Original Article

DISCOVERY OF ANTI-HCV AND NS5B RDRP INHIBITION ACTIVITIES OF NOVEL ANTHRAQUINONES BY IN VITRO AND IN-SILICO APPROACH

Haider Ali Khan[1], Mohtasim Billah[2*], Iftikhar Ali Khan[3]

[1]Department of Biochemistry, Abdul Wali Khan University, Mardan - Pakistan

[2]Department of Pathology, BKMC, Mardan - Pakistan

[3]Department of Nephrology, Khyber Teaching Hospital, Peshawer - Pakistan

ABSTRACT

BACKGROUND: *Hepatitis C virus affects over 170 million people globally. Chronic HCV infection results in major liver disorders for instance hepatocellular carcinoma and cirrhosis finally require liver transplantation. No vaccine is available yet. HCV is a tiny, encapsulated, single-stranded RNA-positive virus that is a member of the Flaviviridae family and belongs to the genus Hepacivirus. Four structural (S) and six non-structural (NS) proteins are created from its RNA genes, which encode a polyprotein precursor with about 3000 amino acids. These proteins are then digested by cellular and viral proteases. Non-structural (NS) protein NS5B, which performs a critical role in RNA-dependent RNA polymerase (RdRp), is a desired target for the development of specialised antiviral medications.*

OBJECTIVE: *In this research study, we studied the activities of the anthraquinone compounds, were found out the active compounds having well anti HCV and NS5B RdRp inhibition activities.*

MATERIALS AND METHODS: *Cassia artemisioides flowers were obtained and purified from their compounds after being harvested in Peshawar in March 2013. After being rinsed to eliminate dust, the flowers were left to air-dry for a few days. big to powdered after that. then methanol was utilized for extract in a soxhlet apparatus. The root barks of C. aretemisioides were crushed to powder the extract of the root barks of the selected plant that is soxhlet extracted andT then evaporated the solvent by using the rotary evaporator under the concentrated pressure. Column chromatography was used to separate the methanol fraction from 1, 6-dihydroxy-8-methoxy-3-methylanthraquinone(2) and 1, hydroxy-8-methoxy-methylanthraquinone (3) Prior to molecular docking, the ligand and protein were made. Then, 2D structures of the compounds were developed, and all of the compounds were then saved in 3D structures in a database. The 3D coordinates of HCV NS5B was retrieved from protein data bank .All the ligands were docked into the pocket having active residues of HCV NS5B using anti-HCV compounds were performed, The cells were produced in 96-well plates and handled with a substance at a concentration of (50 M), whereas the group in control recievwd an equivalent quantity of DMSO. Once the cells had been exposed to the compounds for 48 hours, the relative levels of the luciferase signals in the compound-treated cells compared to the DMSO controls were measured. This was done to ascertain the inhibitory effect of the compounds on HCV replication. On poly rA-U12 template primer in the presence of DMSO or the previously described medicines, HCV NS5B was inhibited by [-32P] UTP and MnCl2 as the divalent cation. Anti-HCV medications were tested for 50% inhibition at dosages of 50 M, which indicated 100% inhibition of NS5B activity in the presence of DMSO (IC50).*

RESULTS: *Our findings indicate that we can also improve anthraquinones and that it may be able to create new anthraquinone analogues with enhanced cellular and enzyme-based activity that have anti-HCV and NS5B RdRp inhibitory capabilities.*

CONCLUSION: *Our research studies focused on the molecular HCV NS5B protein docked with the active residues of the anthraquinone compounds and proved that these compounds were active compounds and then treated with the HCV NS5B RdRp in vitro, Among these compounds, compound 3 is not acting as anti-HCV agent, but has good activity in the NS5B RdRp inhibition and good cell viability. Furthermore, we can also predict new anthraquinone derivatives by in silico approach and as well as can also find out their activities by in vitro process in the lab.*

KEY WORDS: *hepatitis C virus, RNA dependent RNA polymerase, anthraquinones, Molecular docking, NS5B*

inhibitors.

ABSTRACT: *A recognised target for therapeutic study is the NS5B RNA-dependent RNA polymerase (RdRP), which is a vital and distinctive component of the RNA polymerases produced by the hepatitis C virus (HCV), evaluation of 50% inhibition level. In this research study, we report the isolation of compounds, having anti-HCV and NS5B RdRp inhibition activities. Here we studied the activities of the anthraquinone compounds, both by in silico approach and in vitro. These compounds were found out the active compounds having well anti HCV and NS5B RdRp inhibition activities. Our findings also suggest that we can improve anthraquinones and that it would be able to create novel anthraquinone derivatives with enhanced cellular and enzyme activity.*

INTRODUCTION

Infecting roughly 170 million people globally, the hepatitis C virus (HCV) is the primary cause of chronic hepatitis C[1]. Persistent HCV infection causes severe liver problems for instance hepatocellular carcinoma and cirrhosis finally require liver transplantation [1,2]. There is no vaccine available yet [3].

One of the members of the Flaviviridae family and a member of the genus Hepacivirus, HCV is a single-stranded RNA-(+), tiny, enveloped virus[4]. The RNA genome of this organism encodes a polyprotein precursor with over 3000 amino acids, which cellular and viral proteases convert into 4 structural (S) and 6 non-structural (NS) proteins [4]. An attractive target for the creation of selective antiviral drugs is the NS5B protein since it is a crucial enzyme for HCV replication and performs an RdRp function[5]. It belongs to the NS proteins, which are not structural. There are two different kinds of NS5B inhibitors: nucleoside inhibitors (NIs), which work with the active site, and non-nucleoside inhibitors (NNIs), which connect to one of the five recognized allosteric sites[6]. The present system of the allosteric sites is as follows: I thumb site I (TSI); II thumb site II (TSII); III; IV; V; and VI palm site I (PSI); [6a]. It's important to note that academic institutions and pharmaceutical firms are working hard to find efficient and selective inhibitors[6b] that might be used in a therapy that uses various DAAs and is devoid of pegIFN and RBV. The NS5B pathway is the focus of some more recent anti-HCV therapies. A variety of NNIs and NIs have nonetheless been identified in the scholarly literature, FDA has not yet approved any medications that are active against NS5B polymerase. The most advanced medication candidates in clinical trials for the NS5B NIs are mericitabine (RG7128) and sofosbuvir (PSI-7977), whereas the most advanced NNIs are filibuvir (PF-00868554) and setrobuvir (ANA598)[7].

Correspondence:
Prof Dr. Mohtasim Billah
Professor & Chairman
Department of pathology, Bacha Khan Medical college, Mardan Kp Pakistan.
Email: dr.mohtasimbillah@gmail.com
Cell: +92-300-5772024

A research group has been focusing on the production of HCV drugs lately, with a focus on identifying new chemotypes that are potent against the NS5B and HCV[8]. Some of the cassia species have therapeutic properties and are a significant source of anthraquinones and flavonoids[9,10], and[11] for instance hypo-glycemic[12], hepatoprotective[13], hypocholesterolemic[14], antibacterial[15], and anti-diabetic[16] characteristics. Leprosy, syphilis, tuberculous glands, and skin illnesses are all treated with Cassia roots in Ayurveda medicine. Chest discomfort, inflammation, asthma, throat discomfort, liver discomfort, and rheumatism are all treated with Cassia fruits [17]. Here, we report on the anthraquinones derivatives' isolation, anti-NS5B activity, and anti-HCV activity. To determine the location on NS5B polymerase where anthraquinone molecules will bind, we used molecular docking. An significant step in the development of these compounds as NS5B inhibitors is represented by this work.

MATERIALS AND METHODS

Obtaining compound 1 from the Flowers of Cassia artemisioides: In March 2013, Cassia artemisioides flowers were gathered in Peshawar. After becoming scrubbed to eliminate dust, the flowers were given the freedom to air dry for a few days. After that, the flowers were crushed. The chosen plant's powdered flowers were then extracted in a soxhlet device with methanol. The crude residue was produced by focusing the extracts of the chosen plant in a rotary evaporator while under vacuum. Column chromatography was performed on the methanol fraction over silica gel. Various fractions were obtained[18]. One of the fraction was subjected to the column chromatog-

raphy and obtained 3 fractions and by their further purification by the help of utilising hexane:ethyl acetate as the eluting solvent in preparative TLC system which produced compound 1(2,6,8-Trihydroxy-1-methoxy-3-methylanthraquinone).The weight of the compound obtained was 0.85 mg. Molecular weight is 300.26 and it was obtained as yellow needles (Acetone).Its mp is 291-293 °CThe molecular formula is C16H12O6, which was found from the 13C NMR and the EI mass spectrum at m/z 300 (calculated as 300 for C16H12O6).

The ^{13}C NMR spectrum of the compound 2,6,8-Trihydroxy-1-methoxy-3-methylanthraquinone confirmed the presence of sixteen carbon atoms, having one methyl group, one methoxy group, three methane and ten quaternary carbons according to DEPT experiments. Total assignments were achieved according to HMQC and HMBC experiments (Table 1). Extraction and isolation of compound 2 and 3 from root barks of C. aretemisioides: The root barks of C. aretemisioides were crushed to powder and poured methanol in it and shaken well and filtered to remove the extract of the root barks of the selected plant that is soxhlet extracted and then evaporated the solvent by using the rotary evaporator under the concentrated pressure. The methanol fraction was subjected to column chromatography on the silica gel and eluted the methanol and obtained various fractions. Fraction 1 was then subjected to CC followed by the preparative TLC and yielded compound 1, 6-dihydroxy-8-methoxy-3-methylanthraquinone (2) and 1-hydroxy-8-methoxy-3-methylanthraquinone (3) [18].

1- 1,6-dihydroxy-8-methoxy-3-methylanthraquinone: It is Yellow needles (Acetone) and melting point is 300-302 °C. Molecular formula of this compound is $C_{16}H_{12}O_5$andmolecular weight is 284.26. UV (MeOH) λ max (log ε); 225 (4.53) 247 (4.09), 286 (4.43) nm IR (KBr) max; 3427 (OH), 2921, 1680, 1589, 1484, 1441, 1261 cm^{-1} EI-MS; m/z284 [M+] (calcd. 284 for $C_{16}H_{12}O_5$) EI-MS m/z(rel. int.) (%); 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149 (12.64).

2- 1-Hydroxy-8-methoxy-3-methylanthraquinone: Its molecular weight is 268.26 and shown as yellow needles (Acetone), and 0.85mg in weight. Melting point: 195-196 °C. Molecular formula: $C_{16}H_{12}O_4$. UV (MeOH): λmax (log ε) 221 (4.50), 255 (4.30), 280 (4.10), 413 (3.90) nm IR (KBr) max; 3419 (OH), 2945, 1675, 1630, 1586, 1495, 1440, 1373 cm^{-1}

Ligand and protein were prepared prior to molecular docking by using MOE2010.11. 2D structures of the compounds were created by using Chembio draw ultra 12 software and then saved each compound in mol file. Mol files having the compounds are opened in MOE and the energy minimized upto 0.05 gradients by usingMMFF94x force field. The compounds were then recorded in 3D structures in a database that had been built. The 3D coordinates of HCV NS5B was retrieved from protein databank (Pdb id: 5twn). Due to the low resolution of most macromolecular crystal structures, protonation was done, after employing the Protonate 3D Tool to dock in MOE. Upon protonation, energy was lowered up to 0.05 Gradient via the MMFF94x force field. Using the Triangle Matcher docking strategy, all of the ligands were docked into the pocket including the active residues of the HCV NS5B. Each complex's linkages were assessed, and 3D shots of each complex were taken.

Cytotoxicity and Anti-HCV Activity in Cell-Based Assays: Huh7/Rep-Feo1b cells are replicating and having the firefly luciferase reporter were used in cell-based assays to assess the anti-HCV activity of drugs towards sub-genomic HCV genotype 1b (RNA Replicon)[19]. Similar to what was described [19, 20], the analysis of anti-HCV compounds was conducted. On 96-well plates, the cells were cultured and treated with (50 M) concentrations of the chemical and DMSO as a control (DMSO final concentration was kept at 0.5%). In order to determine the inhibitory effect of compounds, the relative quantities of the luciferase activity in contaminant cells relative to DMSO controls were quantified on HCV replication after 48 hours of treatment (Promega). In Huh7/Rep-Feo1b cells, the drugs' impact on cell viability was examined under conditions analogous to antiviral tests. Basically, drugs or DMSO (control) were applied to cells placed in 96-well plates for 48 hours. According to the manufacturer's instructions, CellTiter 96 AQueous One solution was used to measure cell viability (Promega, U.S.).

HCV NS5B RdRp Inhibition Assay: Recom-

binant HCV NS5B with an N-terminal hexa-histidine tag and a deletion of the C-terminus (21 hydrophobic amino acids) was extracted using Ni-NTA column chromatography[21, 22]. Primer for the Poly rA-U12 template, [-32P] To test HCV NS5B suppression in the influence of DMSO or the predetermined medicine, UTP and MnCl2 as the divalent cation were used[21]. After an hour of incubation at 30 °C, the reactions were interrupted by introducing an ice-cold solution of 5% TCA. The radiolabeled RNA was extracted on GF-B filters and weighed with a liquid scintillation analyzer after being precipitated with 5% TCA. Comparing the degree of NS5B activity, in the influence of the chemicals to the fixed-at-100% amount of NS5B action in the presence of DMSO. Tests for 50% inhibition (IC50) of anti-HCV agents were conducted at concentrations of 50 mM, which indicated 50% inhibition. To estimate the IC50 values of various compounds, dose-response graphs at 8–10 comparable strengths of serially diluted compounds are used (Graphpad prism 3.03).

RESULTS AND DISCUSSION

Molecular docking: In MOE software by using the docking protocol, molecular docking was carried out of the compounds with the pocket of the NS5B protein which were isolated from the cassia plants. MOE software is used for the purpose to check the binding of the compounds (inhibitors) with the target protein that is NS5B protein. Docking was done by following the protocol mention in the MOE software. 15 conformations are allowed to be preserved for each ligand using the default MOE parameters. Because the conformations from each docked molecule vary, this was allowed to maintain the top-ranked conformations in a separate database. We observed the interaction of each compound with the target protein by using the LigX in MOE. The compounds which were interacted with the target protein, showed interactions of the residues Tyr195, Arg200, Met414, Ile447, Tyr448, Tyr452 and Phe551 with the pocket for binding of the NS5B protein. Each compound's interactions with the protein were as mentioned as below in Figure 2(A-C).

Binding interactions between the chemical and the NS5B protein. Compounds which were isolated from cassia plants belong to the class anthraquinone. These compounds have shown good interactions with the target protein. From the molecular docking study, the most active compound 1 formed six hydrogen connections with the residues of the active site Tyr 195, Arg200, Tyr452, Phe551, one arene-cation interaction with Tyr448 and one hydrophobic linkage with the Met414.Compound 1 have docking score -14.5721, binding energy -43.17 Kcal/Mol and binding affinity -7.60 Kcal/Mol. Tyr 195, Arg200, Tyr452, Phe551 residues of target protein formed six interactions with oxygen atoms that are polar, of the 5,7-dihydroxynaphthalene-1,4-dione moiety of the compound 1. Met 414 and Tyr 448 showed hydrophobic and arene-cation interactions with the 1-methoxy-3-methylbenzene moiety of the compound (Figure 2A). Compound 2 having the docking score value -13.6515, binding energy -38.14Kcal/Mol and binding affinity -6.96Kcal/Mol. The benzene ring of 3-methoxy phenol of the compound 2 showed polar interaction with Tyr452. Arg200 residue formed hydrogen bonds with the OH group and carbonyl oxygen atoms of the 5-hydroxynaphthalene-1,4-dione moiety of the compound 2. Tyr 448 and Met 414 were observed making hydrophobic contacts with the compound 2 (Figure 2B). Compound 3, an inhibitor of the NS5B protein this compound interacted with binding pocket of protein. Their docking score was -12.4722, binding affinity -6.51Kcal/Mol and binding energy -35.23Kcal/Mol. This compound formed three hydrogen bonds, one hydrophobic and one arene-cation interaction with the Arg200, Tyr 452 and Met 414 residues of the protein respectively. The Arg200 and Tyr 452 residues formed polar bonds with the carbonyl oxygen atoms of the compound 3 (Figure 2C). All these compounds are active and have good inhibition and follow all the criterion of the Lipinski's rule of five and act as good inhibitors of NS5B potein as shown in table 4.

CELLULAR CYTOTOXICITY AND ANTI-HCV ACTION

The compounds obtained from organic sources, and then their activity was tested using an in silico way and the MOE programme. We observed that the compounds can be able to interact with the HCV NS5B target protein and stop both its HCV and RdRp action. The chemicals were initially tested at dosages of 50 M in conventional in vitro RdRp studies to test this notion. Computing the percent restriction of NS5B RdRp expression and is displayed

Table 1: 1H (600 MHz) and 13C (150 MHz) NMRin (CD3)2CO and HMBC Correlations of 2,6,8-trihydroxy-1-methoxy-3-methylanthraquinone (1)

C. No.	13C NMR(δ)	1H NMR (δ) (JHH Hz)	Multiplicity	HMBC Correlation
1	147.20	-	C	
2	155.47	2.86 (b s, OH)	C	
3	131.72	-	C	
4	126.19	7.84, s	CH	C-2, C-9a, C-10
4a	125.86	-	C	
5	107.19	7.16 (d, J = 2.34)	CH	C-7, C-8a, C-10
6	164.53	2.86 (b s, OH)	C	
7	107.67	6.56 (d, J = 2.28)	CH	C-5, C-8a
8	165.49	13.16 (s, OH)	C	
8a	110.99	-	C	
9	187.14	-	C	
9a	123.86	-	C	
10	181.02	-	C	
10a	135.25	-	C	
8-OCH3	61.2	3.90 (3H, s, OCH3)	OCH3	C-8
3-CH3	15.74	2.34 (3H, s, CH3)	CH3	C-2, C-3, C-4

Table 2: 1H (600 MHz) and 13C (150 MHz) NMR in CDCl3 and HMBC Correlations of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone (2)

C. No.	13C NMR (δ)	Multiplicity	1H NMR (δ) (JHH Hz)	HMBC Correlation
1	162.32	C	-	
2	123.94	CH	7.04, s	C-9a, C-4
3	146.83	C	-	CH3 (20.97)
4	119.20	CH	7.48, s	C-2, C-9a
4a	132.59	C	-	
5	107.53	CH	7.23(d, J = 2.3)	C-7, C-8a
6	163.92	C	-	
7	104.45	CH	6.76 (d, J = 2.3)	C-5, C-8a
8	165.79	C	-	
8a	112.75	C	-	
9	187.17	C	-	
9a	114.68	C	-	
10	182.82	C	-	C-4, C-5
10a	137.39	C	-	
3-CH3	20.52	CH3	2.39 (3H, s, CH3)	C-2, C-3, C-4
8-O CH3	55.89	CH3	3.93 (3H, s, OCH3)	C-8

Table 3: 1H (600 MHz) and 13C (150 MHz) NMR* data and HMBC Correlations of 1-hydroxy-8-methoxy-3-methylanthraquinone (3)

C. No.	13C NMR(δ)	Multiplicity	1H NMR (δ) (JHHHz)	HMBC Correlation
1	162.77	C	12.92 s (OH)	OH-C, C-1, C-2, C-9a
2	124.72	CH	7.08 (d, J = 0.96)	C-4, C-9a, 3-CH3
3	147.69	C	-	
4	120.26	CH	7.58, s	C-2, C-9a, 3-CH3
4a	132.47	C	-	
5	120.15	CH	7.36 (d, J = 7.8)	C-7, C-8a, C-10
6	135.89	CH	7.72 (t, J = 7.8)	C-8, C-10a
7	118.21	CH	7.95 (d, J = 7.8)	C-5, C-8a, C-10
8	160.87	C	-	
8a	115.24	C	-	
9	188.64	C	-	
9a	115.06	C	-	
10	183.10	C	-	
10a	135.73	C	-	
3-CH3	22.16	CH3	2.42 (3H, s, CH3)	C-2, C-3, C-4
8-OCH3	56.75	CH3	4.01 (3H, s, OCH3)	C-8

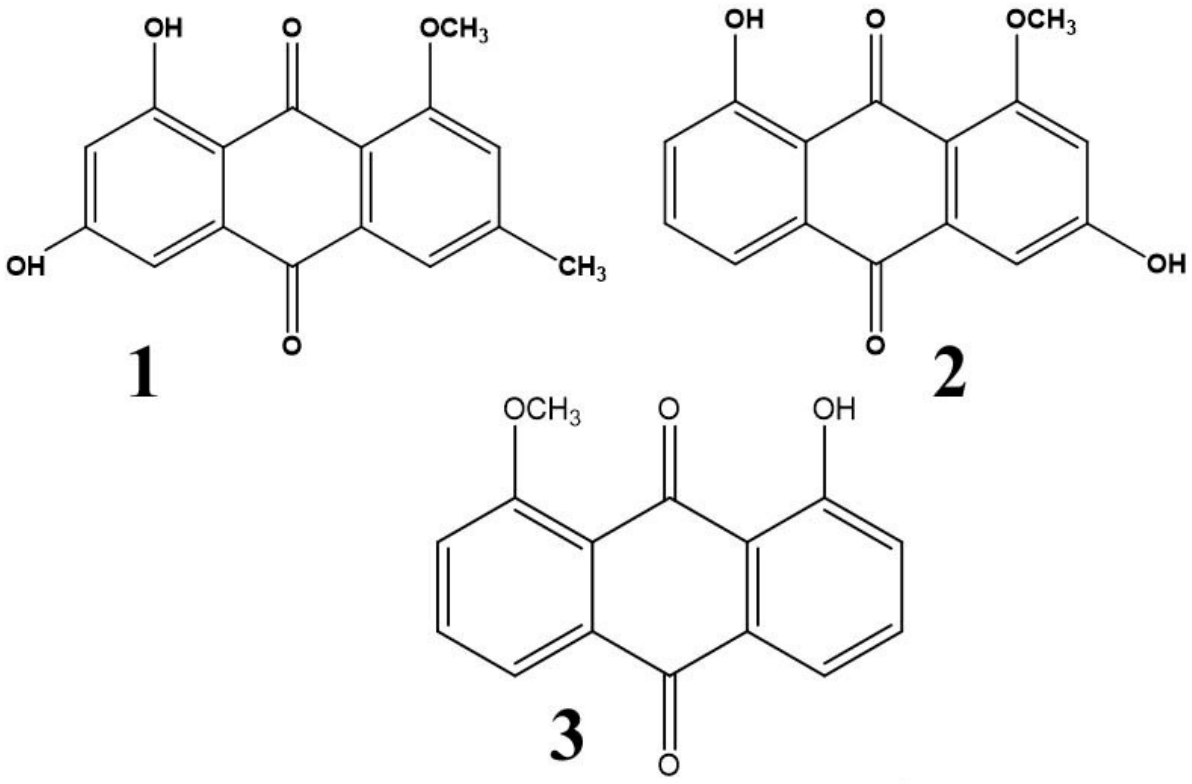

Figure 1: 2D structures of anthraquinone compounds

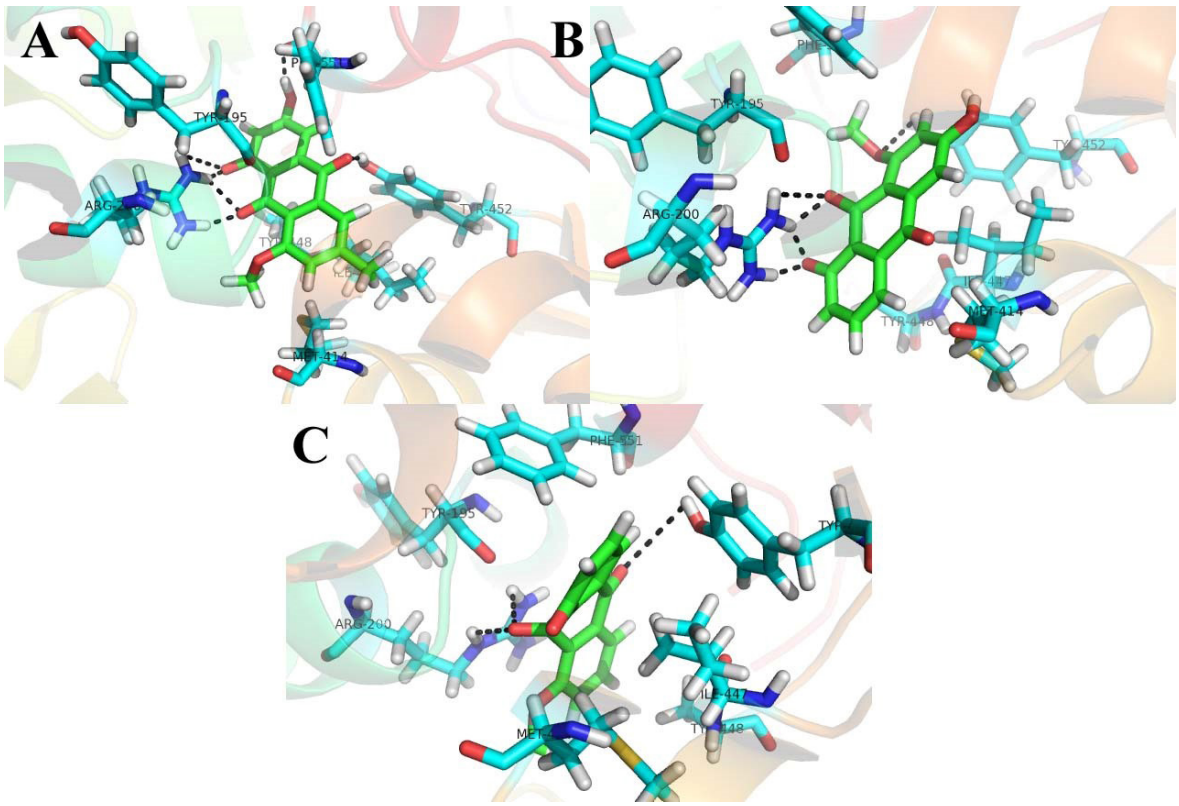

Figure 2: 2 (A-C)shows the binding interaction of the compounds (1-3) with the target receptor.

in table 5. In this research study, we have identified anthraquinones, the novel inhibitors of the HCV NS5BRdRp activity in vitro. From previous studies, it was proved that the natural anthraquinone composites have also the anti-tumor activity [23].Among these compounds, compound 3 showed maximum NS5B RdRp inhibition that is 27.1% but it is inactive in anti HCV activityat fifty μM compound concentration and has no inhibition which is against according to the docking results.

We further examined the cell viability and anti-HCV potential of the isolated compounds employing Huh7/Rep-Feo1b cells which were driven with HCV subgenomic replicons [19,20].In this assay the cell viability of compound 1 and 3 are same and is more than compound 2 .Compound 1 has more anti HCV activity i-e 11% which is more than compound 2 activity and compound 3 has no activity but it was more active in NS5B RdRp inhibition at 50μM concentration.

CONCLUSION

Our research studies focused on the molecular docking and in vitro HCV NS5BRdRp activity. Molecular docking was done by using MOE software and HCV NS5B protein docked with the active residues of the anthraquinone compounds and proved that these compounds were active compounds and followed the criterion of the Lipinski, s rule of five. These composites were then treated with the HCV NS5B RdRp in vitro, but their activity was not exactly like that we obtained by in silico approach. Among these compounds, compound 3 is not acting as anti-HCV agent, but has good activity in the NS5B RdRp inhibition and good cell viability. Furthermore, we can also predict new anthraquinone derivatives by in silico approach and as well as can also find out their activities by in vitro process in the lab.

REFERENCES

1. Lavanchy D,Evolving Epidemiology of Epatitis C Virus. Clin. Microbiol.Infect 2011; 17:107-115.
2. SeeffLB.Natural History of Chronic Hepatitis C. Hepatology2002;36:S35-S46.
3. Fauvelle C, Lepiller Q, Felmlee DJ, Fofana I, Habersetzer F, Stoll-Keller F, Baumert TF,Fafi-Kremer S.Hepatitis

C virus vaccines - Progress and perspectives.Microbial Pathogenesis2013;58:66-72.

4. Pawlotsky JM, Chevaliez S, McHutchison JG. The Hepatitis C Virus Life Cycle as a Target for New Antiviral Therapies. Gastroenterology 2007; 132:1979–1998.
5. Behrens SE, Tomei L, De Francesco R.Identification and properties of the RNAdependent RNA Polymerase of Hepatitis C Virus.EMBO J. 1996;15:12-22.
6. Barreca ML, Iraci N,Manfroni G, Cecchetti V. AllostericInhibitionofthe Hepatitis CVirusNS5B Polymerase:in-Silico Strategies for Drug Discoveryand Development. FutureMed.Chem.2011;3:1027-1055.b)Sofia MJ,Chang W, Furman PA, Mosley RT, Ross BS.Nucleoside, Nucleotide, and Non-Nucleoside Inhibitors of HepatitisCVirusNS-5BRNA-DependentRNA-Polymerase.J.Med. Chem. 2012;55:2481-2531.
7. Garber L, Welzel TM, Zeuzem,S.NewTherapeuticStrategiesinHCV:Polymerase Inhibitors. Liver Int.2013;33:85-92.
8. Manfroni G, Paeshuyse J, Massari S, Zanoli S, Gatto B, Maga G, Tabarrini O, Cecchetti V, Fravolini A, NeytsJ. Inhibition of Subgenomic Hepatitis C Virus RNA Replication by Acridone Derivatives: Identification of an NS3 Helicase Inhibitor. J. Med . Chm. 2009;52:3354-3365. **b)** Manfroni G, Meschini F, Barreca ML, Leyssen P, Samuele A,Iraci N,Sabatini S,Massari S,Maga G, Neyts J, CecchettiV.Pyridobenzothiazole Derivatives as New Chemotype Targeting the HCV NS5BPolymerase.Bioorg.Med.Chem. 2012;20:866-876.**c)**Barreca ML, Manfroni G, Leyssen P, Winquist J, Kaushik-Basu N, Paeshuyse J, Krishnan R, Iraci N, Sabatini S, Tabarrini O, Basu A, Danielson UH, Neyts J,Cecchetti V.Structure-basedDiscoveryofPyrazolobenzothiazineDerivativesas Inhibitorsof Hepatitis C Virus Replication. J. Med. Chem. 2013; 56:2270-2282.
9. Tiwari RD and Singh J. Planta Med.1978 ;34:319.
10. Tiwari RD and Singh J. Phytochemistry1978;18:347.
11. Chopra RN, Chopra IC, Hande KL, and Kapur LD. Indigenous Drugs of India(U.N.Dhur&Sons, Ltd, Calutta, India 1958; p. 499.
12. Bhakta T, Mukherjee PK, Saha K, Pal M, andSaha BP. Hypoglycemic activity of Cassia fistula Linn. (Leguminosae) leaf (Methanol extract) in alloxan-induced diabetic rats.J Ethnobot. 1997;9:35-39.
13. Bhakta T, Mukherjee PK, Saha K, Pal M, andSaha BP and Mandal SC.J. Herbs Spices Med. Plants1999;6: 67 .
14. El-Saadany SS, El-Massry RA, Labib SM and Sitohy MZ.Mol. Nutr. Food Res.1991;35:807.
15. Samy RP, Ignacimuthu S, and Sen A. J. Ethnopharmacol. 1998;62:173.
16. Esposito AM, Diaz A, De Gracia I, De Tello R, and Gupta MP. Rev. Med. Panama 1991;16:39.
17. Yadva RN and Verma V. J. Asian Nat. Prod. Res 2003;5:57.
18. Khair Zaman, Mohammad Rafiullah Khan,Mumtaz Ali & Derek J. Maitland. New anthraquinone dimer from the root bark of Cassia artemisioides (Gaudich.Ex. DC) Randell. Journal of Asian Natural Products Research 2011;13(1): 62-67.
19. Kim K, Kim KH, Kim HY, Cho HK, Sakamoto N, Cheong J. Curcumin inhibits hepatitis C virus replication via suppressing the Akt-SREBP-1 pathway. FEBS Lett. 2010;584:707−712.
20. Nichols DB, Fournet G,Gurukumar KR, Basu A, Lee JC, Sakamoto N, Kozielski F, Musmuca I, Joseph B, Ragno R, Kaushik-BasuN. Inhibition of hepatitis C virus NS5B polymerase by S-trityl-L-cysteine derivatives.Eur. J. Med. Chem. 2012;49:191−9.
21. Kaushik-Basu N, Bopda-Waffo A, Talele TT, Basu A, Costa PR, da Silva AJ,Sarafianos SG, Noel F. Identification and characterization of coumestans as novel HCV NS5B polymerase inhibitors. Nucleic Acids Res. 2008;36:1482−96.
22. Chen Y,Bopda-Waffo A, Basu A, Krishnan R, Silberstein E, Taylor DR, Talele TT, Arora P, Kaushik-BasuN.Characterization of aurintricarboxylic acid as a potent hepatitis C virus replicaseinhibitor.Antiviral Chem. Chemother 2009;20:19−36.
23. Esmat AY, Tomasetto C, Rio MC. Cytotoxicity of a Natural Anthraquinone (Aloin) Against Human Breast Cancer Cell Lines with and without ErbB-2-Topoisomerase IIα Coamplification. Cancer Biology & Therapy 2006;5(1):97-103.

CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE NIL