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SYNTHESIS, CHARACTERISATION AND ANTI-VIRAL ACTIVITY OF CHRYSIN DERIVATIVES

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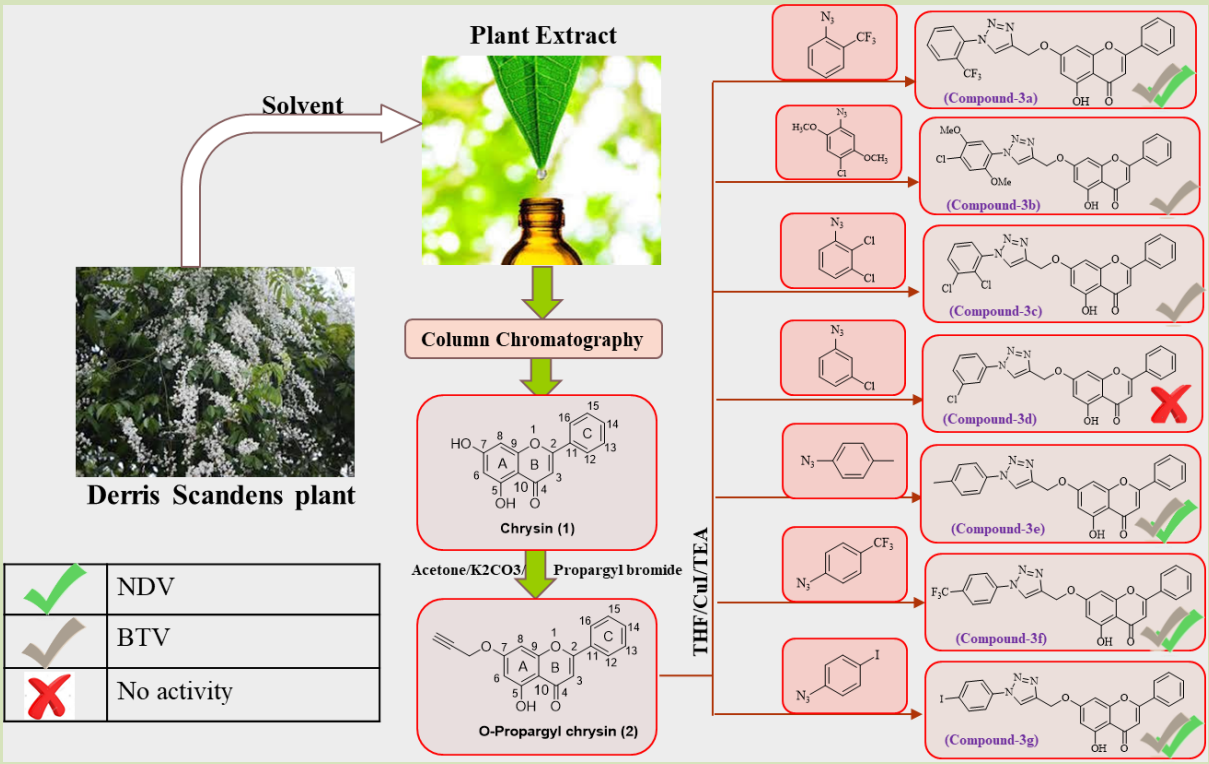
ABSTRACT
This article depicts the isolation of Chrysin (1), which is a significant constituent from hexanes concentrate of Derris scandens. A progression of new 1,2,3-triazole subsidiaries of Chrysin were planned, combined and assessed for their anti-viral movement against NDV and BTV cell lines. These synthetic derivatives have indicated great anti-viral action. All of them (3a to 3g) were distinguished by using Nuclear Magnetic Resonance (NMR) and Mass spectroscopic techniques. Among derivatives, 3a, 3e, 3f and 3g displayed conspicuous action against NDV, then again 3a, 3b, 3c, 3e, 3f and 3g showed remarkable action against BTV than Chrysin.

Graphical Abstract:



Keywords: NDV- Newcastle Disease Virus , BTV- Blue Tongue Virus

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INTRODUCTION:

Plant flavonoids have piqued the public's and scientists' interest in recent years due to their alleged health advantages. Natural products, particularly heterocyclic chemicals such as alkaloids, flavonoids, and their partly synthetic counterparts, have long been recognised as important in drug discovery and development. Flavonoids have been demonstrated to decrease acute HIV-1 infection in H9 and C8166 cultures, as well as evidence of flavonoids inhibiting HIV-1 protease, integrase, and reverse transcriptase. The regular isoflavone Chrysin confined from Derris scandens Benth and is one of the main bioactive constituents of various natural products, vegetables and even mushrooms[1]. Several *in vitro* and *in vivo* research have demonstrated that chrysin has therapeutic properties against a variety of disorders. Heterocyclic nitrogen compounds are omnipresent in the environment, these are present in various proteins, nucleotides, peptides, nutrients and coenzymes, are seen through characteristic items, for example, alkaloids[2-4]. Because of the wide range of qualities displayed by Heterocyclic compounds contain nitrogen atom, individuals from this chemical classification, such as triazoles, are often used in the pharmaceutical industry for these structural blocks [5&6]. The 1, 2, 3-triazole and 1, 2, 4-triazole rings are the most common heterocyclic rings found in different therapeutic specialities [7-9]. Triazole compounds are intriguing interfacing units because they are resistant to metabolic decomposition and can store hydrogen, which is beneficial in the official of biomolecular targets, and so they have increased bioavailability [10-12]. There are numerous compounds containing the Triazole analogue that have a variety of organic properties, such as antiviral activity, disease resistance, antiproliferative and antibacterial activity, and so on...and those were combined before the methodology of Click Chemistry was developed[13-16]. In this situation, a mix of 1,2,3-triazoles and Chrysin should have high action and selectivity, drug-similarity, and great pharmacokinetic properties. The promising biological profiles of flavonoids, notably chrysin, as well as our present research interests in finding and developing antiviral agents from natural resources [17], prompted us to use copper-catalyzed click chemistry to incorporate the novel Triazole derivatives of Chrysin and test their natural characteristics for antiviral activity.

1.0 Experimental

2.1 Plant material:

Whole Plant materials (leaves, stem and bark) of D. scandens were collected from forest located in Andhra Pradesh, India.

2.2 Materials and methods

The NMR spectra for 1H and 13C were recorded separately on a Bruker 300 MHz spectrometer, with TMS as a standard. Chemical shifts are expressed in parts per million (ppm), while coupling constants (J) are expressed in hertz (Hz). An Agilent Technologies 6510 Q-TOF Mass Spectrometer was used to collect mass spectra. Chromatography was carried out on a silica gel (100-200 lattice, Qingdao Marine Chemical, Inc., Qingdao, China), with ethyl acetate and hexane as eluents. Under the nitrogen climate, reactions that required the use of anhydrous, latent environment technologies were accomplished. Without any further refinement, industrially available reagents, solvents, and starting ingredients were used. Scientific TLC was done with the dissolvable framework Ethyl acetate / hexane on recoated Merck plates (60 F254, 0.2 mm), and the mixtures were seen under UV light and showered with 10% H2SO4, followed by heat.

2.3 General procedure

2.3.1 Isolation of Chrysin (1)

Shade dried plant materials (5 kgs) were grinded and extracted with Soxhlet, mechanical assembly in presence hexanes for 72 hours. 1 bar vacuum applied during drying to attain hexanes extract until a syrupy crude (50.0 gm) formed. Collected crude was processed using Silica gel column chromatography (100 – 200 mesh & 150 X 7.5 cm dimensions) using mixture of Petroleum ether and Chloroform (in range of 100: 0 to 5: 95) to attain 20 cumulative fractions around 100 – 250 ml of each portion. TLC evaluation was performed for collected fractions using various mobile phase compositions of Petroleum Ether and Ethyl acetate in ratio of 95:5 to 70: 30 to select four major fractions. F1 fraction further treated using Hexane: ethyl acetate mixture 95:5 using Colum chromatography to obtain Chrysin derivative **1** [27].

2.3.2 Synthesis of propargylation of Chrysin (2)*:*

Potassium carbonate was progressively added at RT under nitrogen environment to a 20 ml DMF solution containing 1.0 mmol chysin. At 0-5°C, propargyl bromide (1.3 mmol) was gently added for 5 minutes. After the addition, the reaction mass is allowed to heat up to 65°C and kept there until the SM is entirely converted, which is monitored by TLC. The reaction mass is concentrated at decreased pressure and purified using hexane: Ethyl acetate column chromatography (80:20) [27].

2.3.3 Chrysin Triazol Derivatives Preparation [18&19]:

Derivative-2 was dropped into a mixture of THF, Triethylamine and Copper (I) iodide. The batch is then heated to 60°C to complete the reaction conversion. The batch is then filtered and extracted with ethyl acetate. The filtrate on evaporation gave solid, these solids on chromatography using Ethyl acetate and Hexane gave pure Triazole derivatives of Chrysin.

3.0 Results and Discussion:

3.1 Chemistry:

Chrysin comprises of two melded rings A and B that are joined to phenyl ring C from the second position of the B ring as given underneath as Fig - 1.

Chrysin is used as starting material for synthesizing corresponding 1,2,3-triazole derivatives, as shown in Scheme - I[19, 20].

Reagent and conditions:

i) Acetone, K_2CO_3, Propargyl bromide, 3-5 hrs, 65^{o}C

ii) CuI, R = aromatic azides, THF, Triethyl amine, 6hrs, 60°C.

Scheme-I

The synthesized compounds were fully characterized by the determination of their physicochemical properties and spectral characteristics. The chemical structures of the synthesized novel Triazole derivatives of Chrysin molecules (3a-3g) were established by ^{1}H/^{13}C-NMR, FT-IR and mass spectral studies. In Chrysin, hydroxyl group at 5th position is relatively less reactive than 7th position, due to chelation and strong intra-molecular H-bonding between hydroxyl at 5th position and the carbonyl oxygen at 4th position (C-4). The ^{1}H-NMR spectrum of compound-2 showed triplet at 2.49 ppm and doublet at 4.61 ppm explained that the propargyl group attachment at 7th position of Chrysin it was evidenced by IR band at 3277 cm^{-1} for terminal alkyne group. The appearance of IR stretching 1650–1671 cm^{-1} in the spectral data of synthesized derivatives (3a-3g) specified the existence of aromatic keto group at C-4 position. The existence of characteristic bands of compounds (3a-3g) at 3064-3109 and 1607-1631 cm−1 indicated the presence of C–H and C=C group in aromatic ring, respectively. The impression of IR absorption band at 3455–3571cm−1 in the spectral data of compounds (3a-3g) displayed the presence of Ar–OH group on the aromatic ring. The singlet signals between 7.90 and 8.30 δ ppm in proton-NMR spectra is indicative of Triazole attached proton of synthesized derivatives. The compounds (3a-3g) showed singlet at 12.73-12.74 δ ppm due to the existence of OH of Ar–OH. Compounds showed additional peaks at 6.50–7.50 δ ppm due to the existence of additional aromatic ring. Compound 3b showed two singlets at 3.93, 3.88 δ ppm due to existence of two OCH_3 groups of Ar–OCH_3. The compound 3e showed singlet at 2.24 δ ppm due to presence of CH3 of Ar–CH_3. The 13C-NMR spectrum of compounds (3a-3g) showed peak at 181-183 δ ppm due to the existence of C=O of aromatic ring. The ^{13}C-NMR spectral data studies of the Triazole derivatives of Chrysin molecules (3a-3g) were found within ± 0.4% of the theoretical results of synthesized compounds are given in the "Experimental section". ^{1}H and ^{13}C NMR spectral data of all individual compounds were in good agreement with the desired structure of the respective compounds.

3.2 Experimental Data

3.2.1 Spectral data of compound-3a-3g

Propargyl Chrysin(2):

IR data: 3423, 3277, 3095, 1638, 1607, 1202, 816.
Colour: light yellow colour solid.
M.P: 163~164°C.
Yield: 87%
^{1}H NMR (400MHz, $CDCl_3$):
δ_h 12.59 (1H, s), 7.69 (2H, m), 7.39 (3H, m), 6.60 (1H, s), 6.44 (d, *J* = 1.9 Hz, 1H),6.29 (d, *J* = 1.9 Hz, 1H), 4.61 (d, *J* = 2.2 Hz, 2H), 2.49 (t, *J* = 2.2 Hz, 1H).
^{13}C NMR (100MHz, $CDCl_3$): δ 181.92, 163.62, 162.87, 161.82, 157.13, 131.32, 130.74, 128.79, 125.79, 105.31, 98.33, 92.96, 76.82, 62.23.
Calculated for HRESI-MS $C_{25}H_{17}F3N_3O_4$: 293.0813 $[M+H]^+$
Observed: 293.0835 $[M+H]^+$

5-hydroxy-2-phenyl-7-((1-(2-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-5-yl)methoxy)-4H-chromen-4-one (3a):
IR data: 3455, 3072, 1663, 1615, 1159, 835.
Colour: light yellow colour solid.
M.P: 165~167°C.
Yield: 85%
^{1}H NMR (400MHz, $CDCl_3$): δ_h 12.74 (1H, s), 7.96 (1H,s), 7.91-7.78 (d, *J* = 8.7 Hz,2H),7.76 (1H, t, *J*= 7.4, 15.1), 7.71 (1H, t, *J*= 7.4, 15.1), 7.60 (d, *J* = 8.7 Hz, 1H), 7.59-7.53 (m, 3H), 6.69 (s,1H), 6.67 (d, J= 2.1 Hz, 1H), 6.48 (d, *J*= 2.1 Hz, 1H), 5.40 (brs, 2H).
^{13}C NMR (100MHz, $CDCl_3$): δ 182.13, 164.14, 163.95, 162.19, 157.77, 143.08, 157.77, 143.08, 133.10, 131.88, 131.19, 130.62, 129.08, 128.99, 127.37, 127.28, 126.31, 125.65, 105.92, 99.07, 93.32, 62.23.
Calculated for HRESI-MS $C_{25}H_{17}F3N_3O_4$: 480.1171 $[M+H]^+$
Observed: 480.1166 $[M+H]^+$

7-((1-(4-chloro-2,5-dimethoxyphenyl)-1H-1,2,3-triazol-5-yl)methoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (3b)
IR data: 3461, 3064, 2925, 1651, 1622, 1351, 1160, 818.
Colour: light yellow colour solid.
M.P: 171~172°C.
Yield: 83%
^{1}H NMR (400MHz, $CDCl_3$): δ 12.73 (1H, s), 8.31(1H,s), 7.89 (2H, dd, *J* = 1.6, 8.2 Hz), 7.55-7.53 (4H, m), 7.15 (1H, s), 6.68 (1H, d, J= 2.2 Hz), 6.69 (1H, s), 6.49 (1H, d, *J* = 2.2 Hz,), 5.38 (brs, 2H), 3.93 (s, 3H), 3.88 (s, 3H).
^{13}C NMR (100MHz, $CDCl_3$): δ 182.48, 164.13, 164.06, 162.17, 157.77, 149.54, 144.37, 142.63, 131.89, 131.21, 129.08, 126.32, 125.21, 124.65, 123.42, 114.91, 108.95, 106.07, 105.92, 99.06, 93.40, 62.29, 56.88, 56.77.
Calculated for HRESI-MS $C_{26}H_{21}ClN_3O_6$: 506.1189 $[M+H]^+$

Observed: 506.1179 $[M+H]^+$

7-((1-(2,3-dichlorophenyl)-1H-1,2,3-triazol-5-yl)methoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**3c**)
IR data: 3474, 3085, 3009, 1657, 1616, 1349, 1158, 820.
Colour: yellow colour solid.
M.P: 160~161°C.
Yield: 82%
^{1}H NMR (400MHz, $CDCl_3$): δ 12.74 (1H, s), 8.09 (1H, s), 7.90 (2H,dd, J= 1.8, 8.2 Hz), 7.67 (1H, dd, *J*= 1.3, 8.0), 7.57-7.53 (4H, m), 7.41 (t, *J*= 8.0, 1H), 6.69 (s, 1H), 6.68 (d, J= 2.2 Hz,1H), 6.49 (d, J= 2.2 Hz,1H), 5.40 (brs, 2H).
^{13}C NMR (100MHz, $CDCl_3$): δ 182.49, 165.10, 164.18, 163.94, 162.24, 157.78, 143.04, 136.23, 134.75, 131.91, 131.20, 130.16, 129.10, 127.94, 126.33, 126.15, 125.05, 105.95, 99.01, 93.34, 62.22.
Calculated for HRESI-MS $C_{24}H_{16}Cl_2N_3O_4$: 480.0519 $[M+H]^+$
Observed: 480.0528 $[M+H]^+$

7-((1-(3-chlorophenyl)-1H-1,2,3-triazol-5-yl)methoxy)-5-hydroxy-2-phenyl-4H-chromen-4-one (**3d**)
IR data: 3489, 3081, 2965, 1661, 1607, 1501, 1365, 1161, 758.
Colour: pale yellow colour solid.
M.P: 158~159°C.
Yield: 80%
^{1}H NMR (400MHz, $CDCl_3$): 12.73 (1H,s),8.01 (1H,s) 7.89-7.86 (1H, m), 7.65 (1H, dd, *J* = 2.2, 9.6 Hz), 7.60 (1H, dd, *J* = 2.2, 9.6 Hz), 7.54-7.52 (2H, m), 7.48-7.31 (3H, m), 7.20 (1H, d, J = 2.9 Hz), 6.69 (1H, s), 6.68 (1H, d, *J* = 2.1 Hz,), 6.49 (1H, d, J= 2.1 Hz,), 5.40 (2H, s).
^{13}C NMR (100MHz, $CDCl_3$): δ 182.46, 164.21, 164.01, 162.17, 157.81, 135.65, 131.87, 131.20, 130.84, 130.80, 129.07, 128.55, 127.96, 127.76, 126.30, 126.24, 125.09, 106.10, 105.89, 99.03, 93.32, 62.26.
Calculated for HRESI-MS $C_{24}H_{16}ClN_3O_4Na$: 468.0727 $[M+Na]^+$
Observed: 468.0735 $[M+Na]^+$

5-hydroxy-2-phenyl-7-((1-(p-tolyl)-1H-1,2,3-triazol-5-yl)methoxy)-4H-chromen-4-one (**3e**)
IR data: 3501, 3075, 2926, 1664, 1622, 1505, 1354, 1163, 821.
Colour: Pale yellow colour solid.
M.P: 167~169°C.
Yield: 85%
^{1}H NMR (400MHz, $CDCl_3$): δ 12.74 (1H, s), 8.29 (1H, s), 7.90 (2H, dd, *J* = 1.8, 8.2 Hz), 7.55-7.53 (3H, m), 7.15 (4H, dd, *J* = 7.3, 1.9 Hz), 7.03 (1H, s), 6.82 (1H, d, *J* = 2.2 Hz), 6.35 (1H, d, J = 2.2 Hz), 5.40 (2H, s), 2.24 (3H,s).
^{13}C NMR (100MHz, $CDCl_3$): δ 182.11, 163.75, 163.69, 157.64, 142.80, 138.2, 131.67, 130.93, 129.07, 128.95, 126.11, 122.40, 105.71, 105.42, 98.29, 93.29, 62.31, 21.33.
Calculated for HRESI-MS $C_{25}H_{19}N_3O_4Na$: 448.1273 $[M+Na]^+$
Observed: 448.1283 $[M+Na]^+$

5-hydroxy-2-phenyl-7-((1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-5-yl)methoxy)-4H-chromen-4-one (**3f**)
IR data: 3487, 3109, 2944, 1671, 1623, 1510, 1171, 845 & 767.
Colour: Pale yellow colour solid.
M.P: 171~172°C.
Yield: 90%
^{1}H NMR (400MHz, $CDCl_3$): *δ* 12.74 (1H, s), 8.09 (1H,s), 7.90 (2H, dd, *J* = 1.8, 8.2 Hz), 7.65 (1H, dd, *J* = 1.8, 8.2 Hz), 7.58 – 7.52 (5H, m), 7.41 (1H, t, *J* = 8.0 Hz), 6.69 (1H, s), 6.68 (1H, d, *J* = 2.2 Hz), 6.50 (1H, d, *J* = 2.2 Hz), 5.40 (2H, s).
^{13}C NMR (100MHz, $CDCl_3$): δ 180.96, 165.10, 163.42, 161.61, 156.27, 144.40, 137.06, 134.35, 133.19, 125.05, 123.46, 121.83, 105.80, 105.27, 98.35, 91.94, 60.34.
Calculated for HRESI-MS $C_{25}H_{16}F_3N_3O_4Na$: 502.0990 $[M+Na]^+$
Observed: 502.0979 $[M+Na]^+$

5-hydroxy-7-((1-(4-iodophenyl)-1H-1,2,3-triazol-5-yl)methoxy)-2-phenyl-4H-chromen-4-one (**3g**)
IR data: 3462, 3121, 3070, 1653, 1631, 1512, 1184, 824 & 683.
Colour: Pale yellow-brown colour solid.
M.P: 155~156°C.
Yield: 89%
^{1}H NMR (400MHz, $CDCl_3$): δ 12.74 (1H, s), 7.94-7.86 (m, 4H), 7.56-7.52 (4H, m), 7.32 (2H, d, *J* = 8.67), 6.69 (2H, d, *J* = 2.18), 6.53 (1H, d, *J* =2.18), 5.29 (2H, s).
^{13}C NMR (100MHz, $CDCl_3$): δ 181.94, 164.84, 163.08, 161.03, 144.24, 134.57, 133.46, 132.66, 131.66, 129.49, 126.94, 125.65, 106.54, 106.30, 104.15, 102.52, 96.62, 62.05.
Calculated for HRESI-MS $C_{24}H_{17}IN_3O_4$: 538.0263 $[M+H]^+$
Observed: 538.0269 $[M+H]^+$

3.3 Results and discussion on biological Activity:
a) *In-ovo* screening of *Anti-viral activity of derivatives*

Chrysin and its derivatives have been produced are (3a-3g) selected to screen their anti-viral activity *In-ovo* in infected embryonated chicken eggs against Newcastle Disease Virus / Bluetongue Virus. The test derivative and virus were mixed in 1:1 ratio and is set up by blending 1 microgram/mL in Dimethyl (oxido) sulfur (DMSO) for the treatment. After hatching, fluid from eggs were collected for testing.

b) Anti-viral effect of compounds against NDV:

In view of the outcomes got by Hemagglutination (HA) test on infection titre estimates and side effects in

premature chicks, the derivatives displayed critical anti-viral activity than that of native molecule (NM). Among 3a-3g, the mixes, for example, 3a, 3e, 3f and 3g showed improved anti-viral activity. All the derivatives showed preferred anti-viral action over that of Chrysin. The anti-viral activity of the mixes against NDV are captured in Table-II.

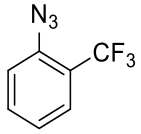
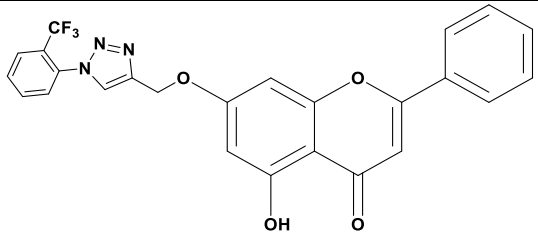
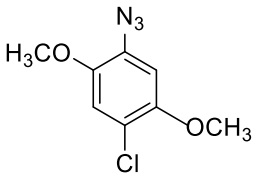
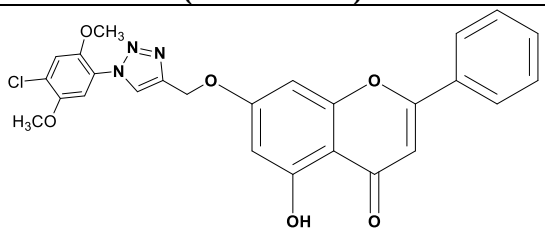
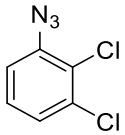
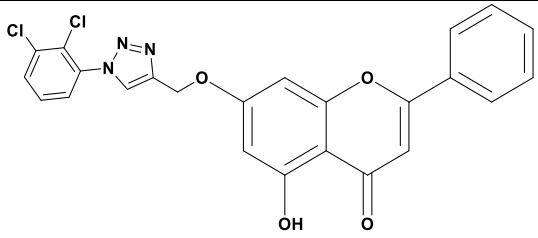
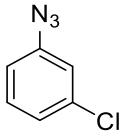
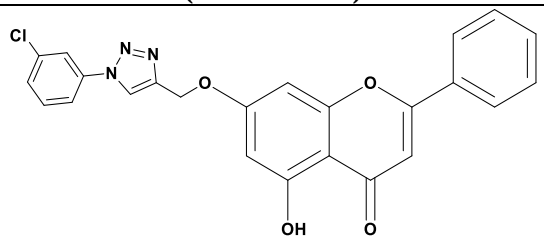
c) **Anti-viral effect of derivatives against BTV:**

The outcomes uncovered that all the derivatives displayed promising anti-viral exercises than that of Chrysin. Among 3a-3g, the 3a, 3c and 3f showed enhanced anti-viral activity by disappearing the infection at different titre interims. Moreover, the mixes 3b, 3d, 3e and 3g additionally displayed promising anti-viral action in terms of killing the infection when contrasted with Chrysin. All the derivatives showed improved anti-viral activity than Chrysin. Table III shows the antiviral characteristics of the mixtures against Blue Tongue Virus.

d) **Derivatives *In-vitro* Anti-viral activity against NDV/ BTV:**

According to the findings of this study, all of the compounds showed potential activity against both infections, such as Blue Tongue Virus, BHK 21 and Newcastle Disease Virus cell lines. They reduced the cell death caused by Newcastle Disease and Blue Tongue Viruses in BHK 21 cell lines. Out of all 3a, 3e, 3f and 3g showed noticeable movement against NDV while 3a, 3b, 3c, 3e, 3f and 3g against BTV with greatest cell feasibility likened to the Chrysin (Table IV and V).

Table-I: Synthesis of derivatives of triazole-connected chrysin derivatives

S. No	Substrate	Structure
1		 **(3a-Derivative)**
2		 **(3b-Derivative)**
3		 **(3c-Derivative)**
4		 **(3d-Derivative)**

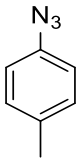
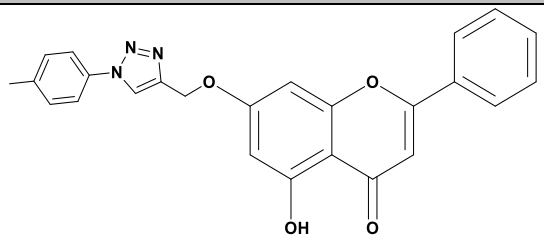
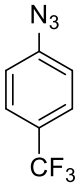
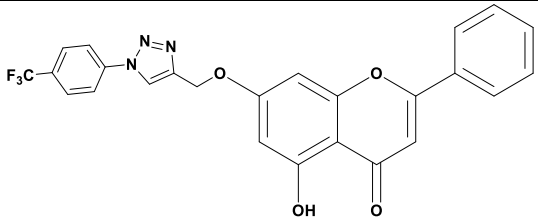
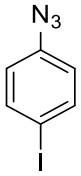
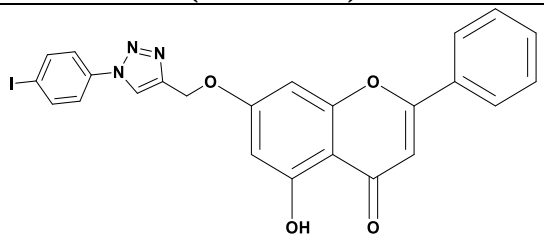
S. No	Substrate	Structure
5		 **(3e-Derivative)**
6		 **(3f-Derivative)**
7		 **(3g-Derivative)**

Table-II: Derivatives (3a-3g) anti-viral activity against Newcastle Disease Virus (NDV) infected embryonated eggs of chicks

Derivatives	No of eggs	Survival				HA Test		Titer value
		24 hours	48 hours	72 hours	96 hours	Positive	Negative	
3a	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 128
3b	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 64
3c	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 64
3d	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 32
3e	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 256
3f	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 256
3g	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 128
NM	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	One	Four	1 of 32

Table III: Antiviral activity of derivatives (3a-3g) against Bluetongue Virus (BTV) infected chicken embryonated eggs

Derivatives	No of eggs	Survival				HA Test		Titer value
		24 hours	48 hours	72 hours	96 hours	Positive	Negative	
3a	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 128
3b	Five	Zero of Five	Zero of Five	Zero of Five	One of Five	One	Four	1 of 64
3c	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 256
3d	Five	Zero of Five	Zero of Five	Zero of Five	One of Five	One	Four	1 of 128
3e	Five	Zero of Five	Zero of Five	Zero of Five	One of Five	One	Four	1 of 64
3f	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	Zero	Five	1 of 128
3g	Five	Zero of Five	Zero of Five	Zero of Five	Zero of Five	One	Four	1 of 32
NM	Five	Zero of Five	Zero of Five	Zero of Five	Two of Five	Two	Three	1of 32

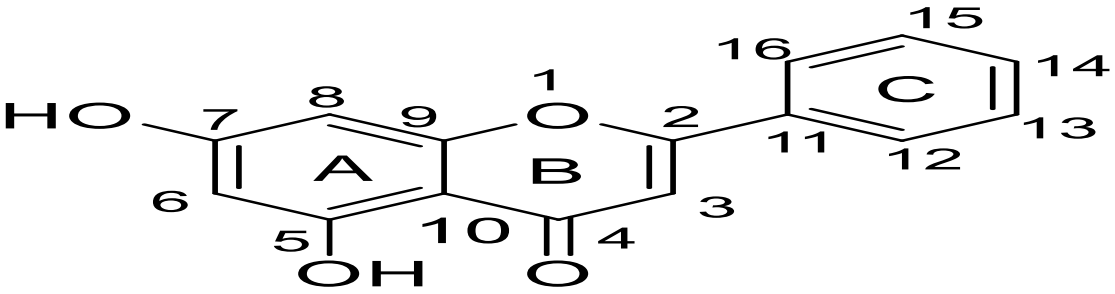
Table-IV: In-vitro antiviral activity against NDV infected BHK 21 cell lines for derivatives (3a-3g).

Derivatives	Newcastle Disease Virus infected BHK 21 Cells (% of live cells)	
	10 µg/mL	20 µg/mL
3a	63	85
3b	60	78
3c	62	80
3d	59	79
3e	64	88
3f	62	87
3g	62	83
NM	45	63

Table-V: Derivatives (3a-3g) In-vitro Anti-viral activity against BTV infected BHK 21 cell lines.

Derivatives	Bluetongue Virus infected BHK 21 Cells (% of live cells)	
	10 µg/mL	20 µg/mL
3a	62	82
3b	54	71
3c	61	81
3d	55	70
3e	59	76
3f	60	81
3g	60	78
NM	41	62

Fig-1



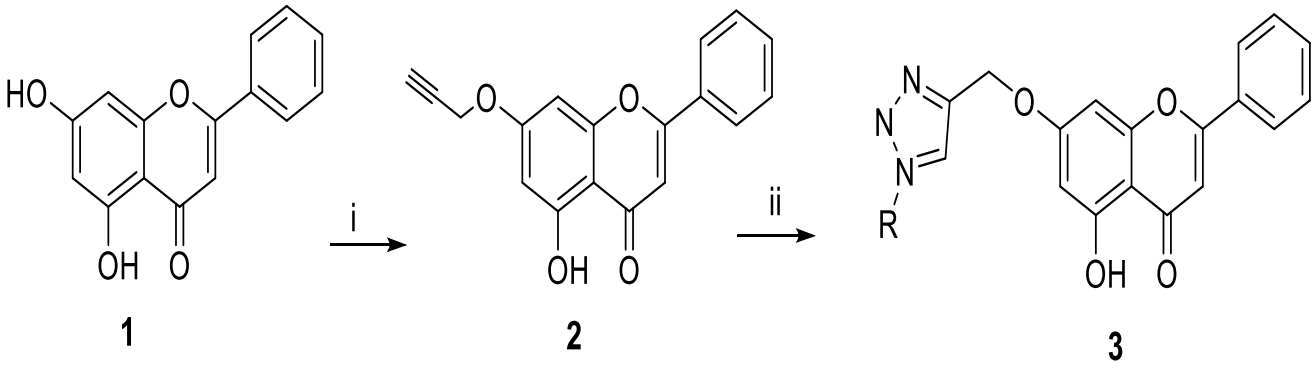
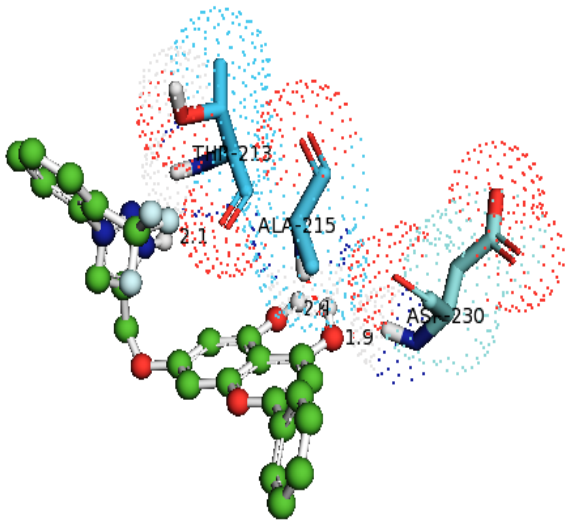
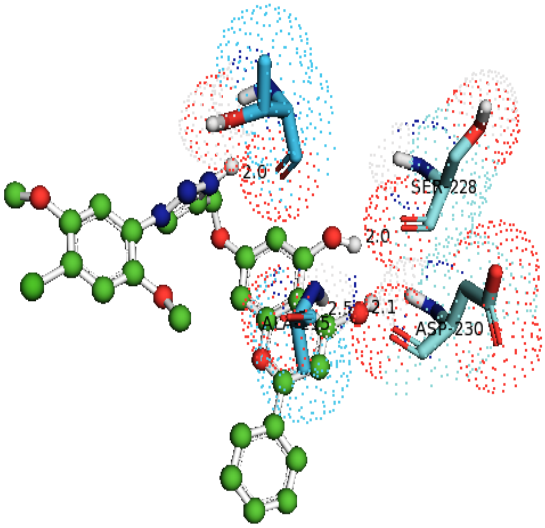


Fig 2: Bonding interactions between derivatives 3a-3g and Native Molecule with VP7 of BTV



(3a-Derivative)



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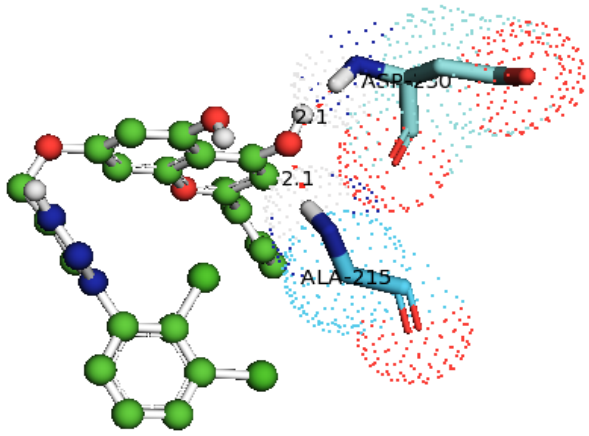
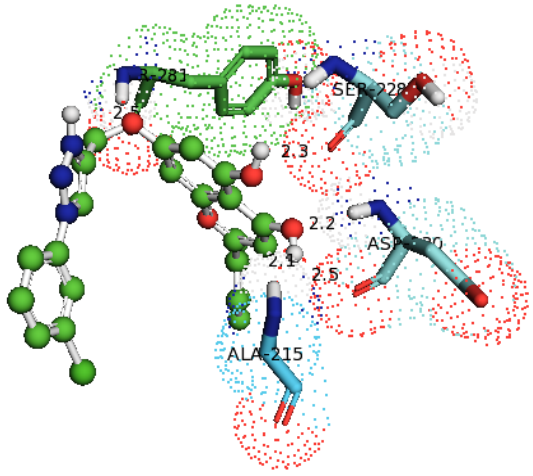
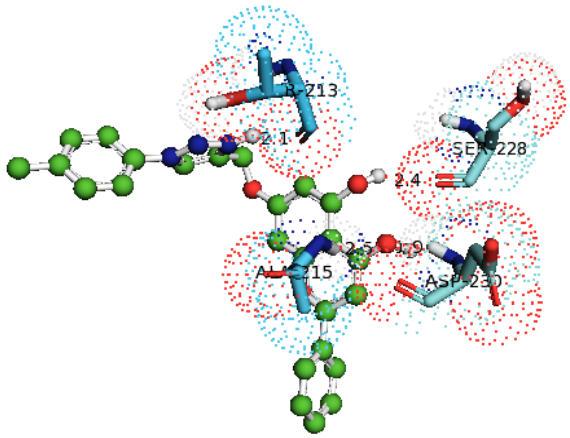


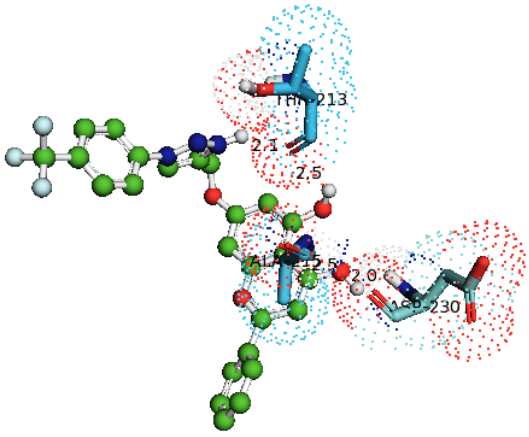
(3c-Derivative)



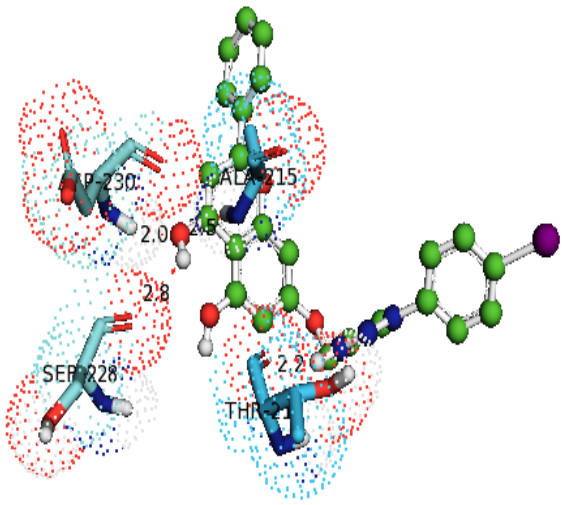
(3d-Derivative)



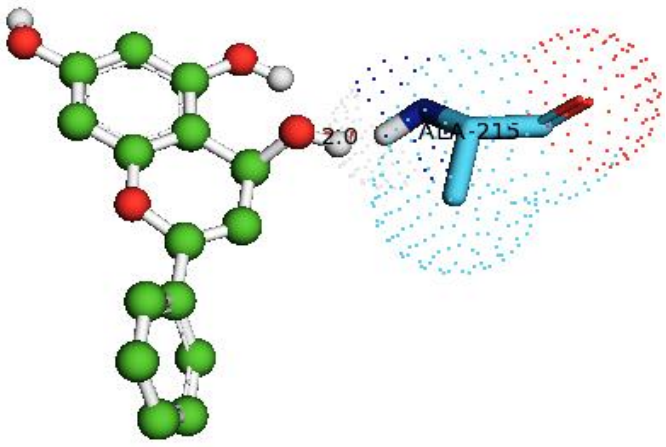
(3e-Derivative)



(3f-Derivative)



(3g-Derivative)



Native Molecule

3.4 Materials and Methods

3.4.1 *In-ovo* Anti-viral activity

A. *Source of Virus and eggs*

NDV, BTV cell lines and embryonated eggs are collected from Virology and poultry departments of Sri Venkateswara University, Andhra Pradesh. The collected eggs were hatched at 37 ºC temperature.

B. *In-ovo* anti-viral activity of derivatives

The mobility of derivatives was assessed using nine-day-old hatching eggs. Cleaning the eggs with 70% liquor and placing them in a sterile plate was done. The eggs were cleaned and used to administer the derivative/virus cocktail via the Allantoic method. In 0.1 mL of NDV / BTV in 0.1 mL of 1 mg / mL derivative in Dimethyl(oxido)sulfur, the derivative / viral mixture was suspended (DMSO). Controls included virus dissolved in saline (without derivative).

9 days of age NDV-injected chick embryos were inoculated using preserved viruses and their controls through CAM and the egg yolk sac in the investigation. Two viral samples were produced in triplicate against each derivative and compared to a control sample. The eggs were sealed with molten wax and then incubated at 37 degrees Celsius. To identify NDV, allantoic fluid from treated eggs were taken[21].

C. *In-ovo* Hemagglutination (HA) test

The airspace in the egg shells was reduced after deceased embryos of eggs were placed in a bio-safety cabinet. Collected blood samples were allowed for centrifugation at 1000 rotations per minute with a period of 10 minutes, then saline was added, the supernatant was removed, and an equal amount of sterile saline was added, centrifuged at 1000 rotations per minute for another 10 minutes. This technique was performed three times, and the packed RBCs were diluted to 1% for use in the Hemagglutination test. 0.025 mL of a 1 percent RBC solution was added to each well. The results were evaluated after 30 minutes of incubation at room temperature[22&23].

3.5. *In-Vitro evaluation*

A. *Cell line collection and preservation*

The National Centre for Cell Sciences in Pune provided BHK-21cell lines. The cell lines were kept at 37°C in Dulbecco's Modified Eagle Medium (DMEM) containing 5% serum, 2% FBS, 100 µg/mL penicillin, and 100 µg/mL streptomycin in water-soaked air containing 5% CO_2. Tripsinization was used to pass the crossing cells once a week [24].

B. Antiviral activity of selected derivatives *in vitro*

A set number (5 X 10^6) of exponentially proliferating NDV/BTV infected cells were planted into 96 well micro titer plates and allowed to proliferate. The virus mixture was made by suspending 0.1 mL of NDV/BTV in 0.1 mL of 1 mg/mL derivative in DMSO, 100 mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) for three hours, followed by 10 mL dissolution solution and mixing vigorously with a pipette/glass rod. The MTT medium was carefully removed from the wells, and the formazan dye was eluted using DMSO. At a wavelength of 570nm, a multi-mode ELISA reader was used to detect absorbance [25].

3.6. *In-Silico* Studies

A. Molecular docking

The structures of HN protein (PDB ID 1USX) and VP7 protein were obtained from the Protein Data Bank (PDB

ID 1BVP). Hetero atoms and H2O molecules were removed from the structures and placed into the MOE working climate, which was followed by protonation and energy minimization using the MMFF94x force field with a cut off value of 0.05. The chemsketch tool was used to create structures and derivatives, and the universal force field (UFF) was employed to reduce energy, which was then converted to PDF for docking. For successful docking of structural derivatives and all other places created by superposition of triplets of receptor sites and their ligand atoms, the alpha triangle placement approach is used. A minimum of ten confirmations were generated for each derivative, which were then inspected and rescored. PyMOL Visualizer was used to assess the interactions of ligands with the target protein. The selected effective compounds of each virus protein were produced and assessed for anti-viral activity after the molecular docking studies were completed, as shown in Fig-2[26].

4.0 Acknowledgements:

The authors wish to thank GIS, IICT and Divi's laboratories for their constant encouragement.

5.0 Conclusion:

Chrysin was isolated naturally from *Derris Scanden* hexanes extract, and Triazole derivatives of Chrysin were synthesised (3a-3g). The overall screening revealed that synthesized derivatives are acted as promising anti-viral agents when compared with native compound. Out of all, 3a, 3e, 3f and 3g exhibited prominent activity against NDV whereas 3a, 3b, 3c, 3e, 3f and 3g against BTV with maximum cell viability akin to the rest of the title derivatives. As a result, the current study will open up new avenues for the identification of new antiviral medications, with a number of the synthesized derivatives standing out as an interesting therapeutic medication candidate for future optimization and development of prospective antiviral medications.

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