In-silico study of anthraquinone derivatives as probable inhibitors of COVID-19

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Abstract

Six previously known anthraquinones with amino derivatives and synthesized new triazene (1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione) were evaluated *in-silico* as inhibitors of COVID-19 main protease. The structures of the synthesized compound were confirmed by IR, ^{1}H, ^{13}C NMR, and LC-MS spectral studies. Chemo-informatics study showed that the compound obeyed Lipinski's rule, PASS, Swiss ADME. Computational docking analysis was performed using PyRx, and AutoDock Vina options based on scoring functions. *In-silico* molecular docking study results demonstrated Greater binding energy and affinity to the active pocket of the N3 binding site of the Coronavirus primary protease.

Keywords. Anthraquinones, triazene, molecular docking, RNA polymerase, protease Covid-19.

1. INTRODUCTION

Coronavirus disease stated December 2019 in Wuhan, China [1]. The epidemic was isolated to a new type strain of coronavirus, the name of which world health organization (WHO) 2019-nCoV or COVID- 19, later renamed the international committee on virus taxonomy to SARS-CoV-2, which was confirmed as the pathogenic source of virus severe acute respiratory syndrome [2]. Coronaviruses contain a positive-sense with single-stranded RNA genome [3]. The genome size of coronaviruses ranges from 26.4 to 31.7 kilobases genome size is one of significant between RNA viruses. The genome has 5-methylated cover and 3-polyadenylated tail. A genome group of four encodesfor a coronavirus is 5′-leader-UTR-replicas (ORF1ab) -spike glycoprotein (S), little envelope protein (E)-membrane, matrix glycoprotein (M), nucleocapsid protein (N), 3′UTR-poly (A) tail [4]. Recent studies suggested that human COVID-19 is a valid target for antiviral drug development based on this aspect, a structure-based virtual screening approach to target the nucleocapsid protein (NP) are made to show goodchemical starting points for medicinal chemistry [5].

Anthraquinones compounds are greatest known for theirvarious biological activities [6], however they have alsosuccessfully treated many diseases such as anticancer [7], and antimalarial [8]. At present, anthraquinone derivatives are extensively studied as therapeutic agents against COVID-19, specifically acting against 3CLpro and PLpro proteases [9]. In this researchpaper, anthraquinones compounds has been evaluated for their potential anti-COVID-19 activity and exploredthrough *in-silico* molecular docking studies.

2. MATERIALS AND METHODS

Chemistry

General

All chemicals were obtained from commercial sources and were used without further purification. The melting points were measured in open capillary tubes. The ^{1}H NMR spectra were recorded on a Varian 400 spectrometer at 400 MHz using DMSO-d6 as solvent unless otherwise stated. The mass spectra were run on an Agilent 1100 Series high-performance liquid chromatograph equipped with a diode array detector and an Agilent mass-selective detector with the possibility of rapidly switching between positive and negative ionization modes. The progress of reactions was monitored by TLC on DC-Fertigfolien Alugram Xtra Sil

G/UV254 silica gel plates (Germany).

1-(Diazyn-1-ium-l-yl)-4-[(2-hydroxyethyl)-amino]anthracene-9,10-dione (2). A 50-mL round-bottom flask equipped with a magnetic stirrer was charged with a solution of anthraquinone **1** (0.1 mmol) in 1 M aqueous HCl (5.0 mL). The solution was cooled to 0–5°C in an ice bath, a solution of sodium nitrite (0.2 mmol) in 0.5 mL of distilled water was added dropwise, maintaining the temperature at 0–5°C, and the mixture was stirred for 5 min at that temperature.

Triazene derivative 3 (general procedure). The mixture containing diazonium salt **2** was allowed to warm up to room temperature, a solution of the corresponding amine (0.15 mmol) in 5 mL of ethanol was added, and the mixture was stirred for ~30 s at room temperature. The progress of the reaction was monitored by change of the color of the reaction mix-ture (the color changed from blue to red after diazotization, and the final product was purple), as well as by RP-TLC using acetone–water (2:3) as eluent. The product was purified by reversed phase column chromatography (RP-18) using water as eluent, to yellow crystalline solid, 61 % yield; IR (KBr, ν_{max}, cm^{-1}): 3488, 3263, 2949, 2843, 1693, 1668, 1598, 1589, 1533, 1431. ^{1}H NMR (400 MHz, DMSO-d6): δ 2.1 s (4H, CH_2), 3.2 t (4H, CH_2), 7.6-8.5 m (3H, H_{arom}), 11.3 s (3H, OH). ^{13}C NMR (100 MHz, DMSO-d6) δ 122.58, 124.42, 127.11, 127.21, 127.40, 131.71, 133.22, 134.83, 135.58, 136.29, 140.89, 152.98 (C_{ar}); 173.58 (C-Br); 180.82, 181.09 (C=O). LC-MS: m/z 588.0 $[M+H]^+$.

Pharmacological/biological assays
ADME analysis

The success of a drug entrant is resolute not only by its most readily useful potential, but in addition to a satisfactory analysis that is ADME that predict of some important capacity to properties *in-silico,* and it is valuable for analysis regarding the excellent qualities of the molecules. Which evaluates necessary Lipinski's rule the important pharmacokinetic parameters such as for instance ADME. The rule is helpful in drug design and development of a potential drug molecule [10].

ADME analysis was carried out using the SWISS ADME and molinsperation predictor for the current investigation [11]. This web servers as a tool to evaluate the ADMET properties, such as molecular weight< 500, number of < 10 Rotatable bonds, hydrogen bond acceptor, hydrogen bond donor (HBDs), molar refractivity, topological polar surface area (TPSA), water solubility (logS), blood brain-barrier, skin permeability (logKp), synthetic accessibility score (SA), percentage absorption, pharmacokinetics, drug-lead likeness and medicinal chemistry friendliness properties of drug molecules. The lipophilicity of molecules by integrating analysis results obtained from several log P prediction programs such as iLOGP, XLOGP3, WLOGP, MLOGP and SILICOS-IT. A portion of lipophilicity of a molecule is the log of the ratio of the concentration of a drug substance in two solvents in a unionized form, lower the log P value the stronger the lipophilicity is good. The aqueous solubility Log S of a compound are important of absorption and distribution properties, low water solubility often leads to bad absorption and therefore general aim is to avoid poorly soluble compounds. The distribution of log S between -1 and -4 will be improved for better absorption and distribution of drugs in the form [12].

Molecular docking
Molecular docking for the total most part used approach in structure-based drug design. The synthesized compound of ligand was drawn with Chem Draw Ultra version 16.0 (Cambridge Software) followed by resulting molecular mechanics (MM2) energy minimization of ligands using ChemBio-3D Ultra version 12.0 with GAMESS Interface by assuring connection error in the bonds. These energy- minimized ligands (structures) MOL, SDF format of that ligands had been converted to mol2 file using open babel and Discovery studio tool and ligand preparation was done using the Chimera software ended up being used in molecular docking study [13]. Crystal structure of 3CLpro-2 (PDB code: 6LU7) [14], was obtained through the Protein Data Bank (http://www.pdb.org) and any heteroatoms, water molecules were eliminated for molecular docking studies. Molecular docking was performed utilizing Autodock Tools 1.5.4 package (http://mgltools.scripps.edu) [15], and Autodock Vina (version 4.2 docking programs) and SAMSON (extended docking programs) to grasp the drug molecule interaction with protein, the potential binding mode and energy and analysis the binding affinity of COVID-19, molecular docking analysis ended up being carried out making use of Autodock 4.2.18. Autodock Vina Wizard approach. The grid box parameters values in VINA search space (X = 10.711, Y = 12.411 and Z = 68.83) had been adjusted using the default

exhaustiveness value = 8 to maximize the binding conformational analysis. We have the adjusted sufficient grid box size on binding pocket residues to allowing the ligand to move freelyinto the search space [16]. The synthesized ligand was docked individually against the target protein (6LU7).In docked complexes, the ligand conformational poses were keenly observed to obtain the useful docking results. The generated docked complexes were evaluated based on the lowest binding energy (kcal/mol) values and structure activity relationship (SAR) analyses the clear presence of hydrogen bond, hydrophobic interaction between ligand compound and good control to each focused-on the receptor. The 3D and 2D graphical depictions of all the docked complexes were accomplished by UCSF Chimera 1.10.1, Discovery Studio (2.1.0), PyMOL software and (https://proteins.plus/) online web server [17].

The torsions of this ligand were groups of detecting the roots in Autodock Vina 1.1.2. Ligand preparation had been finished with adding Gasteiger charges, polar hydrogen. Water molecules and ligandwere removed. Protein and grid preparation were done Autodock tools and auto dock Vina 1.1.2 was utilized to perform molecular docking. The results ofdocking by using AutoDock4 were first converted to *.pdbqt. Docking was done with a Lamarckian genetic algorithm and default parameters. The grid box size was set at 40 Å for x, y and z respectively, and the grid center was set to -11.183, 10.406 and 68.139 for x, y and z respectively, which covered all the amino acid. Docking software AutoDock 4.2 Programsupplied with AutoGrid 4.0 and AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.514 angstroms. The Lamarckian GeneticAlgorithm (LGA) was chosen to search for the best conformers. The first ten top-ranked docking poses were saved for each docking run. To approve the molecular docking protocol, the corresponding reference ligands were initially docked into the crystal structure. The Ramachandran plot and characteristics were gotten to from PDB [18, 19].

3. RESULTS AND DISCUSSION

Chemistry

Synthesis of 1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione **3**

The planned triazene compound was synthesized through two step, aminoanthraquinone **1** was subjected to diazotization with sodium nitrite in aqueous medium in the presence of HCl at 0–5°C [20]. The subsequent azo coupling of diazonium salts **2** with secondary amine afforded triazene **3** (Scheme 1). After normal aqueous workup, thecondensed residue was purified by silica-gel columnchromatography to give the light-yellow needlecrystal solid as 61% yields.

The prominent absorption bands in IR spectrum appeared at 3488 cm^{-1} which is due to the (-OH stretching), 3263 cm^{-1} (N=H stretching), 2949 cm^{-1} (-CH_2-stretching), 1589 cm^{-1} (C=H of aromatic ring), 1668 (C=C stretching of aromatic ring), 1693cm^{-1} due to stretching frequency of ketone functional group respectively, bond at 1598 cm^{-1} is due to C-N group further confirms the structure.

In ^{1}H NMR spectrum of **3** showed a downfield board singlet at δ 11.3 is due to -OH protons. Aromatic protons appeared as multiplet at δ 7.6-8.5, are due to anthraquinone ring, ^{13}C NMR spectra unambiguously matched with the structure of their corresponding 1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione **3**.

Scheme 1: Synthetic pathway of 1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione

4-Substituted derivatives based on bromaminic acid were synthesized and described by us earlier

(Scheme 2) [21, 22].

Scheme 2: Anthraquinone compound

Pharmacology

Biological activity spectrum PASS analysis
The synthesized compounds were determined by online server of PASS. In the pass analysis of compounds **3-9** the Pa showed a (0.3-0.6) value for Antiviral activities (Picornavirus) refer to http://www.way2drug.com/PASSOnline/predict.php.

ADME Analysis
The predicted chemo-informatic properties had been evaluated by computational tool-aided *in-silico* studies demonstrably suggesting that the compounds had drug-like prospect properties. It absolutely was interesting to see that the results associated with the SWISS ADME and Molinspiration predictor values of log P, molar refractivity with the total polar surface area in these molecules were in excellent agreement with the most important rules of drug-likeness. The predicted chemo-informatics properties were evaluated by computational tools. Results exposed that compounds **3-9**, has ADME parameters of H-bond acceptor and H-donor, polar surface area and molar volume (Figure 1 compound **3**, other compounds are listed in Supplementary information). Thought these compounds exhibited good hydrophilicity–lipophilicity stability therefore the predicted bioavailability. Another key property that is related to drug bioavailability. Thus, passively absorbed molecules with TPSA > 134 (only compounds 9) are thought to have low oral bioavailability, the molecular polarsurface area (PSA) is a very useful parameter for drugtransport properties, molecule is well-defined as the surface sum overall polar atoms, primarily oxygens, nitrogen and attached hydrogen atoms. This parameter has been shown to correlate very finely with human intestinal absorption, Caco-2 monolayers, permeability and blood-brain barrier penetration.

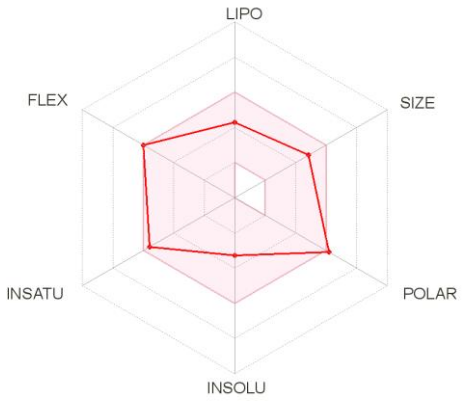

Figure 1: Bioavailability radar graph of 1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione, (pink area reflects the allowed values ofdrug likeness properties of the molecule)

Lipinski's rule RO5 of result 0 violation showed that compounds **3-9**, possess good molecular weight (g/mol), two Hydrogen Bond Accepted and Hydrogen Bond Donor values log P 0.95 - 3.4 which are significantly justified if their drug likeness behaviour were justifiable with the standard values. The results got from theSwiss ADME and Mol Inspiration search engine are listed in Table 1.

Table 1: Physicochemical descriptors and ADME parameters [a]

parameters/compound	**3**	**4**	**5**	**6**	**7**	**8**	**9**
M.W, g/mol	398.41	360.38	374.41	398.39	403.41	360.38	296.32
RB	9	4	5	4	3	3	3
H-A	7	5	5	6	7	5	3
H-D	4	3	3	3	3	3	3
TPSA	134.82 A^2	134.94 A^2	134.94 A^2	148.08 A^2	147.41 A^2	134.94 A^2	92.42 A^2
MR	105.04	92.93	97.74	100.07	103.52	92.93	84.39
WlogP (lipophilicity)	0.96	3.01	3.4	3.24	1.47	3.01	1.57
ESOL logS	-3.29	-4	-4.23	-4.15	-2.21	-4.01	-3.74
BBB Permeant	No	No	No	No	No	No	No
log Kp cm^{-1}	-7.3	-6.43	-6.26	-6.87	-9.04	-6.5	-6.01
Lipinski violations	0	0	0	0	0	0	0
PAINS alerts	3	2	2	2	2	2	2
Synthesis Accessibility	3.67	3.19	3.26	3.29	3.54	3.22	2.69

[a]R bond =Rotatable bond, H-A = Hydrogen bond acceptor, H-D = hydrogen bond donor, TPSA = topological polar surface area, BBB = blood brain-barrier, log P = lipophilicity, log S = water solubility, log Kp = permeability coefficient, PAINS = pan-assay interference structure.

The boiled-egg diagram analysis shows that the compounds strong within the permissible range of standard drugs, blue dot indicates cannot be affected by the P-glycoprotein of the CNS system by P-glycoprotein, point locate in Boiled Eggs yolk is a molecule that passively permeate through the blood-brain barrier (BBB) (figures 2 compound **3**, other compounds are listed in Supplementary information). In the current study, the synthesized ligand and its complexes were initiated to be in a goodpact with the criteria and can be said to possess bioavailability.

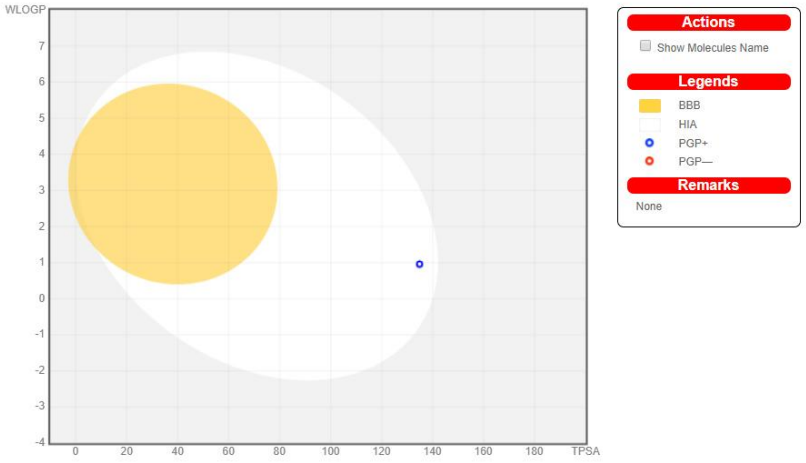

Figure 2: ADME properties of compound 1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione by graphical representation (boiled-egg)

Molecular docking

Molecular docking studies technique in medicinal chemistry has led to advances in drug discovery and design. This technique explores the binding mode and affinity of a small molecule within the binding site of the receptor target protein. The docked ligands were ranked according to their binding affinity in a ligand-receptor (figures 3 and 4). Molecular docking was performed on compound **3**, against the 3CLpro-2(6LU7) main protease to identify the ligand-protein interactions.

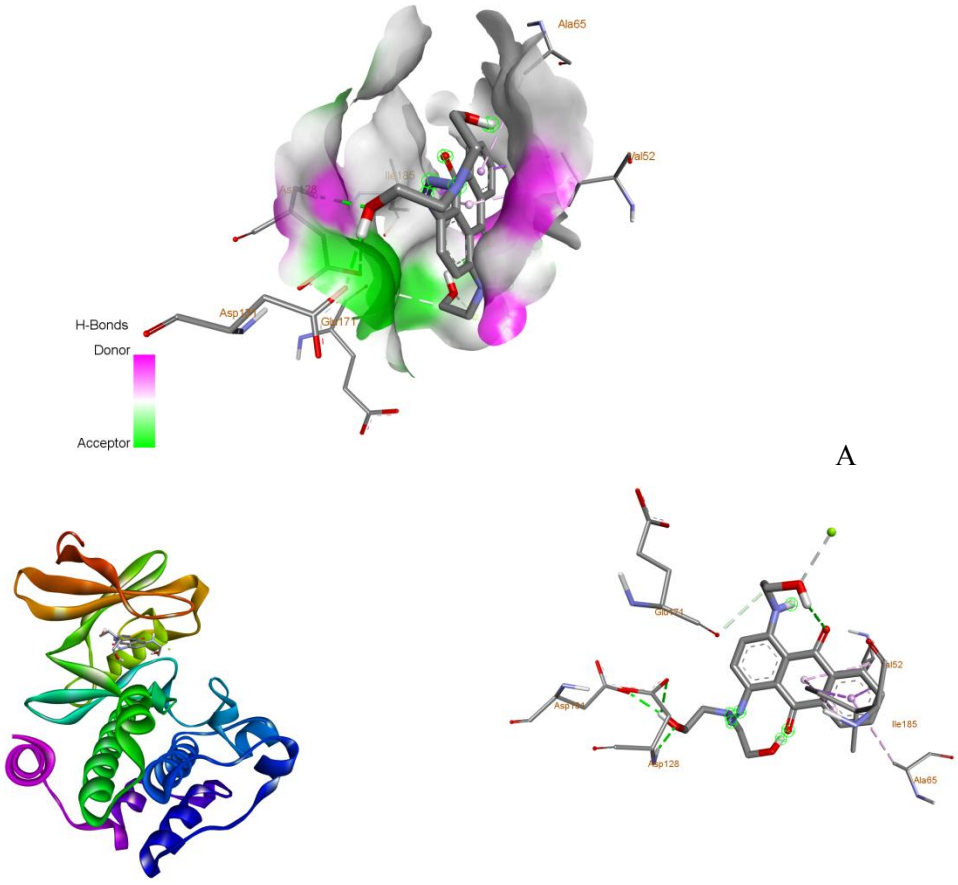

Figure 3: (A) Interaction with the ligand 4 the H-bond surfaces of the receptor COVID-19 inhibitor (B) The docked ligand triazene 4 at the same catalytic site receptor, (C) 3D Docking poses of compound 3 into the N3 binding site of the COVID-19 main protease

The docking result showed that six hydrogen bonds, one electrostatic bond and one hydrophobic interaction were observed in synthesized docked complex (figures 3 compound **3**, other compounds are listed in Supplementary information). Autodock vina According to the got results, it was decided that the docked ligand form a stable complex with COVID-19 inhibitor. For triazene **3** nine representative binding modes have been detected with binding affinities ranging from - 8.2 to -7.2 kcal/mol (table 3). Anthraquinone molecule can be considered as a lead compound for the development of new COVID-19 drugs.

Table 3: The binding affinity values of different poses of compound 1-[(1E)-3,3-bis(2-hydroxyethyl)triaz-1-en-1-ol]-4-[(2-hydroxyethyl)amino]anthracene-9,10-dione predicted by autodock Vina

Mode	**Affinity (kcal/mol)**	**Distance from the bestmode**	
		RMSD1.b.	**RMSD u.b.**
1	-8.2	0.000	0.000
2	-7.8	2.452	4.321
3	-7.5	3.412	6.230
4	-7.7	2.513	3.505
5	-7.4	3.337	6.074
6	-7.4	3.079	6.000
7	-7.4	2.665	5.229
8	-7.2	2.703	5.348
9	-7.2	2.312	2.901

4. CONCLUSION

The *in-silico* inhibition studies of synthesized compounds binding to the (6LU7) main protease showed that

the compounds, and exhibited high binding affinities (-7.8 kcal/mol by AutoDock 4 and -7.0 by AutoDock Vina). These results are, in fact, that configuration can assume in the binding site a favourable orientation. The *in-silico* ADME reporting toxicity, drug-likeness, drug scoring results, PASS analysis and in vitro anti-COVID-19 suggested that the compounds are promising leads for the development of selective, safe and potent COVID-19.

REFERENCES

1. N. Zhu, D. Zhang, W. Wang, X. Li, B. Yang, J. Song, X. Zhao, B. Huang, W. Shi, R. Lu, P. Niu, F. Zhan, X. Ma, D. Wang, W. Xu, G. Wu, G. F. Gao, W. A. Tan. Novel coronavirus from patients with pneumonia in China, N. Engl. J. Med., 2019, 382(8), 727-733.
2. A. Wu, Y. Peng, B. Huang, X. Ding, X, Wang, P. Niu, J. Meng, Z. Zhu, Z. Zhang, J. Wang, J. Sheng, L. Quan, Z. Xia, W. Tan, G. Cheng, T. Jiang. Genome Composition and Divergence of the novel coronavirus (2019-nCoV) originating in China, Cell Host. Microbe., 2020, 27(3), 325-328.
3. A. R. Fehr, S. Perlman. Coronaviruses: An overview of their replication and pathogenesis, Methods. Mol. Biol., 2015, 1282, 1-23.
4. A. D. Reddy, S. B. Suh, R. Ghaffari, N. J. Singh, D. J. Kim, J. H. Han, K. S. Kim. Bioinformatics analysis of SARS proteins and molecular dynamics simulated structure of an alpha-helix motif, Bull. Korean Chem. Soc., 2003, 24, 899-900.
5. P. Zhou, X. Yang, X. G. Wang, B. Hu, L. Zhang, W. Zhang, H. R. Si, Y. Zhu, B. Li, C. L. Huang, H. D. Chen, J. Chen, Y. Luo, H. Guo, R. D. Jiang, M. Q. Liu, Y. Chen, X. R. Shen, X. Wang, X. S. Zheng, K. Zhao, Q. J. Chen, F. Deng, L. L. Liu, B. Yan, F. X. Zhan, Y. Y. Wang, G. F. Xiao, Z. L. Shi. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin, Nature, 2020, 579, 270-273.
6. Malik, E. and Muller, C., Med. Res. Rev., 2016, vol. 36, p. 705.
7. Hussain, H., Al-Harrasi, A., Al-Rawahi, A., Green, I., Csuk, R., Ahmed, I., Shan, A., Abbas, G., Rehman, N., and Ullah, R., Expert Opin. Ther. Pat., 2015, vol. 25, p. 1053.
8. Malik, E.M., Baqi, Y., and Müller, C.E., Beilstein J. Org. Chem., 2015, vol. 11, p. 2326.
9. Khanal, P., Patil, B.M., Chand, J., and Naaz, Y., Nat. Prod. Bioprospect., 2020, vol. 10, p. 325.
10. P. G. Jamkhande, S. K. Pathan, S. J. Wadher. In silico PASS analysis and determination of antimycobacterial, antifungal, and antioxidant efficacies of maslinic acid in an extract rich in pentacyclic triterpenoids, Int. J. Mycobacteriol., 2016, 5, 417-25
11. C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug. Deliv. Rev., 2001, 46, 3-26.
12. A. Daina, O. Michielin V. Zoete. Swiss ADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep., 2017, 7, 42717.
13. S. K. Enmozhi, K. Raja, I. Sebastine, J. Joseph. Andrographolide as a potential inhibitor of SARS-CoV-2 main protease: An in-silico approach, J. Biomol. Struct. Dyn., 2020, doi: 10.1080/07391102.2020.1760136.
14. X. Y. Meng, H. X. Zhang, M. Mezei, M. Cui. Molecular docking: a powerful approach for structure-based drug discovery, Curr. Comput Aid. Drug., 2011, 7, 146-157.
15. S. Forli, R. Huey, M. E. Pique, M. F. Sanner, D. S. Goodsell, A. J. Olson. Computational protein-ligand docking and virtual drug screening with the AutoDock suite, Nature protocols, 2016, 11, 905-919.
16. N. M. Hassan, A. A. Alhossary, Y. Mu. Protein-ligand blind docking using Quick Vina-w with Inter-Process spatio-temporal Integration, Sci. Rep., 2017, 7, 15451.
17. A. Saeed, S. Ur-Rehman, P. A. Channar. Jack bean urease inhibitors, and antioxidant activity based on palmitic acid derived 1-acyl-3-arylthioureas: Synthesis, kinetic mechanism and molecular docking studies, Drug. Res., 2017, 67, 596-605.
18. A. Saeed, P. A. Mahesar, P. A. Channar. Hybrid pharmacophoric approach in the design and synthesis of coumarin linked pyrazolinyl as urease inhibitors, kinetic mechanism and molecular docking. Chem Biodivers, Chem. Biodivers., 2017, 14, 1700035.
19. A. Saeed, S. Rehman, P. A. Channar. Long chain 1- acyl-3-arylthioureas as jack bean urease inhibitors, synthesis, kinetic mechanism and molecular docking studies, J. Taiwan. Inst. Chem. Eng., 2017, 77, 54-63.
20. Baqi, Y. and Muller, C.E., Tetrahedron Lett., 2012, vol. 53, p. 6739.
21. V. Shupeniuk, N. Amaladoss, O. Sabadakh, T. Taras, M. Matkivsky. Synthesis of 4-substituted primary aliphatic aminoanthraquinones and in silico studies. Russ. J. Org. Chem. 2021; 57 (4):582-588.

22. V. Shupenyuk, T. Taras, O. Sabadakh, E. Luchkevich, Y. Kornii. Synthesis some 4-substituted 9,10-anthraquinones. French-Ukrainian Journal of Chemistry. 2020; 8(1):58-65.