Journal of Pharmaceutical Chemistry
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Docking studies of tetra substituted pyrazolone derivatives as potential antiviral agents

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Abstract: In an attempt to find potential antiviral agents, a series of pyrazolones (**PA1**-**PA6** & **PC1**-**PC6**) were designed and evaluated for their DENV NS5 (RNA-dependent RNA polymerase) inhibitory activity. Molecular docking studies of all the designed compounds into the binding site of DENV NS5 (PDB Code: 4C11) were performed to gain a comprehensive understanding into rational binding modes. These compounds were also screened for *in silico* drug-likeliness properties on the basis of the absorption, distribution, metabolism and excretion (ADME) prediction. Among all the synthesized compounds, analogue **PA6** showed superior inhibitory activity against RNA dependent RNA polymerase. SAR study indicated that the presence of an electron withdrawing substitution on pyrazolone derivatives significantly improves its binding interaction with the protein. Results of ADME prediction revealed that most of these compounds showed *in silico* drug-likeliness.

Keywords: Pyrazolone; Antiviral; Autodock 1.5.6; NS5 Protein

1. Introduction

Pyrazolone, a significant class of heterocyclic compound used as a remarkable intermediate for synthesizing pharmaceuticals in various biological activities. It is an important nitrogen containing five-membered heterocyclic compound placed in the center core for many medicinally important derivatives. The pyrazolone function is quite stable and has been inspired medicinal chemists to utilize this established fragment to synthesize new compounds possessing biological activities.[1]

After the work of Fischer and Knovenagel in the late 19th century, the reaction of α, β-unsaturated aldehydes and ketones with hydration became one of the universal methods for the preparation of 2-pyrazolines.[2-3] The chemistry of pyrazolones began in1883, when Knorr reported the first pyrazolone derivative.[4] The reaction of phenyl hydrazine and ethyl acetoacetate resulted in novel structure identified in 1887 as 1-phenyl-3-methyl-5-pyrazolone.[5] The Knorr pyrazole synthesis is the reaction of hydrazine with 1, 3-dicarbonyl compounds to provide the pyrazolone ring system. Pyrazolone is a five membered lactam ring containing two nitrogen at the first and second position and a "one" moiety at fifth position in its ring. Figure 1 list out the drugs with Pyrazolone ring that are available in the market for the treatment of human ailments.

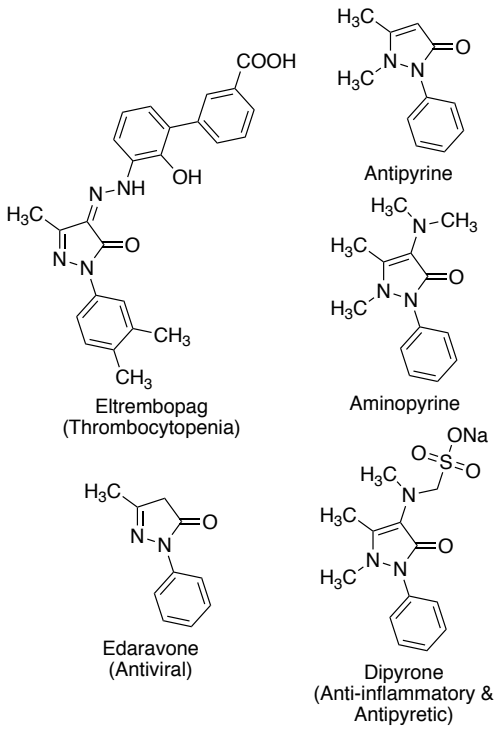

Figure 1. Drugs with pyrazolone ring in market

The process of drug discovery is very complex and requires an interdisciplinary effort to design effective and commercially feasible drugs. The objective of drug design is to find a chemical compound that can fit into a specific cavity on a protein target both geometrically and chemically. The drug discovery process involves the identification and validation of target, identification of

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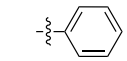
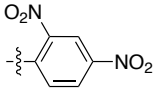
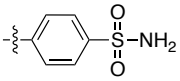
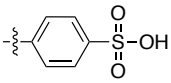
Article info: Submitted on: 07 May 2018; Revised on: 31 Jul 2018; Accepted on: 06 Aug 2018

Table 1. Docking results of designed pyrazolones with Dengue NS5 protein

Code	R	R_1	Binding energy (Kcal/Mol)	Estimated Ki (nM)	H-bonding	Rank
PA1		-	-10.05	42.91	1	2
PA2		-	-9.5	108.39	3	3
PA3		-	-9.22	174.0	1	5
PA4		-	-9.35	140.98	1	4
PA5		-	-8.77	371.13*	1	6
PA6		-	-11.97	1.67	3	1
PC1	-	-H	-8.03	1.3*	0	12
PC2	-	p-Cl	-8.28	859.46	0	8
PC3	-	o-NO_2	-8.22	949.24	0	9
PC4	-	p-OH	-8.21	963.18	2	10
PC5	-	p-CH_3	-8.41	682.54	1	7
PC6	-	-$N(CH_3)_2$	-8.12	1.12*	0	11
Ribavirin	-	-	-5.69	66.94*	3	0

*values in μM

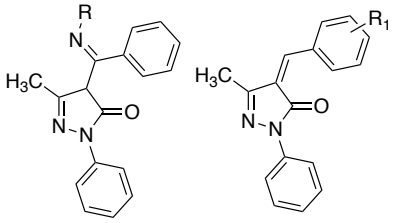
lead, lead optimization, synthesis, screening for its therapeutic efficacy. Once the testing is completed,drug development process will start prior to the clinical trials.[6] After passing the animal tests and human clinical trials, this compound becomes a drug available to patients.[7]

The structural and non-structural proteins of DENV have become the major target of antiviral design.[8-9] The structural proteins (capsid (C), premembrane (prM) and envelope (E)) play vital roles in viral formation and life cycle. While DENV non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) are involved in genome replication, virion assembly and avoiding innate immune responses.[10] Among DENV nonstructural proteins, NS5 is the largest (900 amino acid residues) and the most conserved protein in DENV (67% amino acid sequence identity among dengue serotypes). NS5 has also been an attractive target for antiviral development, as it is required for RNA capping and DENV genome replication. NS5 exhibits RNA-dependent RNA polymerase activity *in- vitro,* although neither function shows template specificity.[11-13]

2. Result and Discussion

2. 1. Molecular Docking Studies

The docking study was performed using the autodock 4.2 with autodock tools 1.5.6.[14] All the twelve pyrazolone derivatives (PA1-PA6 & PC1 – PC6 Figure 2) were docked into the active site of the NS5 (PDB Code: 4c11, RNA dependent RNA polymerase enzyme)[15] protein, which showed significant docking scores compared to that of the reference compound Ribavirin and the results were presented in Table 1. Compounds PA6 and PA1 showed good docking scores of -11.97, -10.05 with NS5 protein (RNA dependent RNA polymerase enzyme). On the other hand, in benzylidine derivatives PC5 has shown a binding energy of -8.41 and PC2 with -8.28 respectively. Thus, both the Schiff base derivatives and benzylidine derivative show a good interaction with the protein with least binding score. The PA1 has a hydrogen bond interaction with the aminoacid Thr783 and PA6 have hydrogen bond interaction with amino acids Asp664, Ser663, Ser661 with pie – pie interaction with Trp795, Ile797. Standard have hydrogen bond interaction with Gln602. PC1 and PA5 have shown least a binding score of -8.03 and -8.77 respectively.



PA1-PA6 PC1-PC6

Figure 2. Designed molecules

The predicted binding and docking energies are the sum of the intermolecular energy and torsional free energy penalty, and the ligand's internal docking energy respectively. The docking approach helps us to predict the binding energy of ligand –receptor complex. It also gives the different conformations possible for the binding (Figure 3).

Among the designed analogs PA6, PA1, PA2 and PA3 have shown significant binding energy of -11.97Kcal/mol, -10.05Kcal/mol, -10.05Kcal/mol, -9.22Kcal/mol respectively with predicted inhibition constant of 1.67nM, 42.91nM, 108.39nM, 174.0nM.

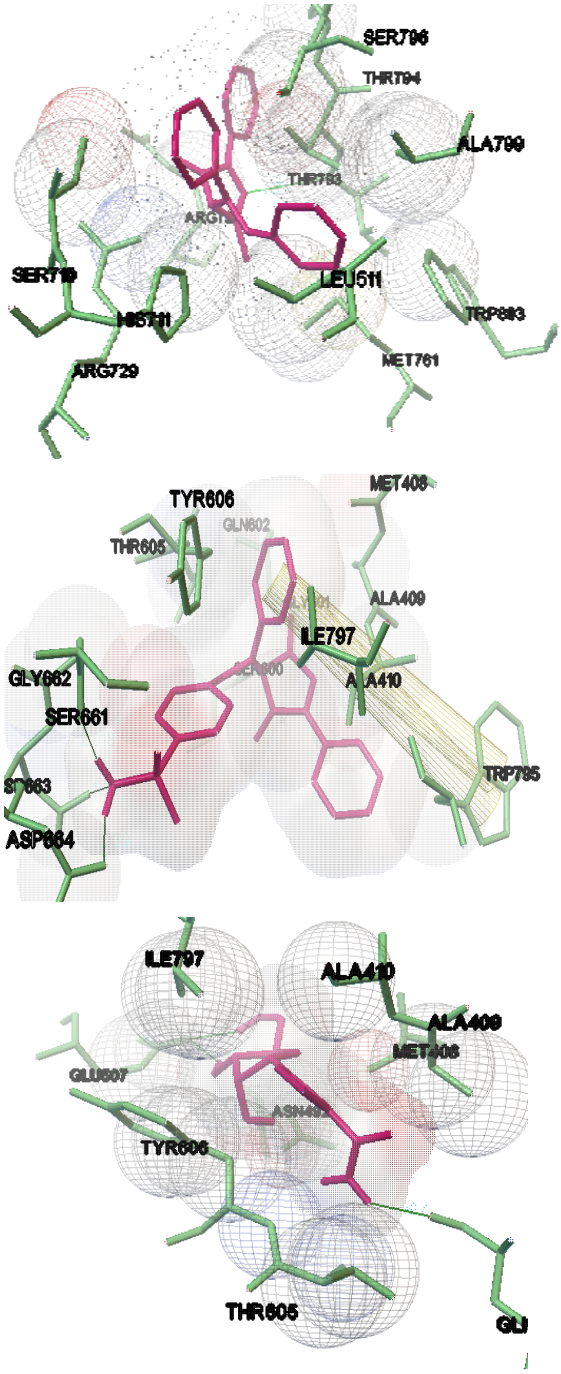


Figure 3. Docking pose of **PA1** (Top)**, PA6** (Middle) & Ribavirin (Bottom) with the NS5 protein

2. 2. ADME Studies

ADME prediction by the software gives out the results that the most of the drugs have good drug likeness property. All the compounds have the good intestinal absorption with a value of greater than 90 %. On the other hand while comparing the distribution factor, all the compounds have moderate distribution score on BBB permeability (blood brain barrier). Moreover none of the designed molecules crosses the BBB according to the values predicted by the corresponding software. The PC5 have slight variation in the values. However in the case of CNS permeability value, none of the compounds crosses the normal values mentioned in the software. The fraction unbound character is also analysed by the software. In that all the designed compounds are found to be in bounded in nature.

3. Experimental

Materials and methods: In the present research study, the molecular docking methodology was implemented by autodock 1.5.6 and MGL tools 1.5.6 packages (ADT; The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA, 92037). Construction and the energy minimization were done with Chemdraw ultra 8.0 and chem3D ultra 8.0 (Cambridge soft. Com, 100cambridge park drive, Cambridge, MA 02140, USA). In this study, autodock 1.5.6 were used to establish a ligand based computer modeling algorithm for the prediction of binding energy and calculation of inhibition constants of the designed antiviral scaffold with the NS5 protein. The hardware used for this study with the Lenovo brand and the processor of Intel (R) Pentium (R) CPU N3710 @ 1.60 GHZ with 4.00 GB RAM, by 64 bit operating systems.

3. 1. Preparation of enzyme

Crystallographic model of NS5 PROTEIN (PDB code: 4C11) was retrieved from www.pdb.org. Within pdb format, therefore the docking tool can assess it. Macromolecule preparation was finished with the marginal options accessible within the autodock tools 1.5.6. Water molecules were deleted, hydrogens were added using polar, assigned AD4 type atoms, Gasteiger charges were added and eventually saved it as macromolecule .pdbqt file format.

3. 2. Preparation of ligand

All the designed ligands were built, 3D optimized and energy minimized using ChemDraw Ultra 8.0 version and saved in pdb formats, which is compatible input file for the autodock tool. The ligands were then imported in ADT for assigning charge and torsion. Finally, it is saved as ligand.pdbqt file format file format file format.

3. 3. Docking methodology

Autodock 1.5.6 was used to implement molecular docking methodologies and to establish a ligand based computer modeling algorithm for the prediction of binding energy and calculation of inhibition constants of the designed antiviral scaffold with the NS5 protein. The torsional freedom of the protein was determined and saved it as a pdbqt format for the further docking procedure. The receptor grids developed by using 60×60×60 grid points in xyz (32.563, 18.812, -16.457 respectively) with grid spacing of 0.375Å. The parameter file was saved as protein.gpf and .glg file was developed by autogrid4. Docking parameter was created with the parameters of the Lamarckian genetic algorithm was used for all molecular docking simulations. Population size of 150, 10 GA runs, GA crossover rate of 0.8 and genetic mutation of 0.02 with a maximum number of generations of 27000 were set as the parameters. The parameter file was saved it as ligand.dpf and docking simulations were done using autodock4. Analyze option in autodock 1.5.6 was used to analyze the docking. Finally the scoring of binding energies determines the good molecule, lesser the energy, better the conformation.

3. 4. ADME Studies

The synthesized compounds (PA1-PA6 & PC1-PC6) were subjected to ADME prediction using the online software from the pkCSM server (http://biosig.unimelb.edu.au/pkcsm/). The SMILES

notation of the molecular structure can be used as a predictor of the pharmacokinetic properties. Since pharmacokinetic properties have a crucial role in the development of potential drug candidates.

4. Conclusion

Pyrazolones are an important class of nitrogen containing heterocyclic ring and acquire a divergent activities, including antiviral activity. An attempt has been made that the compounds combining pyrazolones with schiff base and different aldehydes could maintain the anti-viral activity of the novel scaffolds, but their modes of action are different from that of standard Ribavirin. The present study lays a good foundation for us to find more efficient antiviral agents. The molecular simulation studies of the designed molecules showed a light to the newer areas for the synthesis and experimental evaluation, which will have the antiviral activity compared to that of the standard, Ribavirin.

Acknowledgement

Authors are thankful to the management of Grace College of Pharmacy, Palakkad, Kerala for carrying out the molecular docking studies in the medicinal chemistry and research laboratory.

References

1. Mariappan, G.; Saha, B. P.; Sutharson, L.; Ankits, G.; Pandey, L.; Kumar, D. The diverse pharmacological importance of pyrazolone derivatives: A Review. J Pharm Res 2010**,** 3, 2856-2859.
2. Fischer, E.; Knoevenagel, O. 2) Ueber die Verbindungen des Phenylhydrazins mit Acroleïn, Mesityloxyd und Allylbromid). Justus Liebigs Ann Chem 1887**,** 239 (2), 194-206.
3. Gupta, P.; Gupta, J. K.; Halve, A. K. Synthesis and Biological Significance of Pyrazolones: A Review. Int J Pharm Sci 2015**,** 6 (6), 2291-2310.
4. Knorr, L. Einwirkung von Acetessigester auf Phenylhydrazin. Ber Dtsch Chem Ges 1883**,** 16 (2), 2597-2599.
5. Knorr, L. Synthetische Versuche mit dem Acetessigester. Justus Liebigs Ann Chem 1887**,** 238 (1-2), 137-219.
6. Hughes, J. P.; Rees, S.; Kalindjian, S. B.; Philpott, K. L. Principles of early drug discovery. Br J Pharmacol 2011**,** 162 (6), 1239-1249.
7. Baldi, A. Computational approaches for drug design and discovery: An overview. Sys Rev Pharm 2010**,** 1 (1), 99.
8. Behnam, M. A. M.; Nitsche, C.; Boldescu, V.; Klein, C. D. The medicinal chemistry of dengue virus. J Med Chem 2016**,** 59 (12), 5622-5649.
9. Stevens, A. J.; Gahan, M. E.; Mahalingam, S.; Keller, P. A. The medicinal chemistry of dengue fever. J Med Chem 2009**,** 52 (24), 7911-7926.
10. Nitsche, C.; Holloway, S.; Schirmeister, T.; Klein, C. D. Biochemistry and medicinal chemistry of the dengue virus protease. Chem Rev 2014**,** 114 (22), 11348-11381.
11. Johansson, M.; Brooks, A. J.; Jans, D. A.; Vasudevan, S. G. A small region of the dengue virus-encoded RNA-dependent RNA polymerase, NS5, confers interaction with both the nuclear transport receptor importin-β and the viral helicase, NS3. J Gen Virol 2001**,** 82 (4), 735-745.
12. Selisko, B.; Peyrane, F. F.; Canard, B.; Alvarez, K.; Decroly, E. Biochemical characterization of the (nucleoside-2′ O)-methyltransferase activity of dengue virus protein NS5 using purified capped RNA oligonucleotides 7MeGpppACn and GpppACn. J Gen Virol 2010**,** 91 (1), 112-121.
13. Tan, B.-H.; Fu, J.; Sugrue, R. J.; Yap, E.-H.; Chan, Y.-C.; Tan, Y. H. Recombinant Dengue Type 1 Virus NS5 Protein Expressed inEscherichia coliExhibits RNA-Dependent RNA Polymerase Activity. Virology 1996**,** 216 (2), 317-325.
14. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J Comput Chem 2009**,** 30 (16), 2785-2791.
15. Lim, S. P.; Koh, J. H. K.; Seh, C. C.; Liew, C. W.; Davidson, A. D.; Chua, L. S.; Chandrasekaran, R.; Cornvik, T. C.; Shi, P.-Y.; Lescar, J. A Crystal Structure of the Dengue Virus Non-structural Protein 5 (NS5) Polymerase Delineates Interdomain Amino Acid Residues That Enhance Its Thermostability and de Novo Initiation Activities. J Biol Chem 2013**,** 288 (43), 31105-31114.