-Trp-*ψ*[CO-CH_2-S-Tet] (**5d**)	5	9	3.808	402.47
Fmoc-Arg-*ψ*[CO-CH_2-S-Tet] (**5e**)	5	10	-0.003	495.57

[#] This table signifies the H- Donors, H- Acceptors, and the lipophilicity of the compound represented as log P and molecular weight.

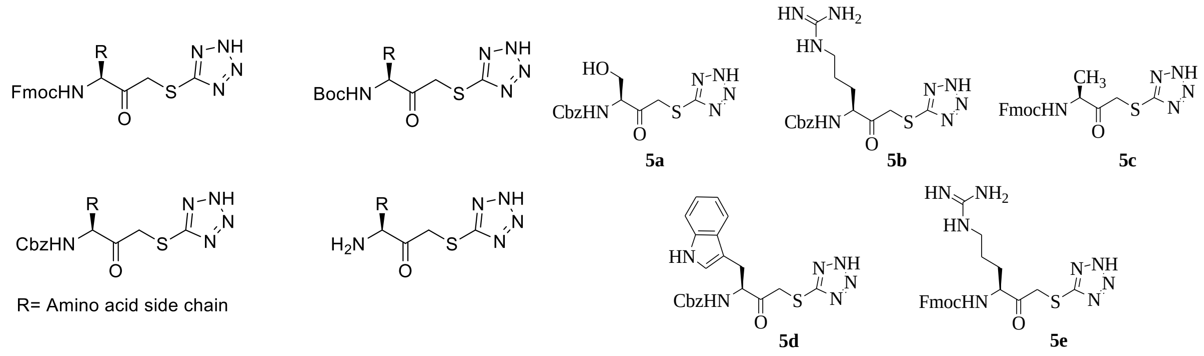


Figure 2. Structures of the screened compounds

Table 2. ADMET properties of 5 compounds.

Compound	**Solubility level**	**Blood-brain barrier level**	**Extension CYP2D6**	**Extension hepatotoxic**	**Extension PPB**
Cbz-Ser-*ψ*[CO-CH_2-S-Tet] (**5a**)	3	4	-3.50	-6.07	-7.855
Cbz-Arg-*ψ*[CO-CH_2-S-Tet] (**5b**)	3	4	-4.2	-6.9	-7.89
Fmoc-Ala-*ψ*[CO-CH_2-S-Tet] (**5c**)	-2.54	4	-6.3589	0.2945	-9.06
Cbz-Trp-*ψ*[CO-CH_2-S-Tet] (**5d**)	1	4	-2.60	0.6413	-13.054
Fmoc-Arg-*ψ*[CO-CH_2-S-Tet] (**5e**)	3	4	-4.54	0.366	-15.99

It is crucial to check the ADMET properties of any drug candidate to ensure its safety. These properties include metabolism, adsorption, excretion, distribution, and toxicity. For a drug to be considered safe, it should have low levels of these properties. The table provided above represents the drug's solubility, BBB (Blood-Brain Barrier) penetration, CYP2D6 (Cytochrome P450 2D6) inhibition, HEPATOX (Hepatotoxicity), and PPB (Plasma Protein

Binding). A solubility score of 0-2 indicates high solubility. BBB penetration score of 1 indicates high penetration, 2 indicates medium penetration, and 3 indicates low penetration. A CYP2D6 score of -ve indicates non-inhibitors, while a positive score indicates inhibition. For HEPATOX, a score of less than 1 indicates nontoxicity. The PPB score indicates the drug's binding capacity; the higher the value, the greater the binding capacity.

3.2. Protein preparation.

Target proteins: ACTIBIND protein (3D3Z) produced in *Aspergillus niger* belongs to the T2 RNase family and has functions such as digestion of extracellular poly ribonucleotide, which in turn accelerates phosphate uptake and also interferes in intracellular actin network structure [42]. Inflammatory proteins are COX1 (1CQE), COX2 (1CX2) and LOX5 (3V99). COX1 and COX2 are involved in the biosynthesis of arachidonic acid to PGG_2, which causes inflammation in organisms [43,44]. An arachidonic acid mediator, leukotriene, in the presence of LOX5, LOX15, and leukotriene A_4, is produced, which promotes inflammation in organisms [45]. Breast cancer proteins are thioredoxin (1H6V), c-kit tyrosine kinase (1T46), and a closely related tyrosine kinase protein, EGFR (2J5F). 3D sketches of target proteins were obtained from the PDB website. Proteins were minimized, and the active location was recognized by reference ligand using Discovery Studio v 3.5.

Docking: Protein-ligand docking was carried out in Lead IT using the FlexX algorithm. FlexX can predict protein-ligand complex geometry accurately and quickly for known protein 3D structures and small ligands. The active region of proteins was allowed to interact with the screened ligands. From the docking scores, we can analyze that the more negative the value, the more interaction there is with the protein.

Synthesis: The next process after docking was to synthesize the compounds based on the docking score. Synthesis of tetrazoles was done *via* mixed anhydride reaction, bromination, thiocyanate reaction, and finally, synthesis of *S*-linked keto tetrazoles.

A mixed anhydride reaction forms diazomethyl ketone, which further reacts with HBr to form bromomethyl ketone. Bromomethyl ketone forms a keto thiocyanate compound when reacted with KSCN and TBAB. Cycloaddition of these keto thiocyanates with azide ion in the presence of a Lewis acid catalyst will be carried out to obtain the *N*-protected keto methylene *S*-linked tetrazoles and partially verified by TLC. Synthesized compounds were evaluated by HRMS for mass and ^{13}C and ^{1}H NMR for structure identification. These results show that the synthesized compounds and the designed compounds' molecular weights are exactly the same, and these results indicate that the synthesized compounds do not have any contamination.

3.2.1. Molecular docking and dynamics.

The target protein 3D structure of *Aspergillus niger* and anti-inflammatory targets like COX1 and COX2 were selected for in silico approach, and the structure (PDB id: 3D3Z, 1CQE, 1CX2) was downloaded from the PDB website (www.rcsb.org/pdb) and cleaned using Discovery Studio 3.5 (DS). The active region residues are tabulated in Table 3.

Table 3. Active site residues of selected target proteins.

Protein name	Active site residues
Antifungal	PHE35, HIS51, ASP97, TYR98, PHE107, HIS110
Anti-inflammation (COX1)	VAL116, ARG120, ILE245, VAL349, GLN350, LEU352, SER353, TYR355, LEU359, TRP387, PHE518, MET522, ILE523, GLU524, GLY526, ALA527, PRO528, SER530, LEU531, LEU534.

Protein name	Active site residues
Anti-inflammation (COX2)	ARG120, GLN192, VAL349, LEU352, SER353, GLY354, TYR355, TRP387, ARG513, PHE518, VAL523, GLU524, ALA527

Protein-ligand docking was performed using the FlexX algorithm in Lead IT software. The algorithm accurately predicts the geometry of the protein-ligand complex within a few seconds for a protein with a known 3D structure and a small ligand molecule. The docking score has been tabulated in Table 4.

Table 4. Docking score and interaction residues for selected proteins.

Target Protein	Ligand name	Docking score	Interacting residues
3D3Z	Fmoc-Ala-ψ[-CO-CH_2-S-Tet] (**5c**)	-13.1587	PHE35, HIS51, ASP97, TYR98
	STD (fluconazole)	-8.2817	PHE35, HIS51, ASP97, TYR98
COX1	Cbz-Trp-ψ[-CO-CH_2-S-Tet] (**5d**)	-17.4948	VAL116, ARG120, ILE245, VAL349, GLN350, LEU352, SER353, TYR355, LEU359
COX2	Fmoc-Arg-ψ[-CO-CH_2-S-Tet] (**5e**)	-11.9524	ARG120, GLN192, VAL349, LEU352
	STD (Ibuprofen)	-10.3762	ARG120, GLN192, VAL349, LEU352

3.3. Antifungal studies.

In this present work, the strain used for the Kirby-Bauer well diffusion method was *Aspergillus niger* (MTCC 2425). The choice of test microorganisms depends on the main purpose of the study. From the above table, it's clear that the synthesized sample, i.e., Fmoc-Ala-ψ[-CO-CH_2-S-Tet] (**5c**), shows better activity when compared to fluconazole. The majority of tetrazoles are susceptible and show their activity when tested at 500 µg/mL [46]. The reason for the susceptible nature of the compounds is due to the interactions with the proteins selected, and also due to the kind of penetration of the compounds into the bacterial cell.

Table 5. The inhibition zones of Fmoc-Ala-ψ[CO-CH_2-S-Tet] against *Aspergillus niger* (MTCC2425).

Sl no.	Sample	Volume and concentration (mg/mL)	Inhibitory zone (mm)
1	Std	100 (7 mg/mL)	7.0
2	Fmoc-Ala-ψ[CO-CH_2-S-Tet] (**5c**)	50 (10 mg/mL)	0.0
3		100 (10 mg/mL)	1.0
4		150(10 mg/mL)	2.0
5		200 (10 mg/mL)	2.5
6		250 (10 mg/mL)	3.0
7		300 (10 mg/mL)	4.0

3.4. Anti-inflammatory studies.

The study aimed to test the anti-inflammatory activity of purified peptides. The method employed was a tail immersion test in Swiss Albino mice of 30 mg. The water bath was maintained at a constant temperature of 55°C. The drug was given half an hour before the immersion of the tail in water.

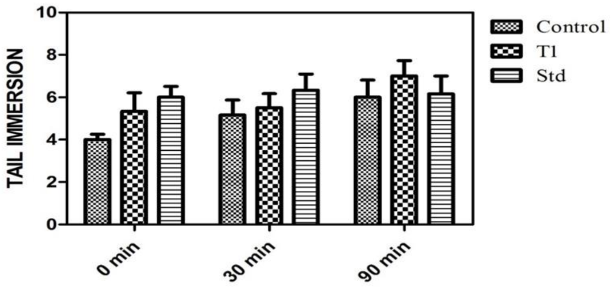


Figure 3. Anti-inflammatory studies for peptide.

The time taken for the first flick by mice when their tail was immersed in water is shown in Figure 3. From the above figure, we can say that if the compound is injected into the body of mice, the time of flicking the tail increases, which shows the compound's activity in reducing pain for a certain period of time. The compound has exhibited a centrally acting analgesic effect through a tail immersion test, which raises the pain threshold of animals towards heat. This elevates the pain threshold of animals towards heat. Hence, we can say our compound is showing analgesic activity.

3.5. Writhing test.

Pure peptides were investigated for their anti-inflammatory activity. The method employed was a writing test in Swiss Albino mice of 30 mg. Acetic acid induces irritation in the abdominal region, which makes mice stretch their abdomen to release the stress in them. Usually, writhing starts within 5 minutes when only acetic acid is given, but when the drug is injected into the mice, it can withstand the pain in their abdominal part, which signifies the action of the drug by increasing the initiation time period of writhing shown in Figure 4.

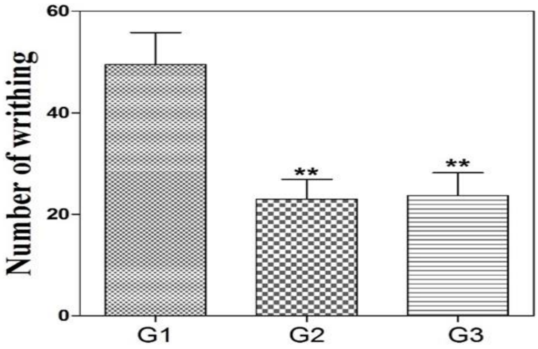


Figure 4. Writhing test for peptide.

Based on the values we observed, the number of writhing in group 2 was reduced by 50% compared to group 1, which was treated with only acetic acid. Our compound is showing the nearest value of IC_{50} to the STD Indomethacin. From this, we can say our compound has analgesic activity and reduces inflammation.

4. Conclusions

In conclusion, we have synthesized 5 lead S-linked tetrazoles derived from Fmoc/Boc/Cbz-amino acids based on the docking scores. Seven target proteins, actibindTRnase protein (3D3Z) from Aspergillus niger, anti-inflammatory proteins (1CQE, 1CX2, 3V99), and breast cancer-causing protein thioredoxin kinase (1H6V, 1T46, 2J5F) were identified, and active sites were predicted. The lead compounds were synthesized by a mixed anhydride reaction and Hantzsch's protocol from *N*-protected amino acids. The keto thiocyanates were synthesized from the respective bromomethyl ketones and subjected to a cycloaddition reaction with azide in the presence of $ZnBr_2$ to obtain the keto *S*-linked tetrazoles. The synthesized compounds were characterized by ^1H NMR, ^{13}C NMR, and HRMS analysis. Finally, the synthesized compounds were evaluated for their anticancer activity on MCF7 cell lines for breast cancer.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

Funding

The present work was supported by REVA University, Kattigenahalli, Yelahanka, Bangalore-560 064, with the grant number: (RU: CONT:0016/2023-24; RU/R&D/SEED/CHE/2023/24).

Acknowledgments

Dr. H.S.L. thanks the Principal, CEO, and Director of SIT, Tumkur, for providing research facilities, and Dr. G.N. and R.M. thank REVA University, Bangalore, for research seed grant support.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Reynard, G.; Lebel, H. Alkylation of 5-Substituted 1*H*-Tetrazoles via the Diazotization of Aliphatic Amines. *J. Org. Chem.* **2021**, *86*, 12452–12459, https://doi.org/10.1021/acs.joc.1c01585.
2. Abdessalam, M.; Sidhoum, M.A.; Zradni, F.-Z.; Ilikti, H. Synthesis of 1,5-Disubstituted Tetrazoles in Aqueous Micelles at Room Temperature. *Molbank* **2021**, *2021*, M1194, https://doi.org/10.3390/M1194.
3. Kamel, M.M.; Ali, H.I.; Anwar, M.M.; Mohamed, N.A.; Soliman, A.M.M. Synthesis, antitumor activity and molecular docking study of novel Sulfonamide-schiff's bases, thiazolidinones, benzothiazinones and their C-nucleoside derivatives. *Eur. J. Med. Chem.* **2010**, *45*, 572–580, https://doi.org/10.1016/j.ejmech.2009.10.044.
4. Abu Mohsen, U.; Yurttaş, L.; Özdemir, A.; Turan-Zitouni, G.; Kaplancikli, Z. Biological evaluation of some tetrazole derivatives as cholinesterase inhibitors. *Clin. Exp. Health Sci.* **2014**, *4*, 1–4, https://doi.org/10.5455/musbed.20140207083648.
5. Strzelecka, M.; Świątek, P. 1,2,4-Triazoles as Important Antibacterial Agents. *Pharmaceuticals* **2021**, *14*, 224, https://doi.org/10.3390/PH14030224.
6. Gao, F.; Xiao, J.; Huang, G. Current scenario of tetrazole hybrids for antibacterial activity. *Eur. J. Med. Chem* .**2019**, *184*, 111744, https://doi.org/10.1016/j.ejmech.2019.111744.
7. Roszkowski, P.; Szymańska-Majchrzak, J.; Koliński, M.; Kmiecik, S.; Wrzosek, M.; Struga, M.; Szulczyk, D. Novel Tetrazole-Based Antimicrobial Agents Targeting Clinical Bacteria Strains: Exploring the Inhibition of *Staphylococcus aureus* DNA Topoisomerase IV and Gyrase. *Int. J. Mol. Sci.* **2022**, *23*, 378, https://doi.org/10.3390/ijms23010378.
8. Myznikov, L.V.; Hrabalek, A.; Koldobskii, G.I. Drugs in the tetrazole series. (Review). *Chem. Heterocycl. Compd.* **2007**, *43*, 1–9, https://doi.org/10.1007/S10593-007-0001-5.
9. Wang, L.; Min, Z.; Jian, G.; Wenzheng, G.; Ni, Z.; Hui, S.; Jinfeng, C.; Zhaoqin, Z.; and Wu, W. In vitro activities of the tetrazole VT-1161 compared with itraconazole and fluconazole against *Cryptococcus* and

non-*albicans Candida* species. *Mycologia* **2021**, *113*, 918-925, https://doi.org/10.1080/00275514.2021.1913949.

10. Azim, T.; Wasim, M.; Akhtar, M.S.; Akram, I. An in vivo evaluation of anti-inflammatory, analgesic and anti-pyretic activities of newly synthesized 1, 2, 4 Triazole derivatives. *BMC Complement Med. Ther.* **2021**, *21*, 304, https://doi.org/10.1186/S12906-021-03485-X.
11. Tatarkiewicz, J.; Bujalska-Zadrożny, M. The antihypertensive drugs and contamination with carcinogenic nitrosamines. *Folia Cardiol.* **2019**, *14*, 564–571.
12. Mekni, N.; Baklouti, A. Synthesis of new 1-substituted 4-perfluoroalkyl tetrazol-5-ones. *J. Fluor. Chem.* **2008**, *129*, 1073–1075, https://doi.org/10.1016/J.JFLUCHEM.2008.06.019.
13. Wei, C.-X.; Bian, M.; Gong, G.-H. Tetrazolium Compounds: Synthesis and Applications in Medicine. *Molecules* **2015**, *20*, 5528-5553, https://doi.org/10.3390/molecules20045528.
14. Nasrollahzadeh, M.; Nezafat, Z.; Bidgoli, N.S.S.; Shafiei, N. Use of tetrazoles in catalysis and energetic applications: Recent developments. *Mol. Catal.* **2021**, *513*, 111788, https://doi.org/10.1016/j.mcat.2021.111788.
15. Swami, S.; Sahu, S.N.; Shrivastava, R. Nanomaterial catalyzed green synthesis of tetrazoles and its derivatives: a review on recent advancements. *RSC Adv.* **2021**, *11*, 39058–39086, https://doi.org/10.1039/D1RA05955F.
16. Patowary, P.; Deka, B.; Bharali, D. Tetrazole Moiety as a Pharmacophore in Medicinal Chemistry: A Review. *Malar. Control Elimin.* **2021**, *10*, 2-11.
17. Sultanat; Abad, A.; Mohd, A.; Asim, R.; Mohd, F.; and Zaman, S. Discovery of a novel oxadiazine derivative of glucocorticoids endowed with DNA binding activities and molecular docking studies. *J. Taibah Univ. Sci.* **2019**, *13*, 536–546, https://doi.org/10.1080/16583655.2019.1603575.
18. Kaushik, N.; Kumar, N.; Kumar, A.; Singh, U.K. Tetrazoles: Synthesis and Biological Activity. *Immunol. Endocr. Metab. Agents Med. Chem.* **2018**, *18*, 3–21, https://doi.org/10.2174/1871522218666180525100850.
19. Sathishkumar, S.; Gayathri, K. Synthesis of Tetrazole Derivatives. *Russ. J. Org. Chem.* **2021**, *57*, 402–416, https://doi.org/10.1134/S107042802103012X.
20. Samanta, S.; Cameron, Z.; Leah, G.; J., B.D.; G., O.A.; Tristan, C.; Logan, G.; and Sandhu, J. Carbazolyl-*bis*(triazole) and Carbazolyl-*bis*(tetrazole) Complexes of Palladium(II) and Platinum(II). *J. Coord. Chem.* **2021**, *74*, 983-1008, https://doi.org/10.1080/00958972.2021.1882674.
21. Maleki, A.; Sarvary, A. Synthesis of tetrazoles *via* isocyanide-based reactions. *RSC Adv.* **2015**, *5*, 60938–60955, https://doi.org/10.1039/C5RA11531K.
22. Kostakis, G.E.; Anson, C.E.; Powell, A.K. Synthesis and Supramolecular Structure of a (5-(3-(1*H*-tetrazol-5-yl)phenyl)-*1H*-tetrazole) Cobalt Complex. *Bioinorg. Chem. Appl.* **2010**, *2010*, 104329, https://doi.org/10.1155/2010/104329.
23. Carpentier, F.; Felpin, F.-X.; Zammattio, F.; Le Grognec, E. Synthesis of 5-Substituted 1*H*-Tetrazoles from Nitriles by Continuous Flow: Application to the Synthesis of Valsartan. *Org. Process Res. Dev.* **2020**, *24*, 752–761, https://doi.org/10.1021/ACS.OPRD.9B00526.
24. Tamoradi, T.; Kal-Koshvandi, A.; Karmakar, B.; Maleki, A. Immobilization of La on THH-$CO_2H@Fe_3O_4$ nanocomposite for the synthesis of one-pot multicomponent reactions. *Mater. Res. Express* **2021**, *8*, 056101, https://doi.org/10.1088/2053-1591/ABFA49.
25. Malik, M.A.; Wani, M.Y.; Al-Thabaiti, S.A.; Shiekh, R.A. Tetrazoles as carboxylic acid isosteres: chemistry and biology. *J. Incl. Phenom. Macrocycl. Chem.* **2014**, *78*, 15–37, https://doi.org/10.1007/S10847-013-0334-X.
26. Vagner, J.; Qu, H.; Hruby, V.J. Peptidomimetics, a synthetic tool of drug discovery. *Curr. Opin. Chem. Biol.* **2008**, *12*, 292–296, https://doi.org/10.1016/J.CBPA.2008.03.009.
27. Bizzarri, B.M.; Pieri, C.; Botta, G.; Arabuli, L.; Mosesso, P.; Cinelli, S.; Schinoppi, A.; Saladino, R. Synthesis and antioxidant activity of DOPA peptidomimetics by a novel IBX mediated aromatic oxidative functionalization. *RSC Adv.* **2015**, *5*, 60354-60364, https://doi.org/10.1039/C5RA09464J.
28. Leopold, A.V.; Shcherbakova, D.M.; Verkhusha, V.V. Fluorescent Biosensors for Neurotransmission and Neuromodulation: Engineering and Applications. *Front. Cell. Neurosci.* **2019**, *13*, 474, https://doi.org/10.3389/fncel.2019.00474.
29. Hüfner, K.; Giesinger, J.M.; Gostner, J.M.; Egeter, J.; Koudouovoh-Tripp, P.; Vill, T.; Fuchs, D.; Sperner-Unterweger, B. Neurotransmitter Precursor Amino Acid Ratios Show Differential, Inverse Correlations

with Depression Severity in the Low and High Depression Score Range. *Int. J. Tryptophan Res.* **2021**, *14*, 11786469211039220, https://doi.org/10.1177/11786469211039220.

30. Welch, B.D.; VanDemark, A.P.; Heroux, A.; Hill, C.P.; Kay, M.S. Potent D-peptide inhibitors of HIV-1 entry. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 16828-16833, https://doi.org/10.1073/pnas.0708109104.
31. Staśkiewicz, A.; Ledwoń, P.; Rovero, P.; Papini, A.M.; Latajka, R. Triazole-Modified Peptidomimetics: An Opportunity for Drug Discovery and Development. *Front. Chem.* **2021**, *9*, 674705, https://doi.org/10.3389/FCHEM.2021.674705.
32. Robinson, J.A. β-Hairpin Peptidomimetics: Design, Structures and Biological Activities. *Acc. Chem. Res.* **2008**, *41*, 1278–1288, https://doi.org/10.1021/AR700259K.
33. Lenci, E.; Trabocchi, A. Peptidomimetic toolbox for drug discovery. *Chem. Soc. Rev.* **2020**, *49*, 3262–3277, https://doi.org/10.1039/d0cs00102c.
34. Oklješa, A.M.; Klisurić, O.R. Synthesis, structural and computational studies of new tetrazole derivatives. *J. Mol. Struct.* **2021**, *1226*, 129341, https://doi.org/10.1016/J.MOLSTRUC.2020.129341.
35. Sun, M.-F.; Chen, H.-Y.; Tsai, F.-J.; Liu, S.-H.; Chen, C.-Y.; Chen, C.Y.-C. Search for Novel Remedies to Augment Radiation Resistance of Inhabitants of Fukushima and Chernobyl Disasters: Identifying DNA Repair Protein XRCC4 Inhibitors. *J. Biomol. Struct. Dyn.* **2011**, *29*, 325–337, https://doi.org/10.1080/07391102.2011.10507388.
36. Qiao, Q.; So, S.S.; Goodnow, R.A. Stereochemical Control Factors in the Hantzsch Thiazole Synthesis: A Hammett Substitution Correlation Analysis. *Org. Lett.* **2001**, *3*, 3655–3658, https://doi.org/10.1021/OL010175T.
37. Sureshbabu, V.V.; Venkataramanarao, R.; Naik, S.A.; Chennakrishnareddy, G. Synthesis of tetrazole analogues of amino acids using Fmoc chemistry: isolation of amino free tetrazoles and their incorporation into peptides. *Tetrahedron Lett.* **2007**, *48*, 7038–7041, https://doi.org/10.1016/j.tetlet.2007.07.129.
38. Tan, V.B.C.; Zhang, B.; Lim, K.M.; Tay, T.E. Explaining the inhibition of cyclin-dependent kinase 5 by peptides derived from p25 with molecular dynamics simulations and MM-PBSA. *J. Mol. Model.* **2010**, *16*, 1–8, https://doi.org/10.1007/S00894-009-0514-1.
39. Dammalli, M.; Chandramohan, V.; Biradar, M.I.; Nagaraju, N.; Gangadharappa, B.S. *In silico* analysis and identification of novel inhibitor for new H1N1 swine influenza virus. *Asian Pac. J. Trop. Dis.* **2014**, *4*, S635–S640, https://doi.org/10.1016/S2222-1808(14)60694-0.
40. Raja Naika, H.; V., K.; K., L.; Vivek, C.; Manjunath, D.; N., N.P.; and Suresh, D. Molecular docking and dynamic studies of bioactive compounds from *Naravelia zeylanica* (L.) DC against glycogen synthase kinase-3β protein. *J. Taibah Univ. Sci.* **2015**, *9*, 41-49, https://doi.org/10.1016/J.JTUSCI.2014.04.009.
41. Chandramohan, V.; Kaphle, A.; Chekuri, M.; Gangarudraiah, S.; Bychapur Siddaiah, G. Evaluating Andrographolide as a Potent Inhibitor of NS3-4A Protease and Its Drug-Resistant Mutants Using *In Silico* Approaches. *Adv. Virol.* **2015**, *2015*, 972067, https://doi.org/10.1155/2015/972067.
42. de Leeuw, M.; González, A.; Lanir, A.; Roiz, L.; Smirnoff, P.; Schwartz, B.; Shoseyov, O.; Almog, O. The 1.8 Å Crystal Structure of ACTIBIND Suggests a Mode of Action for T2 Ribonucleases As Antitumorigenic Agents. *J. Med. Chem.* **2012**, *55*, 1013-1020, https://pubs.acs.org/doi/10.1021/jm1015507.
43. Picot, D.; Loll, P.J.; Garavito, R.M. The x-ray crystal structure of the membrane protein prostaglandin H_2 synthase-1. *Nature* **1994**, *367*, 243–249, https://doi.org/10.1038/367243A0.
44. Kurumbail, R.G.; Stevens, A.M.; Gierse, J.K.; McDonald, J.J.; Stegeman, R.A.; Pak, J.Y.; Gildehaus, D.; iyashiro, J.M.; Penning, T.D.; Seibert, K.; Isakson, P.C.; Stallings, W.C. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature* **1996**, *384*, 644-648, https://doi.org/10.1038/384644a0.
45. Gilbert, N.C.; Rui, Z.; Neau, D.B.; Waight, M.T.; Bartlett, S.G.; Boeglin, W.E.; Brash, A.R.; Newcomer, M.E. Conversion of human 5-lipoxygenase to a 15-lipoxygenase by a point mutation to mimic phosphorylation at Serine-663. *FASEB J.* **2012**, *26*, 3222-3229, https://doi.org/10.1096/FJ.12-205286.
46. Upadhayaya, R.S.; Sinha, N.; Jain, S.; Kishore, N.; Chandra, R.; Arora, S.K. Optically active antifungal azoles: synthesis and antifungal activity of (2*R*,3*S*)-2-(2,4-difluorophenyl)-3-(5-{2-[4-aryl-piperazin-1-yl]-ethyl}-tetrazol-2-yl/1-yl)-1-[1,2,4]-triazol-1-yl-butan-2-ol. *Bioorg. Med. Chem.* **2004**, *12*, 2225-2238, https://doi.org/10.1016/j.bmc.2004.02.014.

Publisher’s Note & Disclaimer