Design, Synthesis, Molecular Docking, And Biological Evaluation Of Tetrazole Derivatives For Antimicrobial Potential

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ABSTRACT: *A series of tetrazol derivatives 1(a-c) and 2(a-c) were synthesized by Mannich base reaction. Synthesized compounds1(a-c), and 2(a-c) were confirmed by IR, ^{1}H NMR, ^{13}C NMR, mass spectral, and elemental analysis. Synthesized compounds 1(a-c), and 2(a-c) were screened for antibacterial and antifungal activity. The compound 1b was highly active against S. aureus in antibacterial screening. The synthesized compounds have been screened for fungal species. The compounds were synthesized and characterized them, and the structure was confirmed It was revealed that some synthesized derivatives show remarkable biological activity against both gram-negative and gram positive bacterial species and fungal microorganisms. Therefore, current study demonstrates the antibacterial and antifungal activity potential of new tetrazole derivatives and provides future insights for developing antibacterial and antifungal drugs.*

Keywords: *Heterocyclic compound, Tetrazole derivatives, Fungal species, Bacterial strain*

INTRODUTION

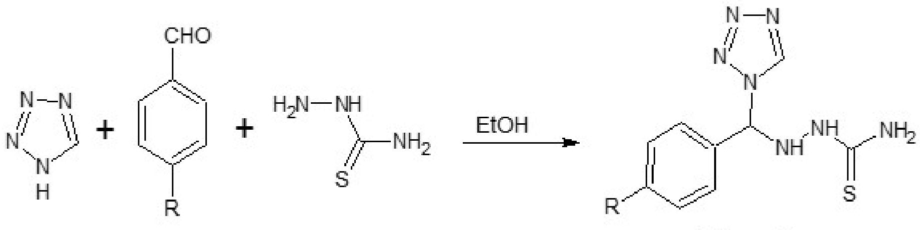
The global development of antibiotic-resistant organisms is making infectious diseases a serious problem once more, which is weakening the progress made in contemporary medicine [1]. When bacteria are treated with antimicrobial substances, their growth, reproduction, or even death can be inhibited. They come in a variety of types, such as antibiotics [2]. Some medications have the ability to significantly change physiological and metabolic processes. The ability of bacteria to endure and multiply in the presence of antimicrobial medications is known as antimicrobial resistance (AMR). The long-term, extensive use of antibiotics has led to the development of AMR in microorganisms [3, 4]. Microbial infections are treated with antibacterial and antifungal drugs. However, the development of antibiotic resistance presents a significant obstacle to the treatment of this kind of infection, which has grown to be a serious issue that endangers human life and has a direct impact on the essence of life. In order to address and overcome antibiotic resistance, it is now imperative that this issue be addressed and that prompt solutions be found. Therefore, it is crucial and urgent to develop and find new antimicrobial medications in order to cure microbial illnesses [5, 6, 7]. It is thought that in order to help fight antibiotic-resistant bacteria and stop their spread, new treatment technologies are needed, and this will take a lot of work from experts in the field. Fungal infections are becoming more widespread worldwide, particularly in developing nations where access to adequate care is lacking, putting lives at risk. More specifically, up to 40% of those who have invasive candidiasis die [9], making it the third most common cause of bloodstream infections [8].

Azoles, which include imidazole, triazole, and tetrazole, have a variety of potent antibacterial properties and are frequently used to treat inner or topical mycoses, especially those associated with AIDS-related mycotic diseases [10]. It has also been discovered that tetrazole has a strong propensity for binding to benzodiazepine receptors 2. Inhibitors of α-mannosidase 3–4 have been reported for mannose mimetics. 1,5-Disubstituted-1H-tetrazoles are appropriate bioisosteres of peptides [11], and some of the tetrazole derivatives have medicinal value and have been reported to have antifungal drugs [12, 13], antinociceptive [14, 15], antimycobacterial [16], anti-inflammatory [17], antiproliferative [18], anticonvulsant activities [19], and antibiotics [12]. The production of physiologically significant chemicals and multidrug compounds depends heavily on the Mannich process [20, 21]. In general, Mannich bases have a variety of biological properties, including cytotoxicity [22, 23], anticonvulsant properties [24], and antibacterial properties [25]. Therefore, bearing in mind the above observation, we decided to synthesize new series of tetrazol derivatives and screening fordental plaque antibacteria, and cytotoxicity activities.

MATERIAL AND METHODS:

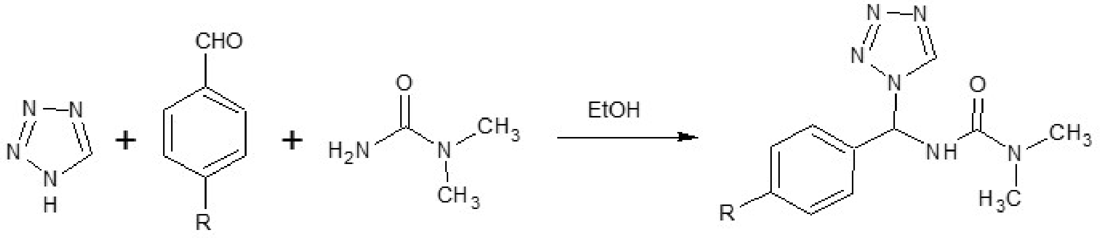
Instrumentation techniques: Melting points were recorded in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr on an FT-IR spectrometer (Shimadzu 8201PC) in the range of 4000-400 cm^{-1}. The 1H-NMR spectra were recorded on a Bruker DRX-300 spectrometer at 300MHz. Elemental analysis (C, H, N, and S) was performed using an elemental analyzer (VarioEL III). The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plates.

Synthesis of 2-[phenyl(1H-tetrazol-1-yl)methyl]hydrazine carbothioamide 1(a-c): A mixture of tetrazole (0.1mol, 7.0g), thiosemicarbazone(0.1mol, 9.1g), and benzaldehyde (0.1 mol, 10mL) in ethanol(30mL), the reaction mixture was taken in R.B flask. The reaction mixture was refluxed and stirred for 2h with help of magnetic stirrer. Final product was purified by column chromatography. [26]



1(a-c)

Synthesis of 1, 1-dimethyl-3-[phenyl (1H-tetrazol-1-yl) methyl] urea 2(a-c): A mixture of tetrazole (0.1mol, 7.0g), 1,1-dimethylurea (0.1mol, 8.8g), and benzaldehyde (0.1 mol, 10mL) in ethanol(30mL), the reaction mixture was taken in R.B flask. The reaction mixture was refluxed and stirred for 2h with help of magnetic stirrer. The reaction mixture cooled and poured into crushed ice. The resulting solid was filtered, dried and recrystallized from ethanol. [27, 28]



2(a-c)

Antimicrobial Activity: Novel synthesized compounds 1(a-c), and 2(a-c) were evaluated were screened for their antimicrobial activity by using different bacterial and fungal microorganisms. The bacterial and fungal strains used for the study of antibacterial activity were Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus megaterium and Aspergillus niger, C. albicans. [29]

Agar Cup Borer Method: Ciprofloxacin and ketoconazole were used as a reference for the bacterial and fungal cultures, respectively, in the test, which was conducted using the agar cup borer method with several changes. In a test tube, combine 0.2 mL of the test culture suspension with 1.5% sterile melted top agar that has been cooled to room temperature. Mix thoroughly, then transfer the mixture into a petri dish filled with sterile base agar medium (autoclaved at 121 °C for 15 minutes) and let it solidify. To sterilise the cup borer, it was dipped in pure ethanol, burned, and then allowed to cool. Three cups in the agar plate were marked with a sterile cup-borer, and 0.1 mL of test solution, 0.1 mL of reference solution, and 0.1 mL of DMSO solvent were injected into each cup, respectively. After that, the dishes were left in the refrigerator at 4-5 °C for 20 minutes to diffuse. After that, the plates were incubated for 24 hours at 37 °C while standing up. Following incubation, a distinct zone of growth inhibition surrounding the cup indicates the microorganism's relative susceptibility to the possible antimicrobial agent. By measuring the inhibition zone that the different substances caused on the microorganisms, the activity was graded according to the size of the inhibition zone. [30, 31]

Determination of the minimal inhibitory concentration (MIC): Dimethylsulfoxide was used to dissolve the compound at a concentration of 64 µg/mL. The solution was produced in two dilutions (64, 32,..., 0.5 µg/mL). The appropriate wells were infected with the microbe suspensions at doses of 10^6CFU/mL (colony forming unit/mL). For 24 hours, the plates were incubated at 36° C. [32]

RESULTS AND DISCUSSION

A series of compounds 1(a-c), and 2(a-c) were synthesized from condensation method and reactions are outline in scheme 1, physicochemical data are given in Table 1.

Table 1: Physical Characterization of Compounds 1(A-C) & 2(A-C)

Deri.	R	M.F	M.W	Yield (%)	Elemental Analysis, Calculated (Found) (%)			
					C	H	N	S
1a	-H	$C_9H_{11}N_7S$	242.56	82	43.36 (54.70)	4.45 (5.14)	39.33 (18.79)	12.86 (12.97)
1b	-Cl	$C_9H_{11}N_7OS$	259.11	83	40.75 (40.23)	4.18 (4.22)	36.96 (36.80)	12.09 (12.20)
1c	-OH	$C_9H_{10}N_7SCl$	273.11	79	38.10 (52.41)	3.55 (3.91)	34.56 (34.61)	11.30 (11.47)
2a	-H	$C_9H_{11}N_7O$	243.67	84	46.35 (46.34)	4.75 (4.71)	42.04 (42.06)	-

2b	-Cl	$C_{11}H_{14}N_6O$	246.65	78	53.65 (53.60)	5.73 (5.71)	34.13 (34.10)	-
2c	-OH	$C_{11}H_{14}N_6O_2$	211.22	82	50.38 (50.29)	5.38 (5.35)	32.04 (32.10)	-

The structures of compounds were characterized from IR, ^{1}H NMR, ^{13}C NMR and Mass spectral analysis. IR spectra of the compounds (1a) shows that the absorption band at NH2, C=S, C=N and N=N corresponding to 3408, 1660, 1315, and 1512 cm-^{1} respectively. ^{1}H NMR spectrum of the compound (1a) shows that signals obtained at δ 9.60, 6.32, and 2.22 corresponding to NH2, -CH-, and NH respectively. The ^{13}C NMR spectrum of the compound (1a) shows that signals obtained at δ 182.86 and 74.11 corresponding to (C=S) and -CH- carbon group respectively. Mass spectra of the compound (1a) shows molecular ion peak at m/z 249.09 corresponding to expected molecule weight of the compound (1a).

2-[phenyl(1H-tetrazol-1-yl)methyl]hydrazinecarbothioamide 1(a): IR (KBr, cm^{-1}): 3408 (NH_2), 3002 (NH), 2926 (CH=str), 1660 (C=S), 1512 (N=N), 1315 (C=N), 947(NH), 701 (ArH). ^{1}H NMR (DMSO-d6), δH (ppm): 9.60 (2H, s, NH_2), 8.72 (1H, s, 5CH-tetrazole), 7.30-7.26 (4H, m, ph), 6.32 (1H, s, -CH-), 2.22(1H, s, NH). 13C NMR (DMSO-d6), δC (ppm): 182.86(C=S), 144.87 (5CH-tetrazole), 138.26-126.67 (Ph), 74.11(-CH-). EI-Ms, m/z (Relative intensity %): m/z 249.09 (M,+ 10%).

2-[(4-chlorophenyl) (1H-tetrazol-1-yl)methyl] hydrazine carbothioamide 1(b): IR (KBr, cm^{-1}): 3377(NH_2), 3023(NH), 2932 (CHstr), 1653(C=S), 1577(N=N), 936(NH), 646(Ar-Cl). ^{1}H NMR(DMSO-d6) δH (ppm): 9.52(NH2,s,2H), 8.82(5CH-teterzole, s,1H), 7.75(2H, dd, Ph, J=5.6Hz, J=6.2Hz), 7.44 (2H, dd, Ph, J=5.8Hz, J=6.4Hz), 6.43(1H, s, -CH-), 2.43(1H, s, NH), 2.14 (1H, s, NH). 13C NMR (DMSO-d6), δ(ppm): 181.12(C=S), 147.67 (5CH-tetrazole), 131.23 (C-Cl), 130.12-129.11 (Ph), 72.11 (-CH-). EI-Ms, m/z (Relative intensity %): m/z 283.65 (M,+ 66%).

2-[(4-hydroxyphenyl) (1H-tetrazol-1-yl)methyl]hydrazine carbothioamide 1(c): IR (KBr, cm^{-1}): 3408(NH2), 2997(NH), 2909(CHstr)1560(N=N), 1660(C=S), 1315(C=N), 942(NH), 942(Ar-OH) . ^{1}H NMR (DMSO-d6), δH (ppm): 9.83 (1H, s, Ph-OH), 9.21 (2H, s, NH2), 8.36 (1H, s, 5CH-tetrazole), 7.43(2H, dd, Ph, J=6.8Hz, J=7.2Hz), 7.22(2H, dd, Ph, J=6.7Hz, J=7.0Hz), 6.32(1H, s, -CH-), 2.43(1H, s, NH). 13C NMR (DMSO-d6), δC (ppm): 180.21 (C=S), 154.07(Ph-OH), 143.67 (5CH-Tetrazole moiety), 138.26-126.67 (Ph), 72.08 (-CH-). EI-Ms, m/z (Relative intensity %):m/z 264.98(M,+ 26%); .

IR spectra of the compound (2a) shows that the absorption band at C=O, C=N, and NH corresponding to 1694, 1376, and 942 cm-1 respectively. ^{1}H NMR spectrum of the compound (2a) shows thatthe proton signals observed 6.43, 9.52, 6.12, and 2.14 corresponding to -CH-, NH2, NH, and (-NH(CH3)2 protons respectively. The ^{13}C NMR spectrum of the compound (2a) shows that the carbon peaks obtained at 154.07, 72.11, and 36.12 corresponding to C=O, CH, and CH2N respectively. Mass spectra of the compound (2a) shows molecular ion peak at m/z 246.88 corresponding to expected molecule weight of the compound (2a).

1,1-dimethyl-3-[phenyl(1H-tetrazol-1-yl)methyl]urea 2(a): IR (KBr, cm^{-1}): 2986(NH), 2936 (CHstr), 1694(C=O), 1512(N=N), 1376(C=N), 942(NH), 919(Ar). ^{1}H NMR (DMSO-d6) δH (ppm): 9.52(2H, s, NH2), 8.82 (2H, s, 5CH-tetrazole), 7.75-7.44 (5H, m, Ph), 6.43 (1H, s, -CH-), 6.12 (1H, s, NH), 2.14 (6H, s, -NH(CH3)2). 13C NMR (DMSO-d6), δ (ppm): 154.07 (C=O), 147.67 (5CH-tetrazole), 137.86-126.21 (Ph), 72.11(-CH-), 36.12 (-N(CH3)2). EI-Ms, m/z (Relative intensity %):m/z 246.88(M,+ 44%).

3-[(4-chlorophenyl) (1H-tetrazol-1-yl)methyl]-1,1-dimethylurea 2(b): IR (KBr, cm^{-1}): 3240(CH_3), 2934 (CHstr), 1683(-CONH), 1565(N=N), 1363 (C=N), 954(Ar), 646(Ar-Cl). ^{1}H NMR(DMSO-d6)δH (ppm): 8.85 (1H, s, 5CH-teterzole), 7.47(2H, dd, Ph, J=5.9Hz, J=6.8Hz), 7.26 (2H, dd, Ph, J=5.7Hz, J=6.6Hz), 7.15(1H, s, -CH-), 6.13 (1H, s, NH), 2.83(6H, s, -NH(CH3)2). 13C NMR (DMSO-d6), δ (ppm): 156.67 (C=O), 143.30 (5CH-teterazole), 132.06(C-Cl), 131.01- 128.30 (Ph), 66.21 (-CH-), 36.12 (-N(CH3)2). EI-Ms, m/z (Relative intensity %): m/z 279.12(M,+ 38%); .

3-[(4-hydroxyphenyl) (1H-tetrazol-1-yl)methyl]-1, 1-dimethylurea 2(c) : IR(KBr, cm^{-1}): 3297 (CH_3), 2922 (CHstr), 1683 (-CONH), 1565 (N=N), 1377 (C=N), 942 (Ar), 942 (Ar-OH),. ^{1}H NMR (DMSO-d6)δH (ppm): 9.83 (1H, s, Ph-OH), 8.84 (1H, s, 5CH-teterzole), 7.31(2H, dd, Ph, J=6.3Hz, J=5.7Hz), 7.22 (2H, dd, Ph, J=6.6Hz, J=5.8Hz), 7.14(1H, s, -CH-), 6.13 (1H, s, NH2), 2.81(6H, s, -NH(CH3)2). 13C NMR (DMSO-d6), δC (ppm): 154.07 (Ph-OH), 156.43 (C=O), 143.12 (5CH-teterazole), 131.42-128.09 (Ph), 66.09 (-CH-), 36.01 (-N(CH3)2). EI-Ms, m/z (Relative intensity %): m/z 262.87 (M,+ 21%);

Antimicrobial activity: The produced substances were examined for antibacterial activity in vitro. Out of the six tetrazole compounds, 1a and 1c shown moderate inhibition against gram-negative bacterial species, particularly Escherichia coli, according to the findings in Table 2, whereas 1b and 2b demonstrated maximum action against the majority of gram-negative organisms. Nearly every molecule in the series demonstrated maximal inhibition against gram-positive organisms, with compounds 1b and 1c demonstrating the strongest inhibition against B. megaterium and 1a demonstrating good inhibition against fungal organisms, particularly C. albicans. While 1a shown good inhibition against fungal species, the other chemicals showed moderate to less suppression.

Table 2: Antimicrobial activity of compound by Zone of Inhibition

Der.	Zone of Inhibition					
	Bacterial strain				Fungal strain	
	Escherichia coli	***Pseudomonas aeruginosa***	***Bacillus subtilis***	***Bacillus megaterium***	***Aspergillus niger***	***Candida albicans***
1a	10	12	-	-	20	19
1b	20	19	22	16	19	18
1c	12	10	17	10	15	12
2a	14	17	10	10	14	16
2b	17	22	10	19	15	18
2c	10	13	10	19	16	19
Std	30	30	30	30	33	33

Antimicrobial activity of compounds at 10 mg% in DMSO.
Ciprofloxacin and ketoconazole used as a standard

The antibacterial and antifungal properties of compounds 1(a-c) and 2(a-c) were examined. When compared to the standard, one of the synthesised compounds (1b) was very active (MIC: 8 µg/mL) against Pseudomonas aeruginosa, and compound (2b) was highly active (MIC: 8 µg/mL) against Pseudomonas aeruginosa when compared to other compounds, but very low active when compared to standards. When combined with conventional ciprofloxacin and ketoconazole, the compound (1c) demonstrated high activity against Escherichia coli (MIC: 4µg/mL). Table 3 summarises the data and shows the antibacterial minimum inhibit concentration.

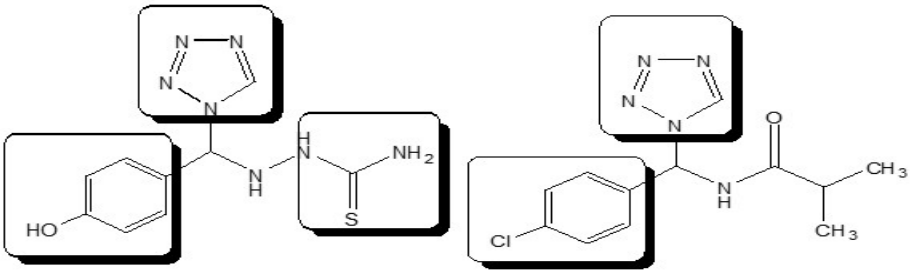
Table 3: Antimicrobial activity of compound by MIC

Der.	MIC					
	Bacterial strain				Fungal strain	
	Escherichia coli	***Pseudomonas aeruginosa***	***Bacillus subtilis***	***Bacillus megaterium***	***Aspergillus niger***	***Candida albicans***
1a	64	64	-	-	32	8
1b	64	8	8	32	16	32
1c	4	32	32	16	>100	>100
2a	64	32	>100	>100	64	32
2b	16	8	>100	32	32	8

2c	64	64	>100	32	>100	32
Std	0.5	0.5	32	0.5	2	8

Ciprofloxacin and ketoconazole used as a standard

Structure activity relationship: From the results of antimicrobial activities, we are discussed in following structure activity relationships:



1c **2b**

Figure 1: Structure Active Relationship Active Compound

Molecular Docking: Compounds 1c and 2b, which contain a tetrazole ring molecule at 1-position through methyl bridged and exhibit good antibacterial and antifungal activity, were investigated in relation to one another using the in silico molecular docking method. The target used in this study was sterol 14-α demethylase (CYP51). According to molecular docking, the compounds' binding sites are similar. As shown in Figure 2, the core of azoles is responsible for the interaction with tetrazole derivative molecules. The molecular docking contact energies of the substances. 2b is the most active molecule and has the highest docking energy, at -8.7430 kcal/mol. Compound 1c exhibits several H-acceptor stacking contacts with Ser 378 (3.26 Å), a 4.28 Å H-pi stacking interaction with heme, and a 4.18 Å hydrogen bond between the benzimidazole core pi-H and Ser 506.

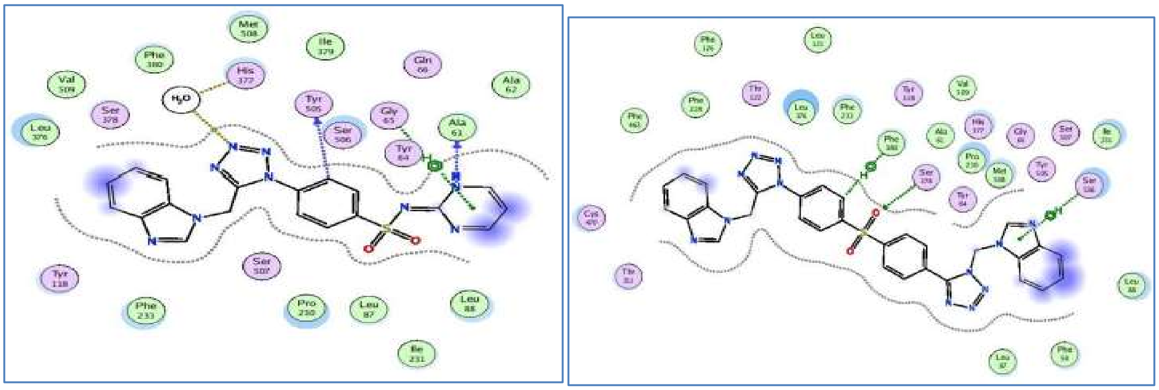


Figure 2. 2D Interaction diagrams representing the docked conformation of ligand 1c and 2b

CONCLUSION: To concluded, we have discovered a productive and useful method for creating tetrazole derivatives. In antibacterial screening, the compound (1b) shown excellent activity against S. aureus. When it came to antibacterial and antifungal screening, the chlorine substituent in the para position of the phenyl rings made the majority of compounds active, while further substitutions on the thiosemicarbazide and tetrazole rings did the same. All things considered, this work shows the potential for antibacterial and cytotoxic activity of novel tretazole derivatives and offers guidance for future research into the development of antibacterial and antifungal medications.

REFERENCES

1. Yapar N. Epidemiology and risk factors for invasive candidiasis. Therapeutics and Clinical Risk Management, 2014, 10:95 [Google Scholar], [Publisher]
2. Bansal Y., Kaur M., Bansal G. Antimicrobial potential of benzimidazole derived molecules. Mini Reviews in Medicinal Chemistry, 2019, 19:624 [Crossref], [Google Scholar], [Publisher]
3. Keri R.S., Rajappa C.K., Patil S.A., Nagaraja B.M. Benzimidazole-core as an antimycobacterial agent. Pharmacological Reports, 2016, 68:1254 [Crossref], [Google Scholar], [Publisher]
4. Friedberg J.W., Cohen P., Chen L., Robinson K.S., Forero-Torres A., La Casce A.S., Fayad L.E., Bessudo A., Camacho E.S., Williams M.E. Bendamustine in patients with rituximabrefractory indolent and transformed nonHodgkin's lymphoma: results from a phase II multicenter, single-agent study. Journal of Clinical Oncology, 2008, 26:204 [Crossref],
5. Vatolkina O., Plotkin A., Libinzon R. Analysis of the relationship between the inhibition of the cyclic AMP phosphodiesterase activity and pharmacological action of drugs. Pharmaceutical Chemistry Journal, 1985, 19:372
6. Kearns G.L., Andersson T., James L.P., Gaedigk A., Kraynak R.A., Abdel-Rahman S.M., Ramabadran K., van den Anker J.N., Network P.P.R.U. Omeprazole disposition in children following single-dose administration. The Journal of Clinical Pharmacology, 2003, 43:840 [Crossref], [Google Scholar], [Publisher]
7. Elslahi R.H., Osman A.G., Sherif A.M., Elhussein A.A. Comparative study of the fungicide Benomyl toxicity on some plant growth promoting bacteria and some fungi in pure cultures. Interdisciplinary Toxicology, 2014, 7:12 [Crossref], [Google Scholar], [Publisher]
8. Gupta P.K. Toxicity of fungicides. Veterinary Toxicology (Third Edition), 2018, 569. [Crossref], [Google Scholar], [Publisher] [15]. Lever R., Hefni A., Moffatt J., Paul W., Page C. Effect of tecastemizole on pulmonary and cutaneous allergic inflammatory responses. Clinical & Experimental Allergy, 2007, 37:909 [Crossref], [Google Scholar], [Publisher]
9. Citarella A., Moi D., Pedrini M., Pérez-Peña H., Pieraccini S., Dimasi A., Stagno C., Micale N., Schirmeister T., Sibille G. Synthesis of SARSCoV-2 M pro inhibitors bearing a cinnamic ester warhead with in vitro activity against human coronaviruses. Organic & Biomolecular Chemistry, 2023, 21:3811 [Crossref], [Google Scholar], [Publisher]
10. Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014 Jun 24:47-55.
11. Vishvakarma P. Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. Journal of the Chilean Chemical Society. 2018 Jun;63(2):3988-93.
12. Vishvakarma P, Mandal S, Verma A. A review on current aspects of nutraceuticals and dietary supplements. International Journal of Pharma Professional's Research (IJPPR). 2023;14(1):78-91.
13. Prabhakar V, Agarwal S, Chauhan R, Sharma S. Fast dissolving tablets: an overview. International Journal of Pharmaceutical Sciences: Review and Research. 2012;16(1):17.
14. Mandal S, Vishvakarma P, Verma M, Alam MS, Agrawal A, Mishra A. Solanum Nigrum Linn: an analysis of the Medicinal properties of the plant. Journal of Pharmaceutical Negative Results. 2023 Jan 1:1595-600.
15. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis of The Most Recent Trends in Flavoring Herbal Medicines in Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98.
16. Mandal S, Vishvakarma P, Mandal S. Future Aspects and Applications of Nanoemulgel Formulation For Topical Lipophilic Drug Delivery. European Journal of Molecular & Clinical Medicine. 2023;10(01):2023.
17. Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-98.

18. Prabhakar V, Agarwal S, Chauhan R, Sharma S. Fast dissolving tablets: an overview. International Journal of Pharmaceutical Sciences: Review and Research. 2012;16(1):17.
19. Parab RH, Dixit BC. Synthesis characterization and anti-microbial activity of imidazole derivatives based on 2-chloro-7-methyl-3- formylquinoline. J Chem 2012;9:1188-95.
20. Mishra R, Ganguly S, Sethi KK, Mazumder PM. Synthesis and anticonvulsant activity of some novel 2-methyl imidazole derivatives. Lett Drug Des Discov 2012;9:402-8.
21. Khaliullin F, Nikitina I, Valieva A, Miftakhova A, Khalilov L, Mescheryakova E. Synthesis and antidepressant activity of 2-bromo-1- (thietan-3-yl) imidazole 4, 5-dicarboxylic acid derivatives. Int J Pharm Pharm Sci 2017;9:154.
22. Li Y. F., Wang G. F., He P. L., Huang W. G., Zhu F. H., Gao H. Y., Tang W., Luo Y., Feng C. L., Shi L. P., Ren Y. D., Lu W., Zuo J. P., Synthesis and anti-hepatitis Bvirus activity of novel benzimidazole derivatives J. Med. Chem, 2006, 49, 479
23. Varvarason A., Tantili-Kakoulidou A., Siatra-Papastasikoudi T., Tiligada E., Synthesis and biological evaluation of indole containing derivatives of thiosemicarbazide and their cyclic 1, 2, 4-triazole and 1, 3, 4-thiadiazole analogs., Arzneim. Forsch, 2000, 50, 48.
24. Gokce M., Cakir B., Earl K., Sahin M., Synthesis and antimicrobial activity of [(2-oxabenzothiazolin-3-yl)-methyl]-4-alkyl/aryl-1, 2, 4-triazoline-5-thiones., Arch. Pharm, 2001, 334, 279.
25. Pintilie O., Profire L., Sunel V., Popa M., Pui A., Synthesis and antimicrobial activity of some new 1, 3, 4-thiadiazole and 1, 2, 4-triazole compounds having a D,L-methionine moiety., Molecules, 2007, 12, 103.
26. Faroumadi A., Mirzaei M., Shafiee A., Synthesis and antituberculosis activity of 2-aryl-1, 3, 4-thiadiazole derivatives., Pharmazie, 2001, 56, 610.
27. Mamolo M. G., Falagiani V, Zanpier D., Vio L., Banfi F., Synthesis and antimycobacterial activity of [5-(pyridin-2-1, 3, 4-thiadiazol-2-yl-thio)]-acetic acid arylidene-hydrazide derivatives., Farmaco, 2001, 56, 587.
28. Zamani K., Faghifi K., Tefighi I., Sharlatzadeh R., Synthesis and potential antimycotic activity of 4-substituted 3-(thiophene-2-yl-methyl)-Δ2-1, 2, 4-triazoline-5-thiones., Turk. J. Chem, 2004, 28, 95.
29. Zan XI Lai L. H., Jin G. Y., Zhong Z. X., Synthesis, fungicide activity and 3D- QSAR of 1, 3, 4-oxadiazoles and 1, 3, 4-thiadiazoles., J. Agric. Food Chem, 2002, 50, 3757.
30. Chem H., Li Z., Han Y., Synthesis and fungicidal activity against Rhizoctonia solani of 2-alkyl (alkylthio)-5-pyrazolyl-1, 3, 4-oxadiazoles (thiadiazoles)., J. Agric. Food Chem, 2000, 48, 5312.
31. Clerici F., Pocar D., Guido M., Loche A., Perlini V., Brufoni M., Synthesis of 2-amino-5-sulphonyl1,3,4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity., J. Med. Chem, 2001, 44, 931.
32. Onkol T., Cakir B., Sahin M. F., Synthesis and antinociceptive activity of 2-[(2-oxabenzothiazolin-3-yl)-methyl]-5-aminoalkyl/aryl-1,3,4thiadiazole., Turk. J. Chem, 2004, 28, 461