

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

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### ABSTRACT

A confirmed target for drug development, the hepatitis C virus (HCV) NS5B RNA-dependent RNA polymerase (RdRp) is a crucial and unique part of the HCV RNA replication machinery. We describe the identification of compounds with anti-HCV and NS5B RdRp inhibitory activity in this research investigation. Here, we investigated the activity of anthraquinone compounds using both an in vitro and an in silico method. These substances were discovered to be active with strong anti-HCV and NS5B RdRp inhibitory properties. Our findings also suggest that anthraquinones may be optimized and that novel anthraquinone derivatives with enhanced cell and enzyme-based activity may be produced.

**Keywords:** RNA-dependent RNA polymerase, anthraquinones, molecular docking, hepatitis C virus, and NS5B inhibitors.

### INTRODUCTION

Almost 170 million individuals worldwide are infected with the hepatitis C virus (HCV), which is the cause of chronic hepatitis C<sup>1</sup>. Major liver illnesses such as cirrhosis and hepatocellular carcinoma are brought on by chronic HCV infection and ultimately need liver transplantation<sup>1,2</sup>. There isn't yet a vaccination available<sup>3</sup>.

HCV is a tiny, enclosed, single-stranded RNA-(+) virus member of the Flaviviridae family's genus Hepacivirus<sup>4</sup>. Its RNA genome codes for a polyprotein precursor with over 3000 amino acids. Viral and cellular proteases break this precursor down into 4 structural

(S) and 6 non-structural (N.S.) proteins<sup>4</sup>. NS5B is a crucial enzyme for HCV replication. It has an RNA-dependent RNA polymerase (RdRp) activity, making it a desirable target for the creation of targeted antiviral drugs among the non-structural (N.S.) proteins<sup>5</sup>. NS5B inhibitors are categorized as either non-nucleoside inhibitors (NNIs) that engage with one of the five known allosteric sites or nucleoside inhibitors (N.I.s) that interact with the active site<sup>6</sup>. As of right now, the allosteric sites are categorized as follows: Thumb sites I (TSI), II (TSII), III (PSI), IV (PSII), and V (PSI) are listed in that order<sup>6a</sup>. It is necessary to identify novel anti-HCV medications that target NS5B. Research teams and pharmaceutical firms are working very hard to identify selective and effective inhibitors<sup>6b</sup> that can be combined with other DAAs to provide a pegIFN- $\alpha$ /RBV-free treatment. Nevertheless, although various NNIs and N.I.s have been documented in the literature, the FDA has not yet authorized effective medications against NS5B polymerase. Memeticitabine (RG7128) and sofosbuvir (PSI-7977) are the most advanced medication candidates for the NS5B NIs in clinical studies, whereas filibuvir (PF-00868554) and

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setrobuvir (ANA598) are the most advanced NNIs<sup>7</sup>.

A research team has been working on finding HCV drugs for the last several years, and they have identified new chemotypes with anti-NS5B and anti-HCV action<sup>8</sup>. Anthraquinones<sup>10</sup> and flavonoids<sup>9</sup> are mostly found in cassia species, and several of these compounds have therapeutic benefits<sup>11</sup>, including hepatoprotective<sup>13</sup>, hypocholesterolemic<sup>14</sup>, antibacterial<sup>15</sup>, and anti-diabetic<sup>16</sup> qualities.

Cassia fruits are used in Ayurvedic medicine to treat rheumatism, throat problems, inflammation, asthma, leprosy, syphilis, and tuberculous glands<sup>17</sup>. On the other hand, chest ailments, inflammation, and asthma are treated using Cassia roots. Here, we describe the anthraquinone derivatives' isolation, anti-NS5B activity, and anti-HCV activity. We used molecular docking to anticipate the anthraquinone compounds' binding location on NS5B polymerase. This is a major step toward developing these compounds as this work represents NS5B inhibitors.

## MATERIALS AND METHODS:

In March 2013, Cassia artemisinin flowers were harvested from Peshawar. After washing to remove dust, the flowers were left to air dry for a few days. After that, the flowers were pulverized into a powder. The chosen plant's powdered flowers were extracted using methanol in a soxhlet system. The selected plant extracts were concentrated under a vacuum in a rotary evaporator to produce the crude leftovers. Column chromatography was applied to the methanol fraction over a silica gel.

Different fractions were acquired<sup>18</sup>. After one of the fractions underwent column chromatography, three particles were obtained. These fractions were then further purified using preparative TLC, which generated compound 1 (2,6,8-Trihydroxy-1-methoxy-3-methylanthraquinone) using hexane: ethyl acetate as the eluting solvent system. The obtained chemical weighed 0.85 milligrams. Acetone has a molecular weight of 300<sup>26</sup>. and was accepted as yellow needles. 291-293 °C is its mp. The molecular formula, C<sub>16</sub>H<sub>12</sub>O<sub>6</sub>, was inferred from the <sup>13</sup>C NMR and the E.I. mass spectrum at m/z 300 (300 for C<sub>16</sub>H<sub>12</sub>O<sub>6</sub>).

According to DEP experiments, the <sup>13</sup>C NMR spectra of the chemical 2,6,8-Trihydroxy-1-methoxy-3-methylanthraquinone verified the existence of sixteen carbon atoms with one methyl group, one

methoxy group, three methane, and ten quaternary carbons. As per the results of the HMQC and HMBC studies, all assignments were completed (Table 1). Compounds 2 and 3 are extracted and isolated from *C. aretemisioides* root barks:

The *C. aretemisioides* root barks were ground into a powder. Methanol was added, the mixture was agitated vigorously, and the resulting extract was filtered out to remove the soxhlet-extracted root bark extract. The solvent was then removed using a rotary evaporator operating under concentrated pressure. Using column chromatography on a silica gel, the methanol fraction was eluted to produce a variety of fractions. Compounds 1, 6-dihydroxy-8-methoxy-3-methylanthraquinone (2), and 1-hydroxy-8-methoxy-3-methylanthraquinone (3) were obtained by subjecting Fraction 1 to CC and preparative TLC<sup>18</sup>. 1,6-dihydroxy-8-methoxy-3-methyl anthraquinone:

The substance is acetone, yellow needles, with a melting point of 300–302 °C. This chemical has the molecular formula C<sub>16</sub>H<sub>12</sub>O<sub>5</sub> and the molecular weight 284.26. U.V. (MeOH) λ max (log ε); 225 (4.53) 247 (4.09), 286 (4.43) nm I.R. (KBr) max; 3427 (O.H.), 2921, 1680, 1589, 1484, 1441, 1261 cm<sup>-1</sup> EI-MS; m/z 284 [M<sup>+</sup>] (calculated as 284 for C<sub>16</sub>H<sub>12</sub>O<sub>5</sub>)- %; 284 (100), 266 (49.09), 238 (75.63), 226 (8.89), 197 (17.89), 149 (12.64); EI-MS m/z(rel. int.) (2). 1-Hydroxy-8-methoxy-3-methylanthraquinone:

It weighs 0.85 mg and has a molecular weight of 268.26. Yellow needles represent Acetone. Point of melting: 195–196 °C. The formula for a molecule: C<sub>16</sub>H<sub>12</sub>O<sub>4</sub>. 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<sup>-1</sup>

Table 3. HMBC Correlations with <sup>1</sup>H (600 MHz) and <sup>13</sup>C (150 MHz) NMR\* data of 1-hydroxy-8-methoxy-3-methyl anthraquinone (3) \*<sup>1</sup>H NMR in acetone-d<sub>6</sub> and <sup>13</sup>C NMR in methanol-d<sub>4</sub>

## Molecular docking:

Before molecular docking, ligands and proteins were produced using MOE2010.11. Chembio Draw Ultra 12 was used to generate the 2D structures of the compounds, and each combination was then saved as a mol file. Mol files containing the compounds are opened in MOE, and the MMFF94x force field is used

to reduce the energy to 0.05 gradients. A database was established that included all of the compounds' 3D structures. HCV NS5B's 3D coordinates were obtained from the Protein Databank (Pdb id:5twn). Because most macromolecular crystal structures have low resolution and lack hydrogen coordinates, protonation was carried out using the MOE software's Protonate 3D Option before docking. After protonation, the MMFF94xforce field minimises energy up to a 0.05 gradient. Using the Triangle Matcher docking approach, every ligand was placed into the pocket containing the active residues of HCV NS5B. Every complex underwent interaction analysis, and three-dimensional images were captured.

**Anti-HCV Activity and Cytotoxicity in Cell-Based Assays:** Using *Il*-based assays, the anti-HCV efficacy of drugs against sub-genomic HCV genotype 1b (RNAReplicon) was assessed in Huh7/Rep-Feo1b reproducing cells, which carry the firefly luciferase reporter<sup>19</sup>. The anti-HCV drugs were evaluated consistently with the reports<sup>19,20</sup>.

In summary, the cells were cultured in 96-well plates and treated with a chemical at a concentration of 50 $\mu$ M, while a control group received an equivalent volume of DMSO (with the final DMSO content being kept at 0.5%). Using a luciferase assay kit (Promega), the relative amounts of luciferase signals in compound-treated cells vs DMSO controls were used to determine the inhibitory impact of drugs on HCV replication after 48 hours of treatment.

Huh7/Rep-Feo1b cells were used to test the drugs' effects on cell viability in an environment similar to that of antiviral experiments. In short, for 48 hours, chemicals were applied to cells plated in 96-well plates, or DMSO was used as a control. CellTiter 96 Aqueous One solution was used to evaluate cell viability in compliance with the manufacturer's procedure (Promega, U.S.).

### HCV NS5B RdRp Inhibition Assay:

Using Ni-NTA column chromatography, recombinant HCV NS5B with an N-terminal hexa-histidine tag and a deletion of the C-terminus (21 hydrophobic amino acids) was isolated<sup>21,22</sup>. Using poly rA-U12 template primer and [ $\alpha$ -<sup>32</sup>P] UTP as the divalent cation, HCV NS5B was inhibited in the presence of DMSO or the drug, as previously reported<sup>21</sup>. After one hour of incubation at 30 °C, the reactions were stopped

by adding an ice-cold 5% TCA solution. The immature radiolabeled RNA was spotted on GF-B filters, precipitated with 5% TCA, and weighed in a liquid scintillation counter. NS5B activity was measured in this control while the compounds were present, and it was set to 100% when DMSO was present. Anti-HCV compounds demonstrating  $\geq$ 50% inhibition at 50 $\mu$ M concentrations were evaluated for 50% inhibition (IC<sub>50</sub>). In two separate trials, the IC<sub>50</sub> values of the compounds were calculated by extrapolating the dose-response curves at 8–10 concentrations of the serially diluted compounds in parallel (GraphPad prism 3.03).

## RESULTS AND DISCUSSION

### Molecular docking:

Using the docking methodology, MOE software did the molecular docking of the chemicals with the NS5B protein pocket that was identified from cassia plants. MOE software aims to verify that chemicals, or inhibitors, bind to the target protein, the NS5B protein. The MOE software's protocol was followed to complete the docking process. Fifteen conformations may be stored for each ligand using the MOE default values.

Since each docked molecule has a unique conformation, retaining the top-ranked conformations in a distinct database was permitted. Using the LigX inMOE, we could see how each chemical interacted with the target protein. The compounds that interacted with the target protein were Tyr195, Arg200, Met414, Ile447, Tyr448, Tyr452, and Phe551. These compounds were shown to interact with the binding pocket of the NS5B protein. Figure 2(A–C) below describes each compound's interactions with the protein. Binding interactions of the compound with the NS5B protein:

All compounds isolated from cassia plants are members of the anthraquinone class. These substances have shown positive interactions with the intended protein. The most active combination 1, according to the molecular docking analysis, generated six hydrogen connections with Tyr 195, Arg200, Tyr452, and Phe551, one anionic contact with Tyr448 and one hydrophobic linkage with Met414. Compound 1 has a binding energy of -43.17 Kcal/mol, a binding affinity of -7.60 Kcal/mol, and a docking score of -14.5721. Six polar contacts were established by the target protein residues Tyr 195, Arg200, Tyr452, and

Phe551 with the oxygen atoms of the chemical 1's 5,7-dihydroxynaphthalene-1,4-dione moiety. The compound's 1-methoxy-3-methylbenzene moiety exhibited hydrophobic and anionic interactions with Met 414 and Tyr 448 (Figure 2A). Compound 2 has a binding energy of -38.14 Kcal/mol, a binding affinity of -6.96 Kcal/mol, and a docking score value of -13.6515. Compound 2's 3-methoxy phenol's benzene ring exhibited polar contact with Tyr 452. The Arg200 residue of compound 2 established hydrogen bonds with the carbonyl oxygen atoms and O.H. group of the 5-hydroxynaphthalene-1,4-dione moiety. Hydrophobic interactions between Tyr 448, Met 414, and compound 2 were detected (Figure 2B). Compound 3, an NS5B protein inhibitor, interacted with the protein's binding pocket. Their binding energy was -35.23 Kcal/Mol, their binding affinity was -6.51 Kcal/Mol, and their docking score was -12.4722. Three hydrogen bonds, one hydrophobic interaction, and one amide contact, were established by this chemical with the protein's Arg200, Tyr 452, and Met 414 residues, respectively. Compound 3's carbonyl oxygen atoms and the residues Tyr 452 and Arg200 created polar interactions (Figure 2C). As seen in Table 4, all these substances are potent

NS5B inhibitors that exhibit excellent inhibition and all five of Lipinski's criteria.

**Anti-HCV Activity and Cellular Cytotoxicity.** The chemicals were extracted from natural sources; then, MOE software was used to conduct an in silico method to determine each compound's activity. We found evidence that the chemicals could bind with the target protein, HIV NS5B, and deactivate its RdRp and HCV activities. The compounds were first screened using conventional in vitro RdRp assays at 50  $\mu$ M doses to verify this assumption. The relative inhibition of NS5B RdRp activity was then computed and is shown in Table 5. Our research work has discovered anthraquinones as the unique in vitro inhibitors of HCV NS5BRdRp activity. It has been shown by earlier research that natural anthraquinone molecules also had anti-tumour properties<sup>23</sup>. Out of all the compounds, compound 3 had the highest NS5B RdRp inhibition of 27.1%; nevertheless, at fifty  $\mu$ M compound concentration, it exhibited no inhibition, indicating a negative outcome regarding docking data.

We conducted further research on the isolated compounds' cell survival and anti-HCV activity using

**Table 1: <sup>1</sup>H (600 MHz) and <sup>13</sup>C (150 MHz) NMRin (CD<sub>3</sub>)<sub>2</sub>CO and HMBC Correlations of 2,6,8-trihydroxy-1-methoxy-3-methylantraquinone (1)**

C. No.	<sup>13</sup> C NMR( $\delta$ )	<sup>1</sup> H NMR ( $\delta$ ) (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-OCH <sub>3</sub>	61.2	3.90 (3H, s, OCH <sub>3</sub> )	OCH <sub>3</sub>	C-8
3-CH <sub>3</sub>	15.74	2.34 (3H, s, CH <sub>3</sub> )	CH <sub>3</sub>	C-2, C-3, C-4

**Table 2: <sup>1</sup>H (600 MHz) and <sup>13</sup>C (150 MHz) NMR in CDCl<sub>3</sub> and HMBC Correlations of 1,6-dihydroxy-8-methoxy-3-methylanthraquinone (2)**

C. No.	<sup>13</sup> C NMR (δ)	Multiplicity	<sup>1</sup> H 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	-	CH <sub>3</sub> (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-CH <sub>3</sub>	20.52	CH <sub>3</sub>	2.39 (3H, s, CH <sub>3</sub> )	C-2, C-3, C-4
8-O CH <sub>3</sub>	55.89	CH <sub>3</sub>	3.93 (3H, s, OCH <sub>3</sub> )	C-8

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

C. No.	<sup>13</sup> C NMR(δ)	Multiplicity	<sup>1</sup> H 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-CH <sub>3</sub>
3	147.69	C	-	
4	120.26	CH	7.58, s	C-2, C-9a, 3-CH <sub>3</sub>
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-CH <sub>3</sub>	22.16	CH <sub>3</sub>	2.42 (3H, s, CH <sub>3</sub> )	C-2, C-3, C-4
8-OCH <sub>3</sub>	56.75	CH <sub>3</sub>	4.01 (3H, s, OCH <sub>3</sub> )	C-8

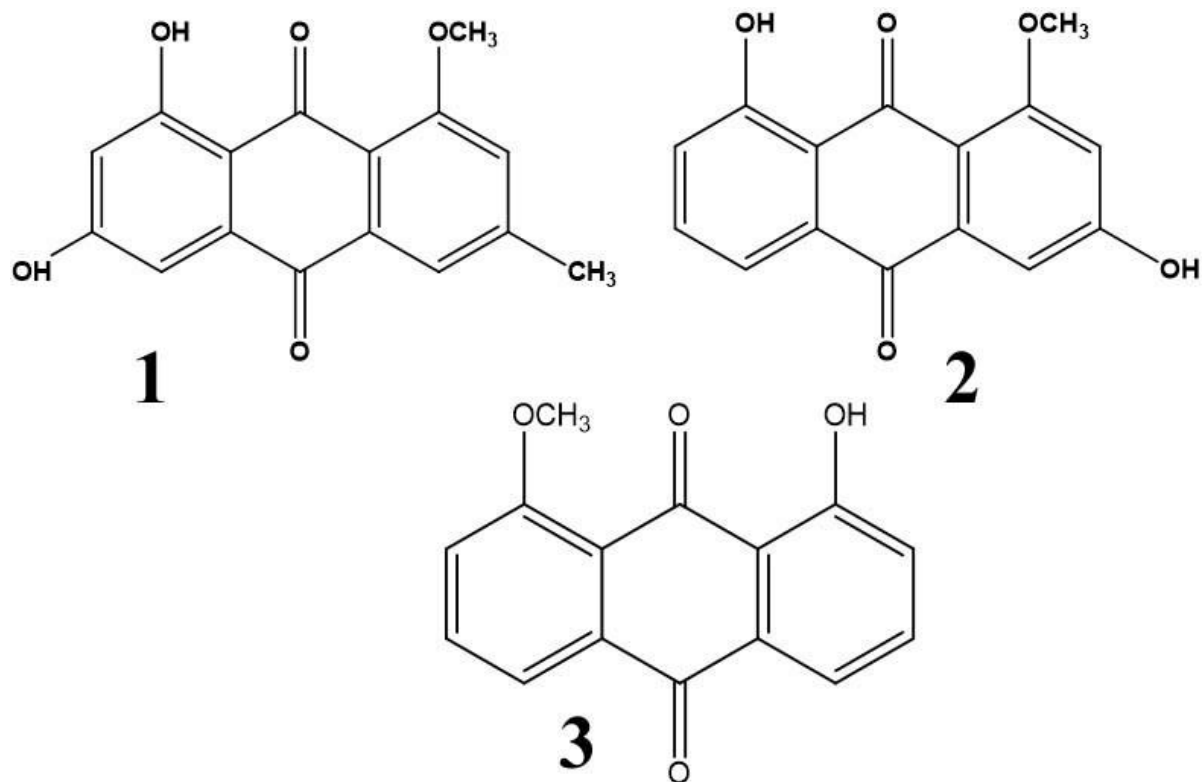


Figure 1: 2D structures of anthraquinone compounds

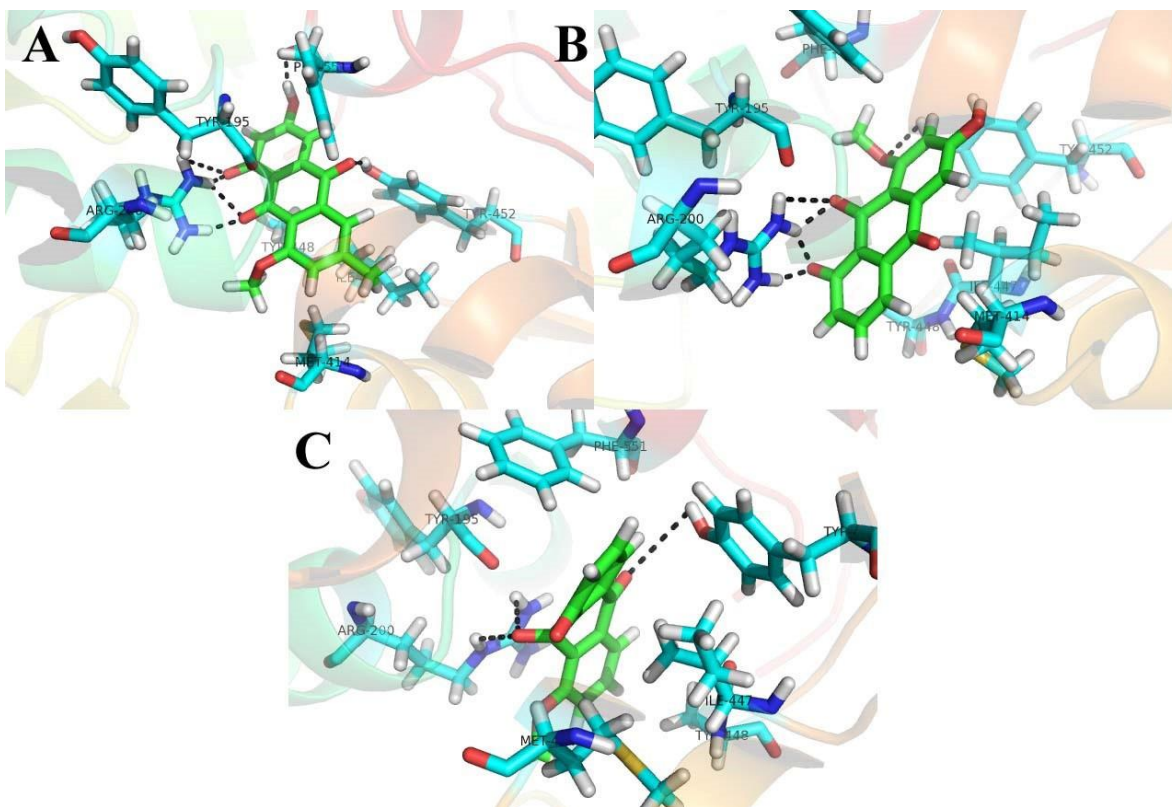


Figure 2: 2 (A-C) shows the binding interaction of the compounds (1-3) with the target receptor.

Huh7/Rep-Feo1b cells that carry the HCV subgenomic replicons in cell-based HCV reporter assays [19, 20]. Compounds 1 and 3 had the same and greater cell viability in this experiment than compound 2. Compound 3 had no action but was more active in NS5B RdRp inhibition at 50 $\mu$ M concentration. Compound 1 has stronger anti-HCV activity (11%), more than compound 2's.

## CONCLUSION

Our investigations focused on the in vitro HCV NS5BRdRp activity and molecular docking. Using MOE software, molecular docking was performed with the HCV NS5B protein docked with the active residues of the anthraquinone compounds.

This demonstrated that the compounds were active and met the requirements of Lipinski's rule of five. After being treated in vitro with HCV NS5B RdRp, these compounds' activity differed somewhat from what we had observed using an in silico method. Compound 3 is not an anti-HCV drug but exhibits excellent cell viability and strong efficacy in suppressing NS5B RdRp.

Moreover, we may use an in vitro procedure in the lab to determine the activity of novel anthraquinone derivatives and anticipate them using an in silico technique.

## REFERENCES

1. Lavanchy D, Evolving Epidemiology of Hepatitis C Virus. *Clin. Microbiol. Infect* 2011; 17:107-115.
2. Seeff LB. Natural History of Chronic Hepatitis C. *Hepatology* 2002;36:S35-S46.
3. Fauvelle C, Lepiller Q, Felmlee DJ, Fofana I, Habersetzer F, Stoll-Keller F, Baumer TF, Fafi-Kremer S. Hepatitis C virus vaccines - Progress and perspectives. *Microbial Pathogenesis* 2013;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 RNA dependent RNA Polymerase of Hepatitis C Virus. *EMBO J.* 1996;15:12-22.
6. a) Barreca ML, Iraci N, Manfroni G, Cecchetti V. Allosteric Inhibition of the Hepatitis C Virus NS5B Polymerase: in-Silico Strategies for Drug Discovery and Development. *Future Med. Chem.* 2011;3:1027-1055. b) Sofia MJ, Chang W, Furman PA, Mosley RT, Ross BS. Nucleoside, Nucleotide, and Non-Nucleoside Inhibitors of Hepatitis C Virus NS-5B RNA-Dependent RNA-Polymerase. *J. Med. Chem.* 2012;55:2481-2531.
7. Garber L, Welzel TM, Zeuzem S. New Therapeutic Strategies in HCV: Polymerase Inhibitors. *Liver Int.* 2013;33:85-92.
8. a) Manfroni G, Paeshuyse J, Massari S, Zanoli S, Gatto B, Maga G, Tabarrini O, Cecchetti V, Fravolini A, Neyts J. Inhibition of Subgenomic Hepatitis C Virus RNA Replication by Acridone Derivatives: Identification of an NS3 Helicase Inhibitor. *J. Med. Chem.* 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, Cecchetti V. Pyridobenzothiazole Derivatives as New Chemotype Targeting the HCV NS5B Polymerase. *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-based discovery of pyrazolobenzothiazine derivatives as an inhibitor of 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. *Phytochemistry* 1978;18:347.
11. Chopra RN, Chopra IC, Hande KL, and Kapur LD. *Indigenous Drugs of India* (U.N. Dhur & Sons, Ltd, Calcutta, India 1958; p. 499.
12. Bhakta T, Mukherjee PK, Saha K, Pal M, and Saha 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, Saha B.P., and Mandal SC. *J. Herbs Spices Med. Plants* 1999;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. Yadav 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. D.C.) 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-Basu N. 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-Basu N. Characterization of aurintricarboxylic acid as a potent hepatitis C virus replicase inhibitor. *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 II $\alpha$  Coamplification. *Cancer Biology & Therapy* 2006;5(1):97-103.

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