IJBPAS, December, Special Issue, 2021, 10(12): 550-562

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DESIGN AND IN SILICO STUDIES OF 2-CYANO-3-(3,5-DI-TERT-BUTYL-4-HYDROXYPHENYL) ACRYLAMIDE DERIVATIVES OF AMINO ACIDS

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Received 19th Aug. 2021; Revised 20th Sept. 2021; Accepted 29th Oct. 2021; Available online 1st Dec. 2021

https://doi.org/10.31032/IJBPAS/2021/10.12.2049

ABSTRACT

Aim: To design and perform *in silico* studies of 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of amino acids (C1 to C24).

Methodology: ChemDraw Ultra 12.0 was used to generate structure, nomenclature and SMILES notations. Molinspiration Cheminformatics, SwissADME and Osiris Property Explorer were used to predict the molecular properties, bioactivity score, ADME properties and toxicity. AutoDock 4.2 was used to perform molecular docking studies by selecting enzymes involved in the process of inflammation COX-1 (PDB ID: 1EQG), COX-2 (PDB ID: 3LN1) and 5-LOX (PDB ID: 3O8Y).

Results and Discussion: All the compounds C1 to C24 were predicted as drug like molecules, except compound C20. All the compounds possess good ADME properties and low toxicity. Most of the compounds were identified as bioactive protease inhibitors and enzyme inhibitors. Molecular docking studies indicated 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of Aspartic acid and Methionine (compounds C8 and C17) as dual inhibitors of COX and 5-LOX enzymes.

Conclusion: The present investigation provided new insights into structure activity relationships in amino acid residues and identified 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide as promising lead moiety comprising bioactive 2,6-di-*tert*-butyl-phenol and 2-cyanoacrylamide.

Keywords: Amino acids, 2,6-di-tert-butylphenol, 2-Cyanoacrylamide, *In silico* studies

INTRODUCTION

Amino acids are compounds with an amine group, a carboxylic acid group and a variable side-chain. Amino acids are indeed a type of cell signaling molecules that are associated in gene regulation and protein phosphorylation pathway as well as in synthesis of hormones and low-molecular nitrogenous compounds having vast biological significance [1]. Commercially, amino acids are modified for the synthesis of human insulin, interferons etc. Amino acids modification is used in a variety of disciplines, including synthetic chemistry, materialistic science, targeted delivery techniques, and biological function investigation [2]. The development of prodrugs using amino acids resulted in improvement of several properties such as increased bioavailability, decreased toxicity of the parent drug, accurate delivery to target tissue and prevention of fast metabolism [3]. Amino acid derived compounds are used in treatment of various diseases such as cancer, inflammation and a variety of infectious diseases. [4 - 8].

Many drugs containing sterically hindered phenolic compounds, particularly 2,6-di-*tert*-butylphenol derivatives, have been reported to have a variety of pharmacological activities including anti-inflammatory, anti-rheumatic, antitumor and antihyperlipidemic properties. It was found that two *tert*-butyl groups adjacent to phenolic hydroxyl group are required to retain *in vivo* anti-inflammatory potency. The anti-inflammatory efficacy of many synthetic derivatives including heterocyclic compounds comprising the butylated hydroxyltoluene (BHT) moiety has been described with dual inhibition of 5-lipoxygenase and cyclooxygenase enzymes. Prifelone, Tebufelone, Tazofelone and Darbufelone, used to treat inflammatory disorders and Eldacimibe used to treat hyperlipidemia consists of 2,6-di-*tert*-butylphenol [9, 10].

Acrylamide derivatives are helpful as therapeutics, especially in the prevention or treatment of various disorders and diseases involving the activity of the mitochondrial permeability transition pore, like ischemia, oxidative or degenerative tissue damage [11]. The growing interest in 2-cyanoacrylamide containing compounds derives from their potential biological characteristics which include anticancer, antibacterial, antioxidant and anti-inflammatory activities [12 - 18]. Based on the above observations, the present investigation aimed to develop 2-cyano-3-(3,5-di-*tert*-butyl-4-

hydroxyphenyl)acrylamide derivatives of amino acids *in silico* and predict the molecular properties, bioactivity score, ADME properties and toxicity using free online software. Further, it is aimed to perform molecular docking studies by selecting enzymes involved in the process of inflammation using AutoDock 4.2 software.

MATERIALS AND METHODS

The software used for the *in silico* studies of 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of amino acids include ChemDraw Ultra 12.0, Molinspiration Cheminformatics, SwissADME, Osiris Property Explorer and AutoDock 4.2.

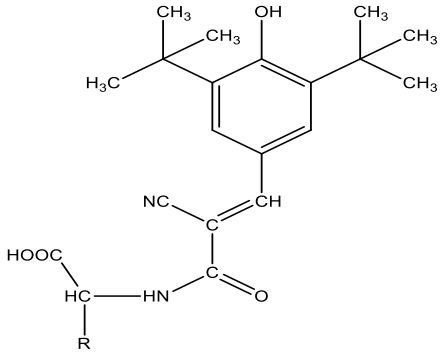
ChemDraw Ultra 12.0 was used for the generation of structure, IUPAC name and SMILES notation. Molinspiration cheminformatics was employed to acquire physicochemical properties such as miLogP (partition coefficient), total polar surface area (TPSA), molecular weight, number of hydrogen bond donors, number of hydrogen bond acceptors, number of heavy atoms, number of violations, number of rotatable bonds and volume. Molinspiration Cheminformatics is also used for the prediction of bioactivity scores of the designed compound. The molecule's bioactivity may be assessed by calculating the activity score as GPCR ligands, ion channel modulators, nuclear receptor ligands, kinase inhibitors, protease inhibitors and enzyme inhibitors. ADME properties such as gastrointestinal absorption, blood brain barrier permeation, permeability glycoprotein substrate specificity, inhibition of cytochrome P450 isoenzymes such as CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4 and skin permeability coefficient were predicted using SwissADME. Toxicity properties such as mutagenicity, tumorigenicity, irritant effect and reproductive effect were predicted using Osiris Property Explorer.

AutoDock 4.2 was used for docking newly designed 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of amino acids. The structures of new compounds (C1 to C24) were downloaded in 3D format from chimera to make it ready for docking. Three-dimensional crystal structures of COX-1 (PDB ID: 1EQG), COX-2 (PDB ID: 3LN1) and 5-LOX (PDB ID: 3O8Y) were retrieved from RCSB in PDB format. All the PDB files were converted to PDBQT files with AutoDock tools. Grid box was generated and saved the Grid Parameter File. Then docking was performed considering the whole protein and results were visualized in UCSF chimera. Most favourable binding

sites were predicted based on Gibbs free energy and bond length between active sites was predicted. The obtained Grid Parameter File and Dock Parameter File converted into log files to analyze docked conformation and viewed the best pose with lowest binding energy in Discovery Studio.

RESULTS AND DISCUSSION

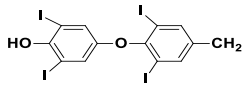
A series of 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of amino acids (C1 to C24) were designed, generated the structure, IUPAC nomenclature and SMILES notation using ChemDraw Ultra 12.0. The generated compounds with substitution at R group in the amino acid residue of 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivative (Compound-C) was shown in Table-1 and the IUPAC nomenclature and SMILES notation were indicated for all the new compounds.

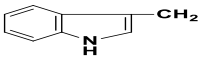
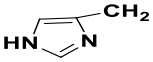


Compound-C: 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivative of amino acid

Table 1: IUPAC nomenclature and SMILES notation of 2-Cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide Derivative of Amino Acids (C1 to C24)

C. No.	R	IUPAC name	SMILES Notation
C1	H	(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl) acrylamido)acetic acid	OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NCC(O)=O)=O)C=C1C(C)(C)C
C2	CH_3	(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)propanoicacid	OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(C)C(O)=O)=O)C=C1C(C)(C)C
C3	$HOCH_2$	(R,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-hydroxypropanoic acid	OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CO)C(O)=O)=O)C=C1C(C)(C)C
C4	$CH_3CH(OH)$	(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-hydroxybutanoic acid	OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(C(O)C)C(O)=O)=O)C=C1C(C)(C)C
C5	$(CH_3)_2CH$	(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-methylbutanoic acid	OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(C(C)C)C(O)=O)=O)C=C1C(C)(C)C
C6	$(CH_3)_2CHCH_2$	(R,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-4-methylpentanoic acid	OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CC(C)C)C(O)=O)=O)C=C1C(C)(C)C
C7	$CH_3CH_2CH(CH_3)$	(2R)-2-((E)-2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-	OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(C(CC)C)C(O)=O)=O)C=C1C(C)(C)C

		methylpentanoic acid	
C8	**$COOHCH_2$**	**(R,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)succinic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CC(O)=O)C(O)=O)=O)C=C1C(C)(C)C**
C9	**$CONH_2CH_2$**	**(R,E)-4-amino-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-4-oxobutanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CC(N)=O)C(O)=O)=O)C=C1C(C)(C)C**
C10	**$COOHCH_2CH_2$**	**(R,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)pentanedioic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CCC(O)=O)C(O)=O)=O)C=C1C(C)(C)C**
C11	**$CONH_2CH_2CH_2$**	**(R,E)-5-amino-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-5-oxopentanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CCC(N)=O)C(O)=O)=O)C=C1C(C)(C)C**
C12	**NH 2 C(NH)NH(CH 2) 3**	**(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-5-guanidinopentanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(N[C@@H](C(O)=O)CCCNC(N)=N)=O)C=C1C(C)(C)C**
C13	**$NH_2(CH_2)_4$**	**(R,E)-6-amino-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)hexanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CCCCN)C(O)=O)=O)C=C1C(C)(C)C**
C14	**$NH_2CH_2CH(OH)(CH2)_3$**	**(2R)-7-amino-2-((E)-2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-6-hydroxyheptanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CCCC(O)CN)C(O)=O)=O)C=C1C(C)(C)C**
C15	**$HSCH_2$**	**(S,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-mercaptopropanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CS)C(O)=O)=O)C=C1C(C)(C)C**
C16	**COOH(NH 2)CHCH 2 S-SCH 2**	**(E)-2-((((carboxy(2-cyano-3-(3,5-di-*tert*-butyl-4hydroxyphenyl)acrylamido)methyl)thio)methyl)amino)-3-mercaptopropanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(SCNC(C(O)=O)CS)C(O)=O)=O)C=C1C(C)(C)C**
C17	**$CH_3SCH_2CH_2$**	**(R,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-4-(methylthio)butanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CCSC)C(O)=O)=O)C=C1C(C)(C)C**
C18	**$C_6H_5CH_2$**	**(R,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-phenylpropanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CC2=CC=CC=C2)C(O)=O)=O)C=C1C(C)(C)C**
C19	**P-$OHC_6H_4CH_2$**	**(R,E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-(4-hydroxyphenyl)propanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(N[C@H](CC2=CC=C(O)C=C2)C(O)=O)=O)C=C1C(C)(C)C**
C20		**(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-(4-(4-hydroxy-2,6-diiodophenoxy)-2,6-diiodophenyl)propanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CC2=C(I)C=C(OC3=C(I)C=C(O)C=C3I)C=C2I)C(O)=O)=O)C=C1C(C)(C)C**
C21	**$CH_2CH_2CH_2$**	**(E)-1-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acryloyl)pyrrol**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(N2C(CCC2)C(O)=O)=O)C=C1C(C)(C)C**

		idine-2-carboxylic acid	
C22	**$CH_2CH(OH)CH_2$**	**(E)-1-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acryloyl)-4-hydroxypyrrolidine-2-carboxylic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(N2C(CC(O)C2)C(O)=O)=O)C=C1C(C)(C)C**
C23		**(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-(1H-indol-2-yl)propanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CC2=CC(C=CC=C3)=C3N2)C(O)=O)=O)C=C1C(C)(C)C**
C24		**(E)-2-(2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamido)-3-(1H-imidazol-5-yl)propanoic acid**	**OC1=C(C(C)(C)C)C=C(/C=C(C#N)/C(NC(CC2=CN=CN2)C(O)=O)=O)C=C1C(C)(C)C**

Prediction of molecular properties and bioactivity score using Molinspiration Cheminformatics:

Molecular properties of all the compounds C1 to C24 were predicted using Molinspiration cheminformatics and the results obtained were shown in Table-2. The drug likeness of title compounds was identified based on Lipinski's rule of five, which states that the molecular weight must be less than or equivalent to 500, the Log P value must be less than or equivalent to 5, the number of hydrogen bond donors must be less than or equivalent to 5 and the number of hydrogen bond acceptors must be less than or equivalent to 10. It is mentioned that an orally active drug has no more than one violation of Lipinski's rule [19]. The data indicated that most of the designed compounds obeyed Lipinski's rule of five and therefore they are considered to be drug like compounds. However, compound C20 violated the rule as log P value was more than 5 and molecular weight was greater than 500.

Table 2: Prediction of Molecular properties of Compounds C1 to C24 using Molinspiration Cheminformatics

Compound	milogP	TPSA	n atoms	M.Wt	n ON	n OHNH	n violations	n rotb	Volume
C1	**2.88**	**110.42**	**26**	**358.44**	**6**	**3**	**0**	**6**	**343.9**
C2	**2.66**	**110.42**	**27**	**372.46**	**6**	**3**	**0**	**6**	**360.49**
C3	**1.69**	**130.65**	**28**	**388.46**	**7**	**4**	**0**	**7**	**368.75**
C4	**2.05**	**130.65**	**29**	**402.49**	**7**	**4**	**0**	**7**	**385.33**
C5	**3.44**	**110.42**	**29**	**400.52**	**6**	**3**	**0**	**7**	**393.88**
C6	**3.97**	**110.42**	**30**	**414.55**	**6**	**3**	**0**	**8**	**410.68**
C7	**3.94**	**110.42**	**30**	**414.55**	**6**	**3**	**0**	**8**	**410.68**
C8	**1.83**	**147.72**	**30**	**416.47**	**8**	**4**	**0**	**8**	**387.73**
C9	**2.54**	**153.51**	**30**	**415.49**	**8**	**5**	**0**	**8**	**391**
C10	**2.11**	**147.72**	**31**	**430.5**	**8**	**4**	**0**	**9**	**404.53**
C11	**1.59**	**153.51**	**31**	**429.52**	**8**	**5**	**0**	**9**	**407.8**
C12	**1.72**	**172.32**	**33**	**457.57**	**9**	**7**	**1**	**11**	**440.32**
C13	**2.17**	**136.44**	**31**	**429.56**	**7**	**5**	**0**	**10**	**422.42**

C14	**1.52**	**156.67**	**33**	**459.59**	**8**	**6**	**1**	**11**	**447.27**
C15	**2.64**	**110.42**	**28**	**404.53**	**6**	**3**	**0**	**7**	**378.39**
C16	**2.16**	**159.74**	**35**	**523.68**	**9**	**5**	**1**	**12**	**469.55**
C17	**3.12**	**110.42**	**30**	**432.59**	**6**	**3**	**0**	**9**	**412.22**
C18	**4.12**	**110.42**	**33**	**448.56**	**6**	**3**	**0**	**8**	**432.14**
C19	**3.64**	**130.65**	**34**	**464.56**	**7**	**4**	**0**	**8**	**440.15**
C20	**8.89**	**139.88**	**45**	**1060.24**	**8**	**4**	**2**	**10**	**616.51**
C21	**2.97**	**101.63**	**29**	**398.5**	**6**	**2**	**0**	**5**	**383.87**
C22	**2.05**	**121.86**	**30**	**414.5**	**7**	**3**	**0**	**5**	**391.92**
C23	**4.58**	**126.21**	**36**	**487.6**	**7**	**4**	**0**	**8**	**461.11**
C24	**2.35**	**139.1**	**32**	**438.53**	**8**	**4**	**0**	**8**	**412.96**

(miLogP: n-octanol-water partition coefficient, TPSA: Topological Polar Surface Area, n atoms: number of atom excluding hydrogens, M.Wt: Molecular weight, n ON: Hydrogen bond acceptors, n OHNH: Hydrogen bond donors, n violations: Number of violations, n rotb: Number of rotatable bonds)

Bioactivity scores of the title compounds C1 to C24 were calculated using Molinspiration Cheminformatics and the results obtained were shown in Table-3. For a compound, the likelihood is that if the bioactivity score is greater than 0, it is active, if the score is between -5.0 and 0.0, it is moderately active and if less than -5.0, it is inactive. The results revealed that all the compounds (excluding compound C20) were active as protease inhibitors and enzyme inhibitors. Among the series, compounds C12 to C16, C21, C22 and C24 were more active as protease inhibitors. Hence, there is a scope for the development of these compounds as antiviral drugs. The data indicated that the compounds C12 to C15, C23 and C24 were more active as enzyme inhibitors. The data also indicted that all the compounds were active or moderately active as GPCR ligand, ion channel modulator, kinase inhibitor and nuclear receptor ligand. The compound C20 was found to be inactive as ion channel modulator and kinase inhibitor.

Table 3: Prediction of Bioactivity Scores of Compounds C1 to C24 using Molinspiration Cheminformatics

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
C1	**-0.17**	**-0.11**	**-0.26**	**0.10**	**0.01**	**0.02**
C2	**-0.14**	**-0.08**	**-0.24**	**0.02**	**0.05**	**0.02**
C3	**-0.04**	**-0.01**	**-0.19**	**0.07**	**0.12**	**0.13**
C4	**-0.09**	**-0.11**	**-0.28**	**0.10**	**0.07**	**0.10**
C5	**-0.12**	**-0.06**	**-0.26**	**0.00**	**0.09**	**0.04**
C6	**-0.04**	**0.02**	**-0.28**	**0.14**	**0.18**	**0.09**
C7	**-0.12**	**-0.04**	**-0.30**	**-0.04**	**0.11**	**0.06**
C8	**-0.00**	**0.01**	**-0.25**	**0.11**	**0.14**	**0.12**
C9	**-0.05**	**-0.06**	**-0.18**	**0.08**	**0.19**	**0.10**
C10	**-0.01**	**-0.01**	**-0.23**	**0.13**	**0.12**	**0.13**
C11	**-0.02**	**-0.02**	**-0.17**	**0.10**	**0.19**	**0.12**
C12	**0.20**	**0.20**	**-0.17**	**-0.14**	**0.45**	**0.23**
C13	**0.08**	**0.11**	**-0.10**	**0.10**	**0.27**	**0.20**
C14	**0.07**	**0.10**	**-0.12**	**0.01**	**0.30**	**0.23**

C15	**-0.04**	**-0.06**	**-0.24**	**0.07**	**0.32**	**0.25**
C16	**-0.09**	**-0.09**	**-0.31**	**-0.10**	**0.37**	**0.17**
C17	**-0.13**	**-0.11**	**-0.49**	**0.02**	**0.12**	**0.14**
C18	**0.01**	**0.01**	**-0.19**	**0.10**	**0.13**	**0.08**
C19	**0.02**	**0.02**	**-0.17**	**0.14**	**0.13**	**0.09**
C20	**-0.2**	**-0.61**	**-0.54**	**-0.12**	**-0.04**	**-0.37**
C21	**-0.00**	**0.07**	**-0.20**	**0.14**	**0.24**	**0.11**
C22	**0.10**	**0.08**	**-0.11**	**0.17**	**0.32**	**0.18**
C23	**0.14**	**0.1**	**0.01**	**0.07**	**0.20**	**0.21**
C24	**0.16**	**0.12**	**-0.01**	**-0.27**	**0.22**	**0.28**

Prediction of ADME properties using SwissADME:

ADME properties of the compounds C1 to C24 were calculated using SwissADME and the results obtained were shown in Table-4. The results revealed that the title compounds C1 to C7, C18, C21 and C22 has high gastrointestinal absorption and the other compounds showed low gastrointestinal absorption. All the title compounds not showed blood brain barrier permeability. Except C1, C18, C19, C20, C21 and C23, all the compounds showed interaction with P-glycoprotein. All the title compounds were found to be non-inhibitors of CYP1A2 and CYP2D6 enzymes. Compounds C15 and C17 were found to be inhibitors of CYP2C19 enzyme and compounds C6, C7, C21 and C23 were found to be inhibitors of CYP2C9 enzyme. Compounds C13, C15, C17, C18, C19, C21, C23 and C24 were found to be inhibitors of CYP3A4 enzyme. All the compounds were less permeable through skin as their skin permeability coefficient values were between -4.67 to -8.11.

Table 4: Prediction of ADME Properties of Compounds C1 to C24 using SwissADME

C. No.	GI absorption	BBB permeation	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log Kp (cm/s)
C1	**High**	**No**	**No**	**No**	**No**	**No**	**No**	**No**	**-5.55**
C2	**High**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-5.35**
C3	**High**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-6.19**
C4	**High**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-5.97**
C5	**High**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-5.97**
C6	**High**	**No**	**Yes**	**No**	**No**	**Yes**	**No**	**No**	**-4.67**
C7	**High**	**No**	**Yes**	**No**	**No**	**Yes**	**No**	**No**	**-4.67**
C8	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-6.23**
C9	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-6.69**
C10	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-6.06**
C11	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-6.52**
C12	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-6.45**
C13	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**Yes**	**-7.49**
C14	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-8.11**
C15	**Low**	**No**	**Yes**	**No**	**Yes**	**No**	**No**	**Yes**	**-5.6**
C16	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-7.92**
C17	**Low**	**No**	**Yes**	**No**	**Yes**	**No**	**No**	**Yes**	**-5.26**
C18	**High**	**No**	**No**	**No**	**No**	**No**	**No**	**Yes**	**-4.68**
C19	**Low**	**No**	**No**	**No**	**No**	**No**	**No**	**Yes**	**-5.04**
C20	**Low**	**No**	**No**	**No**	**No**	**No**	**No**	**No**	**-5.74**

C21	**High**	**No**	**No**	**No**	**No**	**Yes**	**No**	**Yes**	**-5.29**
C22	**High**	**No**	**Yes**	**No**	**No**	**No**	**No**	**No**	**-6.08**
C23	**Low**	**No**	**No**	**No**	**No**	**Yes**	**No**	**Yes**	**-4.81**
C24	**Low**	**No**	**Yes**	**No**	**No**	**No**	**No**	**Yes**	**-5.92**

Prediction of toxicity using Osiris Property Explorer:

Toxicity of compounds C1 to C24 was calculated using Osiris Property Explorer and the results presented in Table-5. Except few, all the compounds have low risk of mutagenic, tumorigenic, irritant and reproductive effect. Compound C16 was found to have high risk of mutagenic effect and compounds C18 to C20 have medium risk of reproductive effect. This study indicated that all the designed compounds may be developed into acceptable drug candidates with minimum toxicity.

Table 5: Prediction of Toxicity of Compounds C1 to C24 using Osiris Property Explorer

Compound	Mutagenic	Tumorigenic	Irritant	Reproductive effect
C1	**Low**	**Low**	**Low**	**Low**
C2	**Low**	**Low**	**Low**	**Low**
C3	**Low**	**Low**	**Low**	**Low**
C4	**Low**	**Low**	**Low**	**Low**
C5	**Low**	**Low**	**Low**	**Low**
C6	**Low**	**Low**	**Low**	**Low**
C7	**Low**	**Low**	**Low**	**Low**
C8	**Low**	**Low**	**Low**	**Low**
C9	**Low**	**Low**	**Low**	**Low**
C10	**Low**	**Low**	**Low**	**Low**
C11	**Low**	**Low**	**Low**	**Low**
C12	**Low**	**Low**	**Low**	**Low**
C13	**Low**	**Low**	**Low**	**Low**
C14	**Low**	**Low**	**Low**	**Low**
C15	**Low**	**Low**	**Low**	**Low**
C16	**High**	**Low**	**Low**	**Low**
C17	**Low**	**Low**	**Low**	**Low**
C18	**Low**	**Low**	**Low**	**Medium**
C19	**Low**	**Low**	**Low**	**Medium**
C20	**Low**	**Low**	**Low**	**Medium**
C21	**Low**	**Low**	**Low**	**Low**
C22	**Low**	**Low**	**Low**	**Low**
C23	**Low**	**Low**	**Low**	**Low**
C24	**Low**	**Low**	**Low**	**Low**

Molecular docking Studies:

The previous literature revealed that the balanced inhibition of 5-LOX and COX (both types 1 and 2) block the formation of all the enzymatically arachidonic acid derived metabolites [20]. Therefore, in the present study molecular docking studies were performed to predict and understand the interaction of enzymes involved in the process of inflammation and the designed ligands using Auto Dock 4.2. All the titled compounds were docked with the target enzymes COX-1 (PDB id: 1EQG), COX-2 (PDB id: 3LN1) and 5-LOX (PDB id:

3O8Y). The docking results of title compounds were compared with the results obtained using standard drugs Ibuprofen (COX-1), Celecoxib (COX-2) and Licofelone (5-LOX). The data presented in Table-6. The results revealed that the compounds C14, C8, C4, C17, C11, C20, C10, C19 and C7 have high binding energy values and low binding affinity towards COX-1 enzyme (constitutive) than the standard compound Ibuprofen. Therefore, it can be inferred that these compounds may possess fewer side effects than Ibuprofen. Compounds C4, C7, C8, C10, C11, C14, and C17 - C20 were found to have low binding energy values and better affinity with COX-2 (inducible) than COX-1 enzyme, indicating these compounds may inhibit COX-2 enzyme. However, the binding energy value of standard compound Celecxib was lower than all the evaluated compounds. The 5-LOX docking data revealed that the compounds capable of inhibiting the COX-2 enzyme, compounds C8 and C17, have comparable affinity towards the 5-LOX enzyme. Additionally, compounds C21 to C23 showed good affinity towards 5-LOX enzyme, but not greater than the standard compound Licofelone. Finally, the docking studies indicate that there is a possibility of development of compounds C8 and C17 as dual inhibitors of COX and 5-LOX enzymes.

Table 6: Molecular Docking of Compounds C1 to C24 with COX-1 (1EQG), COX-2 (3LN1) and 5-LOX (3O8Y)

S. No.	Compound Code	Docking Score (kcal/mol)		
		COX-1 PDB id: 1EQG	COX-2 PDB id: 3LN1	5-LOX PDB id: 3O8Y
1	C1	-9.64	-8.81	-4.96
2	C2	-8.68	-8.37	-4.79
3	C3	-8.26	-8.13	-4.62
4	C4	-5.35	-7.29	-4.16
5	C5	-9.14	-8.38	-4.89
6	C6	-7.22	-7.03	-4.95
7	C7	-6.68	-9.48	-4.73
8	C8	-5.03	-7.86	-5.02
9	C9	-9.54	-7.89	-4.61
10	C10	-6.48	-7.95	-4.92
11	C11	-6.30	-10.09	-4.45
12	C12	-6.05	-4.65	-3.66
13	C13	-8.38	-4.57	-3.63
14	C14	-3.02	-5.13	-2.15
15	C15	-6.61	-6.41	-3.94
16	C16	-4.48	-2.83	-2.12
17	C17	-6.20	-8.41	-5.24
18	C18	-8.47	-10.01	-4.49
19	C19	-6.58	-8.88	-4.36
20	C20	-6.46	-7.33	-4.08
21	C21	-8.00	-9.87	-6.21
22	C22	-9.99	-7.73	-6.44
23	C23	-7.14	-6.83	-5.45
24	C24	-8.87	-5.92	-4.37

25	**Standard Drug**	**-6.95** **Ibuprofen**	**-10.60** **Celecoxib**	**-6.57** **Licofelone**

CONCLUSION

A series of 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of amino acids (C1 to C24) were identified as drug like molecules, except Compound C20. Among the series, 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of Arginine, Cystine, Cysteine, Hydroxyproline, Hydroxylysine, Lysine, Proline and Histidine (compounds C12, C16, C15, C22, C14, C13, C21 and C24) were exhibited better bioactivity score as protease inhibitors. 2-Cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of Histidine, Cysteine, Arginine, Hydroxylysine, Tryptophan and Lysine (compounds C24, C15, C12, C14, C23 and C13) were also displayed good bioactivity score as enzyme inhibitors. All the compounds possess good ADME properties and low toxicity. 2-Cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide derivatives of Aspartic acid and Methionine (compounds C8 and C17) were found to be dual inhibitors of COX and 5-LOX enzymes from the molecular docking studies. Overall, the *in silico* studies revealed new information about structure activity relationships in the amino acid side chain and identified 2-cyano-3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)acrylamide as favourable lead moiety consisting bioactive 2,6-di-*tert*-butylphenol and 2-cyanoacrylamide. Hence, these compounds may develop into new drugs for the treatment of various infectious diseases, inflammation and cancer.

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