RESEARCH ARTICLE

Exploring Novel Ester Linked 1,4-Disubstituted 1, 2, 3 Triazole Derivatives: Design, Regioselective Synthesis and Antiviral Activity as Hemagglutinin and Neuraminidase Inhibitors

Chopade Pavankumar D*, Shaikh Anwar Rafique

Department of Pharmaceutical chemistry, M. C. E. Society's Allana College of Pharmacy, Azam Campus, Camp, Pune-411001, Maharashtra, India.

Received: 3rd Oct, 2024; Revised: 1st Nov, 2024; Accepted: 5th Dec, 2024; Available Online: 25th Dec, 2024

ABSTRACT

1, 2, 3 triazoles and its derivatives plays crucial role in medicinal chemistry. They emerged as a pharmacologically significant scaffold in several viral infections due to its broad spectrum and potent activity. Severe viral Diseases like Covid-19, Ebola virus, Swine Flu are emerging now a day and are found fatal. Currently a very few of antiviral chemotherapeutic agents available, It is essential to prioritize the development of potential antiviral drugs to combat the spread of deadly viral infections. The current study involves the combination of Alkyne and Azide through the convenient Copper catalysed 1,3-dipolar cycloaddition of Huisgen's reaction approach to form 1, 4-disubstituted (regioselective) ester linked 1,2,3-triazoles. The reaction was mediated by Sodium Ascorbate, whereas Cu(I) was used as an efficient, non-toxic and easily available inorganic catalyst. Newly prepared derivatives are identified and assured by thin layer Chromatography, Physical constant and other spectroscopic methods like Infrared, NMR ^{1}H and ^{13}C and Mass. Raised atom economy, good yields, mild reaction condition and low environmental impact makes this process is of considerable synthetic advantages. Assessment of the synthesized compounds for antiviral activity demonstrated their optimum effectiveness against the (H1N1) influenza Swine Flu virus.

Keywords- 1, 2, 3 Triazoles, Synthesis, Alkynes, Azides, (H1N1) influenza virus, Antiviral activity.

How to cite this article: Chopade Pavankumar D, Shaikh Anwar Rafique. Exploring Novel Ester Linked 1,4-Disubstituted 1, 2, 3 Triazole Derivatives: Design, Regioselective Synthesis and Antiviral Activity as Hemagglutinin and Neuraminidase Inhibitors. International Journal of Pharmaceutical Quality Assurance. 2024;15(4):2364-71. doi: 10.25258/ijpqa.15.4.33

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

In the modern age, the prevalence of severe viral infections has made them the most prevalent cause of both disease and mortality in humans. Antiviral chemotherapy drugs are now hard to come by for the prevention and treatment of these illnesses. There is a pressing demand for the creation of effective antiviral treatments to address a wide array of hazardous and fatal viral infections.[1] A global concern is the ongoing evolution of drug-resistant influenza viruses. Nevertheless, in contrast to the 1918 pandemic (H1N1), which occurred during a time when global travel was less frequent and slower, the world is now potentially even more vulnerable to the next pandemic. The influenza virus pandemics of the previous century caused more harm and occurred more frequently than the current severe global COVID-19 pandemic.[2,3] As viruses are inevitably becoming resistant to the antiviral medications that are currently on the market, the effectiveness of these therapies in the future is questionable. As of right now, certain semisynthetic derivatives are also effective choice for obtaining and separating naturally occurring remedy for viruses.[4] The influenza viruses are categorized into four separate genera and are part of the Orthomyxoviridae family. These genera consist of influenza A, B, C, D viruses. The swine flu virus, also known as the H1N1 virus, is classified under the influenza A virus family. A distinct combination of influenza viruses has been discovered, capable of infecting pigs, birds, and humans. IAV infections are the primary cause of all known seasonal epidemics and sporadic influenza pandemics.[2,5] Through a genetic process known as "antigenic shift or reassortment," viral RNA segments from distinct IAV subtypes frequently exchange genetic material, leading to the creation of pandemic IAV variants. On the other hand, during viral replication, "antigenic drift"-a constant alteration in the viral RNA genome in many hosts is how endemic IAV variations evolve.[2,6.] The potential of these triazole analogues as therapeutic moiety for so many disorders have garnered attention due to their capacity to interact with receptor/protein targets.[7,8] The utilization of innovative synthetic approaches, such as green chemistry and click chemistry, has played a pivotal role in the synthesis of diverse derivatives possessing improved biological action. Due to these investigations synthetic chemists have collaborated to develop a thorough knowledge of the structure-activity relations influencing these compounds' activities. [9,10]

Studying the effect of structural transformations on biological characteristics of 1,2,3-triazole derivatives have made possible by investigation of quantitative SAR

**Author for Correspondence: chopadepavan@gmail.com*

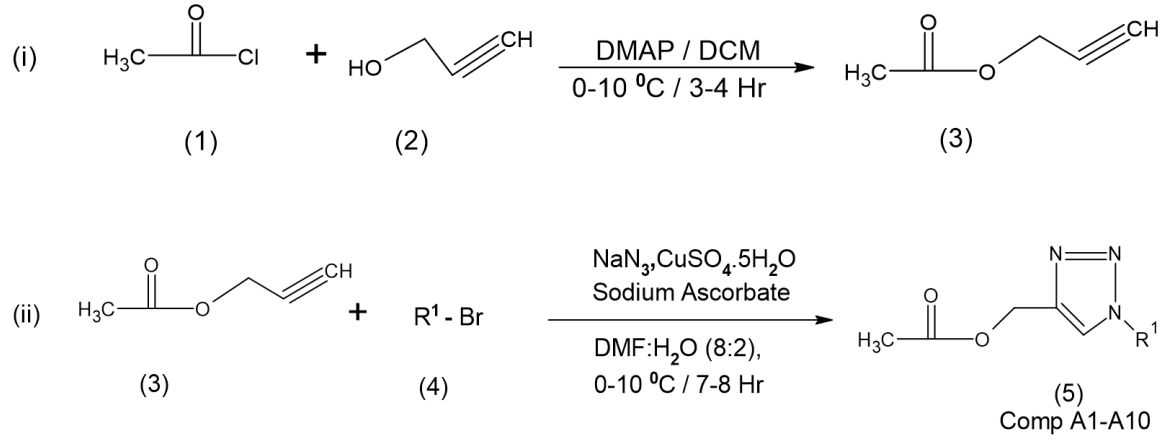


Figure 1**:** Scheme for Synthesis of 1,4-disubstituted 1,2,3-triazoles via Alkyne Azide Cycloaddition.

studies. Development and advancement of innovative drugs with improved biological profiles has been influenced by this information.[11] Cycloaddition reaction of organic azides with alkynes yields 1,2,3-triazoles which are substituted at 1,4 and 1,5 position demonstrating a Proven methods for synthesizing 1,2,3-triazoles, On the other hand biological processes find it challenging to create molecules featuring a five-membered ring structure with three adjacent nitrogen atoms. Therefore, it is essential to create synthetic strategies for producing triazoles and their analogues.[12,13] The triazole ring, a fundamental structural component of these drugs, exhibits a range of properties such as antiviral, antiretroviral, antifungal, anticancer, antidepressant, tranquilizing, anti-allergic, anxiolytic, sedative and many more.[14] The current study involved the synthesis of a derivative of 1-2-3-triazoles, followed by the evaluation of all compounds for their antiviral effectiveness against the H1N1 influenza virus. Our goal is to support the exploration of novel methods for identifying and improving the efficacy of antiviral medications.

MATERIALS AND METHODS

As per the designed Synthetic scheme raw materials, chemicals and apparatus of best standard are received from SRL India and used as it is. Melting points are determined using an electronic melting point device. Purity of the synthesized compounds were assessed using TLC analysis on silica gel G-coated plates, with ethyl acetate and petroleum ether (1:1) as a solvent mixture for elution and iodine chamber was used to examine the plates that had developed. Bruker Advance II operating at a frequency of 400 MHz in CdCl3 solutions is used for the 1H NMR spectra determination. Tetramethylsilane served as the internal standard for NMR spectrometer analysis. LCMS data was measured on mass spectrophotometer. The compounds were prepared according to the procedure and it is purified by column chromatography and/or recrystallization.

Chemistry: Scheme 1 depicts the synthetic process of compounds (A1-A10). Herein we disclose the preparation of novel 1,2,3-triazoles and that are ester-linked. This is alkyne azide reaction via 1,3 dipolar cycloaddition mechanism. In order to circumvent the challenges associated with the instability and handling of low molecular weight organic azides, we adopted a strategy that alkynes and azides, which being catalysed by Cu(I) catalyst.[18] This is a regioselective synthesis targeting the 1 and 4 positions of 1,2,3-triazoles, as 1,4-disubstituted 1,2,3-triazoles have been identified as the most potent antiviral agents.[19] Utilizing techniques like 13C NMR, HRMS, IR, and 1H NMR the synthesized derivatives were cnfirmed and assessed for antiviral properties against the (H1N1) influenza virus in vitro. An analysis of the active compounds was performed through a molecular docking study, and the findings were subsequently interpreted.[20] The docking studies demonstrated a close alignment with the experimental results of bindings for antiviral activity.

Scheme 1 illustrates our overall process for synthesizing 1,2,3-triazoles derivatives which are 1,4 disubstituted . first step involves a preparation of propargyl acetate **(3)** from the reaction of acetyl chloride **(1)** and propargyl alcohol **(2)** ith the catalyst N, N−dimethyl amino pyridine. second step involves propargyl acetate **(3)** rection with Aryl bromides **(4)** and sodium azide with the catalyst copper sulphate and sodium ascorbate. DMF in water is used as solvent to form regioselective 1,4-substituted 1,2,3-triazoles **(5)**. All compounds were characterized by techniques like Infrared, NMR 1H, 13C and Mass. 1,2,3-triazoles skeleton was assured by absorption peaks in the space of 1570-1630 (N=N bond., triazole moiety), 1250-1350 (C-N bond, triazole moiety), 1050–1150 cm−1(C–O sym bond str., ester), 1250–1350 cm−1 (C–O asym. bond str., ester) in IR region. In the 1H NMR spectra the presence of Protons of the triazole ring at δ 7.0-8.5 ppm, Methylene (CH2) protons of the methoxy group at δ 5.34–5.14 ppm, Methyl protons of acetate group at δ 2.0-2.2 ppm is found. In the 13C NMR spectra δ 56.8–57.4 (OCH2), δ 110-150 ppm for C-atom of triazole ring is obtained. Confirmation of these target compounds' formation was retrieved by examining the 1H NMR and 13C NMR spectra. Molecular weights by HRMS spectral

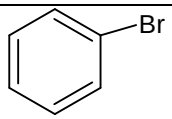
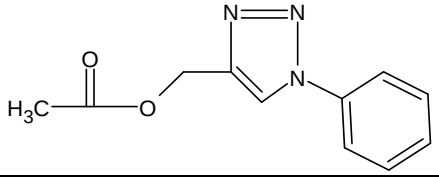
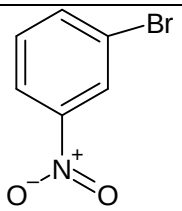
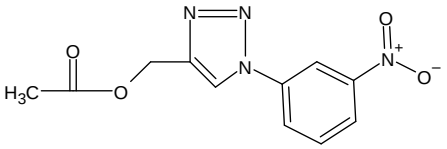
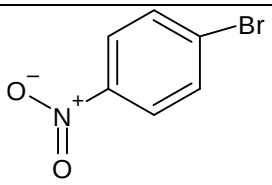
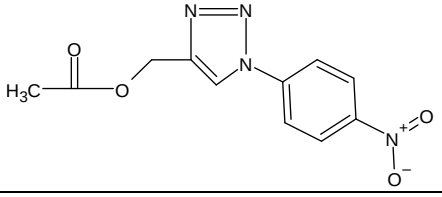
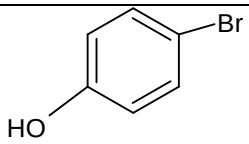
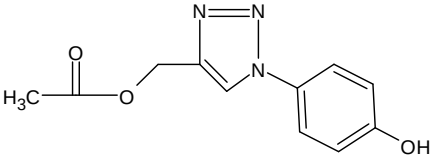
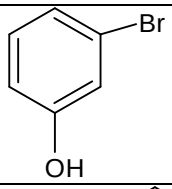
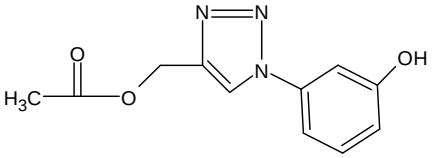
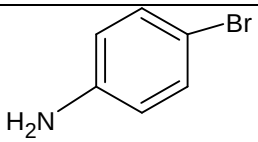
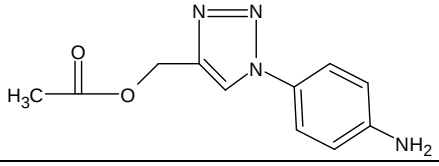
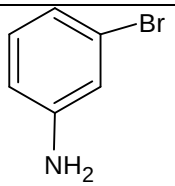
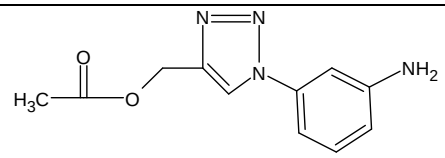
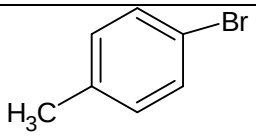
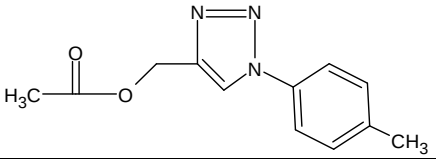
analysis coincides with the molecular weights as envisaged theoretically.

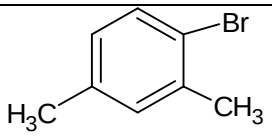
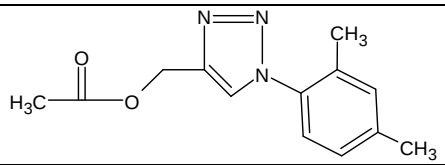
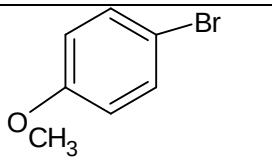
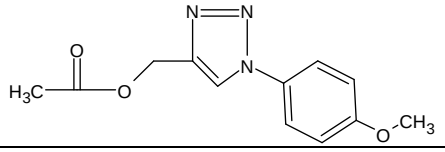
Antiviral Activity

Cell culture and virus propagation

Virus vaccine strain of A/Puerto Rico/13/38 (H1N1) influenza virus, obtained from National Institute of Virology, pune, Maharashtra, India was then cultured into MDCK cells. virus is then cultured in MDCK cells with addition of 1 µg/ml of trypsin- Tosylamide Phenylethyl Chloromethyl Keton-treated Trypsin (TPCK). 48 hours later of incubation, virus-containing medium was collected then performed virus titration using the Reed and Muench formula.[21]

Table 1. Details for the synthesised compounds (A1-A10)

Comp. No.	(R^1-Br)	Structure of Final Compounds	IUPAC name of Final Compounds	M.W.
A1			(1-phenyl-1*H*-1,2,3-triazol-4-yl)methyl acetate	217.2673
A2			[1-(3-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl acetate	262.1672
A3			[1-(4-nitrophenyl)-1*H*-1,2,3-triazol-4-yl]methyl acetate	262.1251
A4			[1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]methyl acetate	233.2736
A5			[1-(3-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]methyl acetate	233.1837
A6			[1-(4-aminophenyl)-1*H*-1,2,3-triazol-4-yl]methyl acetate	232.2357
A7			[1-(3-aminophenyl)-1*H*-1,2,3-triazol-4-yl] methyl acetate	232.0695
A8			[1-(4-methylphenyl)-1*H*-1,2,3-triazol-4-yl] methyl acetate	231.1687

A9			[1-(2,4-dimethyl phenyl)- 1*H*-1,2,3-triazol-4-yl] methyl acetate	245.1256
A10			[1-(4-methoxy phenyl)- 1*H*-1,2,3-triazol-4-yl] methyl acetate	247.0851

Determination of cell viability

Synthesized compound's cytotoxicity was assessed on MDCK cells using the methyl thiazolyl tetrazolium (MTT) assay. MDCK cells were planted in a 96-well plate for 24 hours then incubated at 37°C with 5% Carbon dioxide and a range of component concentrations were generated using serial dilutions by two times and poured in wells in tripple manner. At the conclusion of a two-day incubation period, remove the medium with the compounds and supplement the wells with MTT reagent (5 mg/mL). Keep in a dark environment for 4 more hours to develop further. The media was taken out, and 100 µL of DMSO then introduced in the each well. Plates are shaken at room temp. for 5 minutes to assist in dissolution of formazan crystals. After that, ELISA reader was used to read the plate at 470 nm.

Assessment of antiviral activity

Compounds at 10 mg/ml concentrations were put to a petri plate with 0.1 ml volume in each well. A/Puerto Rico/13/38 (H1N1) influenza virus (m.o.i. 0.01) were then added into MDCK cells for causing infection. Plates were incubated for 73 hours at 37 °C at 5% Carbon dioxide. To analyse cell vitaklity, the MTT test was conducted, and the cytoprotective impacts of compounds were evaluated based on their capacity to increase the OD values in comparison to control wells. The IC50 values, which indicates the concentration of compounds leading to 50% cell protection, was calculated based on the gathered data using Graph Pad Prism software. By dividing the CC50 by the IC50, the selectivity index (SI) for each compound was established. Standard Ribavirin was applied for the H1N1 virus. [22]

RESULTS AND DISCUSSION

All newly synthesized compounds (A1-A10) have been evaluated for their antiviral activity. In vitro, the MDCK cell culture technique was utilized to determine the viral activity of synthesized compounds for influenza A/Puerto Rico/13/38 (Swine Flu H1N1). Assessments were carried out on the compounds with the concentration of 10 mg/mL, resulting in the determination of their CC50 and IC50 values, along with selectivity indices. Oseltamivir served as the reference standard drug. As we see in above Table 2, EC50 values (half-maximal effective concentration) of the synthesised compounds are within 30-39 indicates that the drug's potency against the virus is moderate, as it requires a concentration of 30 units to achieve 50% inhibition of viral replication or infectivity. A higher EC50 value denotes that a bigger concentration of drug is needed to have intended antiviral effect. The drug's antiviral properties are somewhat modest, as a larger dose is needed to reach a 50% reduction in viral replication or infectivity. The synthesized compounds all exhibit SI 50 values above 1. A selectivity index above 1 suggests that synthesized compounds A1-A10 have moderate antiviral activity while being less toxic to host cells. This shows that synthesized compound have a therapeutic window by which they are able supress the multiplication of viruses while inflicting minimal damage on host cells. The examination for cytotoxic effects indicated a complete lack of cytotoxicity effects even with the highest concentration used. Meanwhile, the synthesized compounds showed moderate inhibitory effects on the H1N1 virus. Reviewing the data of the antiviral assay revealed that there is a need of structural modification and activity enhancement to obtain more active and potent

Table 2. Cytotoxicity and anti-influenza data of the Synthesised Compounds A1-A10

Comp. no	Virus screened	Assay name	EC50 mg/ml	CC50 mg/ml	SI50 mg/ml
A1	H1N1	Visual	38	>100	>1.5
A2	H1N1	Visual	34	>100	>1.7
A3	H1N1	Visual	33	>100	>1.8
A4	H1N1	Visual	39	>100	>3.5
A5	H1N1	Visual	36	>100	>3.8
A6	H1N1	Visual	38	>100	>2.7
A7	H1N1	Visual	34	>100	>1.9
A8	H1N1	Visual	35	>100	>4.1
A9	H1N1	Visual	39	>100	>3.56
A10	H1N1	Visual	37	>100	>2.37
Oseltamivir	H1N1	Visual	10	>320	>32

compounds.

Proposed procedure for synthesis of 1,4-disubstituted 1,2,3-triazoles

Step 1: Formation of propargyl Acetate **(3)**

Acetyl chloride solution (1.2 mmol) is magnetically stirred and then was added with DMAP (1.2 mmol) solution and DCM as a solvent maintaining temp. of 0-10 ^{0}C. After that Propargyl alcohol (1 mmol) was incorporated to it with several increments. The reaction mixture was stirred continuously for 3-4 hours for 0-10 ^{0}C. Formation of product was confirmed by TLC. When the reaction finished, hydrochloric acid was poured in the reaction mixture, the final product was extracted with dichloromethane (30x2 mL). Brine solution was used to wash the organic layer and dried using sodium sulphate (anhydrous) and evaporated the solvent using rota vapour for under negative pressure to get desired terminal alkynes (propargyl acetate).

Step 2: Formation of 1,4-disubstituted 1,2,3-triazoles **(5) (A1-A10)**

Sodium azide (3.0 mmol) in its aqueous solution was added to the solution of Aryl bromides in dimethyl formamide and the reaction contents were stirred for half an hour at 25-30 ºc. Thereafter, to the above mixture propargyl acetate (1.0 mmol) was added followed by sodium ascorbate (0.40 mmol) and copper sulphate pentahydrate (0.10 mmol) then stirring was continued at 30-40 ºc for 7 to 8 hours. Formation of product was confirmed by TLC. When the reaction finished, cold water (35 ml) was poured in the reaction mixture , the precipitated product was filtered, washed with aqueous ammonia solution followed by water. Recrystallization was performed on some crude product utilizing a solvent combination of ethyl acetate and hexane, resulting in the isolation of pure 1,4-disubstituted 1,2,3-triazoles. Some compounds were purified by column chromatography for their further analysis.

Characterization of synthesized compounds (A1-A10)

A1, (1-phenyl-1H-1,2,3-triazol-4-yl)methyl acetate, Appearance: Pale yellow solid, Yield : 81%, m. p. : 322-324 ºC, FTIR (KBr): 3108 (C - H str., triazole ring), 1256. (C-N Stretch., triazole ring), 1570 (N=N Stretch., triazole ring), 3108 (C - H str., aromatic ring), 2752. (C-H str., aliphatic), 430 (C=C str., aromatic ring), 1256 (C - O asym. str., ester), 1098 (C-O sym.str., ester), 1 H NMR (400 MHz, DMSO), δ 2.22 (s, 3H), δ 5.34-5.14 (s, 2H,), δ 7.55-7.48 (m, 2H, ArH), δ 7.36–7.29 (m, 2H, ArH), δ 7.12-7.05 (s, 1H, ArH), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 122.8 (2C, s), 127.8 (1C, s), 128.2 (2C, s), 138.1 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C11H11N3O2 [M+H] + :217.2239, found: 217.2673 .

A2, [1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl]methyl acetate, Appearance: Pale orange solid, Yield : 68%, m. p. : 294 296 ºC, FTIR (KBr): 3190 (C - H str., triazole ring), 1300 (C-N Stretch., triazole ring), 1636 (N=N Stretch., triazole ring), 2924 (C - H str., aromatic ring), 2853 (C-H str., aliphatic), 1594 (C=C str., aromatic ring), 1300 (C - O asym. str., ester), 1183 (C-O sym.str., ester), 1556 (N=O Stretch., NO_2), 1300 (N=O Stretch., NO_2), 1 H NMR (400 MHz, DMSO), δ 2.20 (s, 3H), δ 5.30 (s, 2H,), δ 8.30(dd, 1H, ArH), δ 8.10 (d, 1H, ArH), δ 7.85 (dd, 1H, ArH), δ 7.65 (d, 1H, ArH), δ 7.80 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 115.0 (1C, s), 117.7 (1C, s), 122.8 (1C, s), 129.6 (1C, s), 138.1 (1C, s), 139.5 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C11H10N4O4 [M+H] + :262.2215, found: 262.1672

A3, [1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl]methyl acetate, Appearance: Pale orange solid, Yield : 73%, m. p. : 308 310 ºC, FTIR (KBr): 3067 (C - H str., triazole ring), 1347 (C-N Stretch., triazole ring), 1620 (N=N Stretch., triazole ring), 3067 (C - H str., aromatic ring), 2851 (C-H str., aliphatic), 1553 (C=C str., aromatic ring), 1347 (C - O asym. str., ester), 1178 (C-O sym.str., ester), 1553 (N=O Stretch., NO_2), 1347 (N=O Stretch., NO_2), 1 H NMR (400 MHz, DMSO), δ 2.20 (s, 3H), δ 5.30 (s, 2H,), δ 8.05 (d, 2H, ArH), δ 8.25 (d, 2H, ArH), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 117.7 (2C, s), 121.7 (2C, s), 138.1 (1C, s), 139.5 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C11H10N4O4 [M+H] + : 262.2215, found: 262.1251

A4, [1-(4-hydroxyphenyl)-1*H*-1,2,3-triazol-4-yl]methyl acetate, Appearance: Pale orange solid, Yield : 71%, m. p. : 328 330 ºC, FTIR (KBr): 3067 (C - H str., triazole ring), 1347 (C-N Stretch., triazole ring), 1620 (N=N Stretch., triazole ring) 3067 (C - H str., aromatic ring), 2851 (C-H str., aliphatic), 1467 (C=C str., aromatic ring), 1347 (C - O asym. str., ester), 1178 (C-O sym.str., ester), 3360 (O-H Stretch., OH), 1428 (O-H Bend., OH), 1 H NMR (400 MHz, DMSO), δ 2.20 (s, 3H), δ 5.30 (s, 2H,), δ 7.20 (d, 2H, ArH), δ 6.80 (d, 2H, ArH), δ 9.50 (s, 1H, OH), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 115.2 (2C, s), 128.3 (2C, s), 138.1 (1C, s), 157.4 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C11H11N3O2 [M+H] + : 233.22334, found: 233.2736

A5, [1-(3-hydroxyphenyl)- 1*H*-1,2,3-triazol-4-yl]methyl acetate, Appearance: Pale orange solid, Yield : 69%, m. p. : 343 345 ºC, FTIR (KBr): 3269 (C - H str., triazole ring), 1260 (C-N Stretch., triazole ring), 1632 (N=N Stretch., triazole ring), 3081 (C - H str., aromatic ring), 2876 (C-H str., aliphatic), 1532 (C=C str., aromatic ring), 1260 (C - O asym. str., ester), 1097 (C-O sym.str., ester), 3464 (O-H Stretch., OH), 1435 (O-H Bend., OH), 1 H NMR (400 MHz, DMSO), δ 2.20 (s, 3H), δ 5.30 (s, 2H,), δ 7.15 (m, 1H, ArH), δ 6.90 (m, 2H, ArH), δ 6.80 (d, 1H) δ 9.30 (s, 1H, OH), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 101.4 (1C, s), 114.5 (1C, s), 122.8 (1C, s), 129.7 (1C, s), 138.1 (1C, s), 157.4 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C11H11N3O3 [M+H] + : 233.22334, found: 233.1837

A6, **[1-(4-aminophenyl)-1*H*-1,2,3-triazol-4-yl] methyl acetate,** Appearance: Pale orange solid, Yield : 76%, m. p. : 357 359 ºC, FTIR (KBr): 3046 (C - H str., triazole ring), 1261 (C-N Stretch., triazole ring), 1613 (N=N Stretch., triazole ring), 3046 (C - H str., aromatic ring), 2925 (C-H str., aliphatic), 1541 (C=C str., aromatic ring),

1261 (C - O asym. str., ester), 1133 (C-O sym.str., ester), 3323 (N-H Stretch., NH_2), 1655 (N-H Bend., NH_2), 1150 (C-N Stretch., NH2), 1 H NMR (400 MHz, DMSO), δ 2.22 (s, 3H), δ 5.30 (s, 2H,), δ 6.70 (d, 2H, ArH), δ 6.40 (d, 2H, ArH), δ 5.50 (s, 2H, NH_2), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 114.1 (2C, s), 121.7 (2C, s), 138.1 (1C, s), 148.4 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C11H12N4O2 [M+H] + : 232.23858, found: 232.2357

A7, [1-(3-aminophenyl)-1*H*-1,2,3-triazol-4-yl] methyl acetate, Appearance: Pale yellow solid, Yield : 67%, m. p. : 237 239 ºC, FTIR (KBr): 2979 (C - H str., triazole ring), 1258 (C-N Stretch., triazole ring), 1626 (N=N Stretch., triazole ring), 2927 (C-H str., aliphatic), 1536 (C=C str., aromatic ring), 1258 (C - O asym. str., ester), 1094 (C-O sym.str., ester), 3477 (N-H Stretch., NH_2), 1626 (N-H Bend., NH_2), 1153 (C-N Stretch., NH2), 1 H NMR (400 MHz, DMSO), δ 2.22 (s, 3H), δ 5.30 (s, 2H,), δ 6.95 m, 2H, ArH), δ 7.10 (m, 1H, ArH), δ 6.75 (d, 1H, ArH), δ 4.80 (s, 2H, NH_2), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 113.6 (1C, s), 115.0 (1C, s), 122.8 (1C, s), 129.6 (1C, s), 138.1 (1C, s), 147.5 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C11H12N4O2 [M+H] + : 232.23858, found: 232.0695

A8, [1-(4-methylphenyl)-1*H*-1,2,3-triazol-4-yl] methyl acetate, Appearance: Pale yellow solid, Yield : 83%, m. p. : 318 320 ºC, FTIR (KBr): 3089 (C - H str., triazole ring), 1220 (C-N Stretch., triazole ring), 1638 (N=N Stretch., triazole ring), 3089 (C - H str., aromatic ring), 1551 (C=C str., aromatic ring), 1220 (C - O asym. str., ester), 1096 (C-O sym.str., ester), 3089 (C - H asym. str., CH_3), 1 H NMR (400 MHz, DMSO), δ 2.01 (s, 3H), δ 5.25 (s, 2H,), δ 7.1 (d, 2H, ArH), δ 7.3 (d, 2H, ArH), δ 2.35 (s, 3H, Ar-CH3), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 21.3 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 123.2 (2C, s), 129.6 (2C, s), 138.1 (1C, s), 141.5 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C12H13N3O2 [M+H] + : 231.25052, found: 231.1687

A9, 1-(2,4-dimethylphenyl)- 1*H*-1,2,3-triazol-4-yl] methyl acetate, Appearance: Pale yellow solid, Yield : 88%, m. p. : 253 254 ºC, FTIR (KBr): 1260 (C-N Stretch., triazole ring), 1658 (N=N Stretch., triazole ring), 2781 (C-H str., aliphatic), 1524 (C=C str., aromatic ring), 1260 (C - O asym. str., ester), 1097 (C-O sym.str., ester), 2781 (C - H asym. str., CH_3), 1 H NMR (400 MHz, DMSO), δ 2.01 (s, 3H), δ 5.25 (s, 2H,), δ 7.10 (m, 1H, ArH), δ 7.30 (d, 1H, ArH), δ 7.50 (d, 1H, ArH), δ 2.25 (s, 3H, Ar-CH3), δ 2.30 (s, 3H, Ar-CH3) δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 17.7 (1C, s), 21.0 (1C, s), 21.3 (1C, s), 49.1 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 117.7 (1C, s), 125.8 (1C, s), 129.4 (1C, s), 129.6 (1C, s), 134.8 (1C, s), 140.4 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C13H15N3O2 [M+H] + : 245.2771, found: 245.1256

A10, [1-(4-methoxyphenyl)- 1*H*-1,2,3-triazol-4-yl] methyl acetate, Appearance: Pale orange solid, Yield : 66%, m. p. : 253 255 ºC, FTIR (KBr): 3050 (C - H str., triazole ring), 1240 (C-N Stretch., triazole ring), 1620 (N=N Stretch., triazole ring), 3050 (C - H str., aromatic ring), 2988 (C-H str., aliphatic), 1519 (C=C str., aromatic ring), 1240 (C - O asym. str., ester), 1085 (C-O sym.str., ester), 1175 (O-CH3 Stretch), 1046 (C-O Stretch., O-CH3), 1 H NMR (400 MHz, DMSO), δ 2.01 (s, 3H), δ 5.25 (s, 2H,), δ 7.2 (d, 2H, ArH), δ 6.8 (d, 2H, ArH), δ 3.73(s, 3H, Ar-OCH3), δ 7.75 (s, 1H), 13C NMR (100 MHz, DMSO): δ 21.0 (1C, s), 49.1 (1C, s), 56.0 (1C, s), 56.6 (1C, s), 67.3 (1C, s), 114.5 (2C, s), 128.3 (2C, s), 138.1 (1C, s), 159.8 (1C, s), 170.6 (1C, s), HRMS (m/z) calculated for C12H13N3O3 [M+H] + : 247.24992 , found: 247.0851

Docking procedure

A few of the ligands were got from the PubChem drug databases and docked against Hemagglutinin (3AL4) and Neuraminidase (4B7Q) with high AutoDock V4.2 software. For the selection of ligands in the current examination, the existing ligands for the mentioned proteins were chosen from a series of literature review. The existing quantities of ligands are 10 which are chosen to promote perceptions. The inhibitors' 3-D structures, along with their corresponding PubChem CIDs, were retrieved and stored in SDF format. Moreover, the process of ligand preparations included using the 3-D structure of all drugs and importing them into Pymol software to convert the 3-D structure from SDF to PDB format. Pymol software was used to remove metals from the ligand structures to facilitate an accurate docking study.. The prepared drug ligands were saved in PDB format for docking studies. The crystal structures of the target proteins were sourced from the Protein Data Bank (PDB) with PDB ID 3AL4 for Hemagglutinin and PDB ID 4B7Q for Neuraminidase and was carried again for more studies of docking.[23,24] AutoDock 4.2.6 program was used for docking study, using the empirical free energy and the Lamarckian Genetic Algorithm . T AutoGrid calculated the grid maps. In all dockings, a grid map with 48 x40 x 68 points and a grid-point spacing of 1.000 Å was applied. Among the results of the docking search, the conformation with the least docked energy was chosen as the most favourable option. The analysis of complex protein-ligand conformations, which encompassed hydrogen bonds and bond lengths, was performed with the assistance of Pymol software, UCSF Chimera and Accelrys Discovery Studio Visualizer software.

Docking Outcomes

The best conformation with the lowest docked energy was chosen from the docking search. The permissible range selected for torsions is 0 to 6, with any ligand exceeding this value being adjusted to 6. Stable interactions between a ligand and protein are indicated by the presence of H-bond interactions, which are calculated and depicted. For visualizing Hydrogen bonds Discovery studio 2020 Client and Chimera software is employed. The visualization of the docking is significantly improved by obtaining 2D images and images that depict protein-ligand interactions. The three types of interactions are hydrogen bonding, hydrophobic attraction and electrostatic interactions. The negative binding energy resulting from molecular docking experiments serves as an indicator of the stability of the complex formed between the ligand or molecule and the

protein, with a higher negative value indicating greater stability.[25,26] On performing the docking of the Hemagglutinin (3AL4) and Neuraminidase (4B7Q) with A1-A10 it was observed that the Interaction shown by the protein and ligand is good for both the proteins Hemagglutinin (3AL4) and Neuraminidase (4B7Q). On performing the docking of the Hemagglutinin (3AL4) and Neuraminidase (4B7Q) with A1-A10, It was found that the binding energy between protein and ligand is good at A9 (-6.44kcal/mol) for Hemagglutinin (3AL4) and binding energy between protein and ligand is good at A2 (-6.03kcal/mol) for Neuraminidase (4B7Q). These results reveal that these compounds have the potential to be antiviral agent prospects and are suitable for additional pharmacological research.

CONCLUSION

In conclusion some of the ester-linked derrivatives of 1,2,3-triazoles with regioselectivity for the 1 and 4 positions are successfully synthesized in high yield. A safe and efficient method is developed, eliminating the need to isolate unstable or explosive azides. This method is found good for its convenience of use, cost-economical and less environmental impact. The findings of this investigation indicate that compounds A1–A10 suppress the proliferation and infection of the influenza A virus in MDCK cells. This finding offers crucial information on the structures of substances that can be employed to create influenza medications that target the host cell's hemagglutinin (3AL4) and neuraminidase (4B7Q) proteins. As a result, these findings contribute to establish the chemical structures of compounds that could be used in developing influenza medicines. The docking investigations showed that the reported compounds have quite good binding affinities (-4.37 to -6.44 kcal/mol) with the proteins. The docking studies demonstrated a close alignment with the experimental results of bindings. There is potential for the reported compounds to be utilized as antiviral medications in the near future. Nevertheless, Additional research is required to discover the full potential of these compounds as an antiviral agent, apparently offering fresh opportunities for fighting influenza and other viral infections.

Acknowledgements

The authors are thankful to M. C. E. Society's Allana College of Pharmacy, Department of Pharmachemistry, Azam Campus, Camp, Pune-411001, Maharashtra, India for providing necessary facilities. The authors thank them for providing lab research facilities and for extending their support in whole research work.

REFERENCES

1. PavanKumar D. Chopade* and Anwar Rafique Shaikh, "advancements and future perspectives of 1, 2, 3 triazole scaffold as promising antiviral agent in drug discovery", IJPSR (2022), Volume 13, Issue 12, 4805-4818.
2. Javanian M, Barary M, Ghebrehewet S, Koppolu V, Vasigala V, Ebrahimpour S. A brief review of influenza virus infection. J Med Virol. 2021 Aug;93(8):4638-4646. doi: 10.1002/jmv.26990. Epub 2021 Apr 14. PMID: 33792930.
3. Bhalerao, U., Mavi, A. K., Manglic, S., Sakshi, Chowdhury, S., Kumar, U., & Rohil, V. (2023). An Updated Review on Influenza Viruses. *Emerging Human Viral Diseases, Volume I*, 71–106. https://doi.org/10.1007/978-981-99-2820-0_2
4. Ti, H., Zhuang, Z., Yu, Q. & Wang, S. Progress of plant medicine derived extracts and alkaloids on modulating viral infections and inflammation. Drug Des. Dev. Ther. 15, 1385 (2021).
5. Monsalve-Escudero, L. M. et al. Indole alkaloids inhibit zika and chikungunya virus infection in different cell lines. BMC Complement. Med. Ther. 21, 1–17 (2021).
6. Monto, A. S. & Fukuda, K. Lessons from influenza pandemics of the last 100 years. Clin. Infect. Dis. 70, 951–957 (2020).
7. Nehra, N.; Tittal, R.K.; Ghule, V.D. 1, 2, 3-Triazoles of 8-Hydroxyquinoline and HBT: Synthesis and Studies (DNA Binding, Antimicrobial, Molecular Docking, ADME, and DFT). ACS Omega 2021, 6, 27089–27100. doi:10.1021/acsomega.1c03668
8. Kumar, S.; Khokra, S.L.; Yadav, A. Triazole Analogues as Potential Pharmacological Agents: A Brief Review. Futurure J. Pharm. Sci. 2021, 7 (1), 106. doi:10.1186/ s43094-021-00241-3.
9. Ferreira, S.B.; Sodero, A.C.; Cardoso, M.F.; Lima, E.S.; Kaiser, C.R.; Silva, F.P.; Ferreira, V.F. Synthesis, Biological Activity, and Molecular Modeling Studies of 1H-1,2,3- Triazole Derivatives of Carbohydrates as Alpha- Glucosidases Inhibitors. J. Med. Chem. 2010, 53 (6), 2364–75. doi:10.1021/jm901265h.
10. Gonnet, L.; Baron, M.; Baltas, M. Synthesis of Biologically Relevant 1,2,3- and 1,3,4-Triazoles: From Classical Pathway to Green Chemistry. Molecules 2021 Sep 18, 26 (18), 5667. doi:10.3390/molecules26185667.
11. Gupta, O.; Pradhan, T.; Chawla, G. An Updated Review on Diverse Range of Biological Activities of 1,2,4-Triazole Derivatives: Insight Into Structure Activity Relationship. J. Mol. Struct. 2023, 1274, 134487. doi:10.1016/j. molstruc.2022.134487.
12. Santiago, J.V.; Burtoloso, A.C.B. Synthesis of Fused Bicyclic [1,2,3]-Triazoles from γ-Amino Diazoketones. ACS Omega 2019, 4 (1), 159–168. doi:10.1021/ acsomega.8b02764.
13. Forezi, L.D.S.; Lima, C.G.; Amaral, A.A.; Ferreira, P.G.; de Souza, M.C.B.; Cunha, A.C.; da Silva, F.D.C.; Ferreira, V.F. Bioactive 1,2,3-Triazoles: An Account on Their Synthesis, Structural Diversity and Biological Applications. Chem. Records 2021, 21 (10), 2782–2807. doi:10.1002/tcr.202000185.
14. Kharb, R.; Sharma, P.C.; Yar, M.S. Pharmacological Significance of Triazole Scaffold. J. Enzyme Inhib. Med. Chem. 2011, 26 (1), 1–21. doi:10.3109/1475636090352 4304.
15. Brase S,Gil C, Knepper K, ZimmermannV(2005) Organic azides: an exploding diversity of a unique

class of compounds. Angew Chem Int Ed 44:5188–5240. doi:10.1002/anie.200400657.
16. Scriven EFV, Tumbull K (1988) Azides: their preparation and synthetic uses. Chem Rev 88:297–368. doi:10.1021/cr00084a001.
17. L'Abbe'G (1969) Decomposition and addition reactions of organic azides. Chem Rev 69:345–366. doi:10.1021/cr60259a004.
18. C. P. Kaushik, Raj Luxmi, Dharmendra Singh, Ashwani Kumar, Synthesis and antimicrobial evaluation of ester-linked 1,4-disubstituted 1,2,3-triazoles with a furyl/thienyl moiety, February 2017, Molecular Diversity 21(1), doi:10.1007/s11030-016-9710-y
19. Nasrin Jahan, Arkadip Pal, Inul Ansary, Synthesis of Bioactive 1,2,3-Triazole Fused Macrocycles via Azide-Alkyne Cycloaddition, January 2024, SynOpen, DOI: 10.1055/a-2212-0996
20. Vitor Rabelo, Veronica Diniz da Silva, Maria Leonisa Sanchez Nunez, Izabel Paixão, Antiviral evaluation of 1,4-disubstituted-1,2,3-triazole derivatives against Chikungunya virus, October 2023, Future Virology, DOI: 10.2217/fvl-2023-0142
21. Mehrbod P, Abdalla MA, Fotouhi F, Heidarzadeh M, Aro AO, Eloff JN, McGaw LJ, Fasina FO. Immunomodulatory properties of quercetin-3-O-α-L-rhamnopyranoside from Rapanea melanophloeos against influenza a virus. BMC Complement Altern Med. 2018;18:1–10.
22. Shigeta S, Mori S, Watanabe J, Soeda S, Takahashi K, Yamase T. Synergistic anti-influenza virus A (H1N1) activities of PM-523 (polyoxometalate) and Ribavirin in vitro and in vivo. Antimicrob Agents Chemother. 1997;41:1423–7.
23. Sowmya, H., & Setti Sudharshan, M. (2023, March 6). Molecular modeling and docking analysis of Swine flu (H1N1) Neuraminidase protein against the phytochemicals of andrographis paniculata. Journal of microbiology, biotechnology and food sciences, e4751. https://doi.org/10.55251/jmbfs.4751
24. Bogoyavlenskiy, A., Zaitseva, I., Alexyuk, P., Alexyuk, M., Omirtaeva, E., Manakbayeva, A., Moldakhanov, Y., Anarkulova, E., Imangazy, A., Berezin, V., Korulkin, D., Hasan, A. H., Noamaan, M., & Jamalis, J. (2023, December 8). Naturally Occurring Isorhamnetin Glycosides as Potential Agents against Influenza Viruses: Antiviral and Molecular Docking Studies. ACS Omega, 8(50), 48499–48514. https://doi.org/10.1021/acsomega.3c08407
25. Shi, Y., Zhang, X., Yang, Y., Cai, T., Peng, C., Wu, L., Zhou, L., Han, J., Ma, M., Zhu, W., & Xu, Z. (2023, September). D3CARP: a comprehensive platform with multiple-conformation based docking, ligand similarity search and deep learning approaches for target prediction and virtual screening. Computers in Biology and Medicine, 164, 107283. https://doi.org/10.1016/j.compbiomed.2023.107283
26. Yildirim, M., & Celik, I. (2024, April 29). Applications of Molecular Docking Studies in SARS-CoV-2 Targeted Drug Discovery and the Gains Achieved through Molecular Docking. Unravelling Molecular Docking - From Theory to Practice [Working Title]. https://doi.org/10.5772/intechopen.1004804