BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 8(20)

Molecular docking studies on inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids

Anil Kumar[1] & Utpal Bora[1, 2]*

[1]Computational Biology Laboratory, Department of Biotechnology, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India; [2]Biotech Hub, Centre for the Environment, Indian Institute of Technology Guwahati, Guwahati-781039, Assam, India; Utpal Bora – Email: ubora@iitg.ernet.in, ubora@rediffmail.com; *Corresponding author

Received October 04, 2012; Accepted October 05, 2012; Published October 13, 2012

Abstract:
Stat3 is a mammalian transcription factor which regulates various genes involved in cell growth, proliferation, cell survival and other biological processes. Its constitutive activation promotes dysregulated growth, survival and immune responses which contribute to tumor progression and carcinogenesis. Inhibition of Stat3 dimerization which prevents its binding to DNA is a rational strategy that could be translated to potential therapeutic applications. The present computational study provides insights into the inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. The involvement of residues like LYS-591, ARG-609, SER-611, GLU-612, SER-613, SER-636 and VAL-637 seems to play an important role in binding of curcumin natural derivatives and its amino acids conjugates with Src Homology (SH2) domain of Stat3 monomer. Demethoxycurcumin followed by hexahydrocurcuminol were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives and known inhibitors (FLLL32, Sta21 and Stattic). Curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) was predicted to be the most potent inhibitor of Stat3 dimerization amongst the curcumin-amino acid conjugates and known peptide based inhibitor (Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn).

Keywords: Curcumin natural derivatives, Curcumin–amino acid conjugates, Stat3 dimerization, Src Homology (SH2) domain, Molecular docking

Background:
Mammalian signal transducers and activators of transcription (STAT) is a family of 7 transcription factors (Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6) **[1]**. These transcription factors are activated in response to cytokines and growth factors including interferons (IFNs), epidermal growth factor (EGF), interleukin 5 (IL5), IL6, hepatocyte growth factor (HGF), leukemia inhibitory factor (LIF) and bone morphogenetic protein 2 (BMP2) which regulate various genes involved in cell growth, proliferation, cell survival and other biological processes **[2]**. The transcription factors of this family are activated by growth factor receptor tyrosine kinases, Janus kinases or Src family kinases through the phosphorylation of a critical tyrosine residue which leads to the dimerization of two phosphorylated monomers **[3]**. Phosphorylated dimers are translocated to the nucleus where they bind to specific DNA-response elements in the promoter region of target genes, and induce gene expression **[4, 5]**. It has been found that constitutive activation of certain STAT family members, particularly of Stat3 promote dysregulated growth, survival and immune responses which contribute to tumor progression and carcinogenesis **[6, 7]**. Stat3 dimerization relies on the reciprocal binding of Src Homology (SH2) domain-binding peptide (Pro-pTyr-Leu-Lys-Thr-Lys) of one monomer to another **[8]**. It is a critical step in Stat3 activation which presents an attractive target to abrogate Stat3 DNA-binding and to

inhibit its aberrant transcriptional activity **[9]**. Interest in development of small molecule and peptide based inhibitors of Stat3 dimerization in the last few years has led to the discovery of inhibitors like Stattic, Sta21 and FLLL32 **[10, 11]**.

Curcumin (diferuloylmethane) is a principal component of Asian spice turmeric with wide range of pharmacological properties which includes antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities **[12]**. Curcumin has been reported to inhibit the Stat3 phosphorylation and DNA binding activity in human cancer cells **[13, 14]**. It has been found that curcumin is extremely safe even at very high doses in various studies with animal models and human **[15]**. In addition to curcumin, turmeric plant contains several other curcuminoids with broad spectrum of pharmacological properties in which demethoxycurcumin and bisdemethoxycurcumin are abundant **[16]**. In order to improve the pharmacological properties, curcumin was conjugated with various functional groups. Curcumin-amino acids conjugates were also synthesized using different substitution schemes which were tested for antioxidant, antimicrobial, antiviral, antiproliferative and proteasome inhibition activities **[17-20]**. In the present study we investigate the interaction of curcumin natural derivatives and its conjugates with amino acids in the pursuance of potential lead molecule for inhibition of Stat3 dimerization using molecular docking over the SH2 domain of a Stat3 monomer.

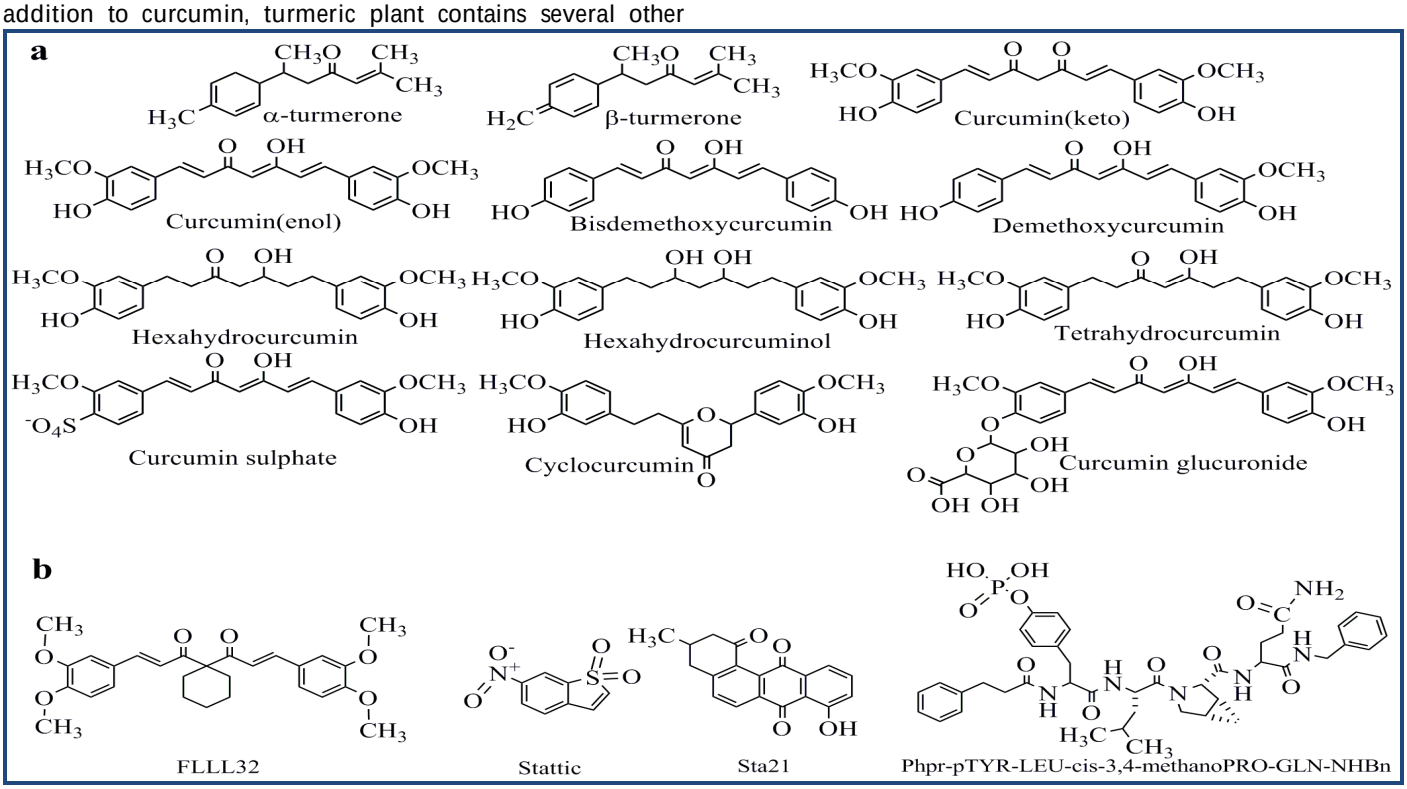


Figure 1: Structures of curcumin natural derivatives and known Stat3 dimerization inhibitors used in the study.

Methodology:

Preparing small molecules

Curcumin natural derivatives **(Figure 1a)**, its conjugates with amino acids **Table 1 (see supplementary material)** and known Stat3 dimerization inhibitors **(Figure 1b)** were drawn and 3D optimized by MarvinSketch (Free Academic License) and saved in Protein Data Bank (PDB) file format. These small molecules were prepared for molecular docking by merging non-polar hydrogens, assigning Gastegier charges, and saving them in PDBQT file format using AutoDock Tools (ADT) 1.5.6.

Preparing Target molecule

To investigate the interaction of curcumin natural derivatives and its amino acid conjugates, X-ray crystal structure of Stat3β complexed with DNA (PDB ID: 1BG1) was obtained from the Protein Data Bank (http://www.rcsb.org/pdb). For molecular docking DNA and other hetero-atoms (water, ions, etc.) were removed using PyMOL 0.99, Gasteiger charges were assigned and macromolecule was saved in PDBQT file format using ADT.

Molecular docking

Grid and docking parameter files were prepared using ADT and molecular docking was performed with AutoDock 4.2.1 (Scripps Research Institute, USA) considering all the rotatable bonds of small molecules as rotatable and macromolecule as rigid. Grid box size of 80 x 80 x 80 Å with 0.375 Å spacing was selected that include the whole SH2 dimerization domain of Stat3 monomer. Empirical-free energy function and Lamarckian Genetic Algorithm, with an initial population of 150 randomly placed individuals, a maximum number of 2,500,000 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80 were used to perform molecular docking with macromolecule. Hundred independent docking runs were performed for each small molecule. Protein– small molecule complex for lowest free energy of binding (ΔG) confirmation from the largest cluster was saved in PDBQT format and converted to PDB file format using UCSF Chimera 1.6.1. Docking results were analyzed using PyMOL 0.99 for possible polar and hydrophobic interactions. Docking studies were performed at Intel(R) Xeon(R) CPU (3.2 GHz) with Linux-based operating system Fedora 15.

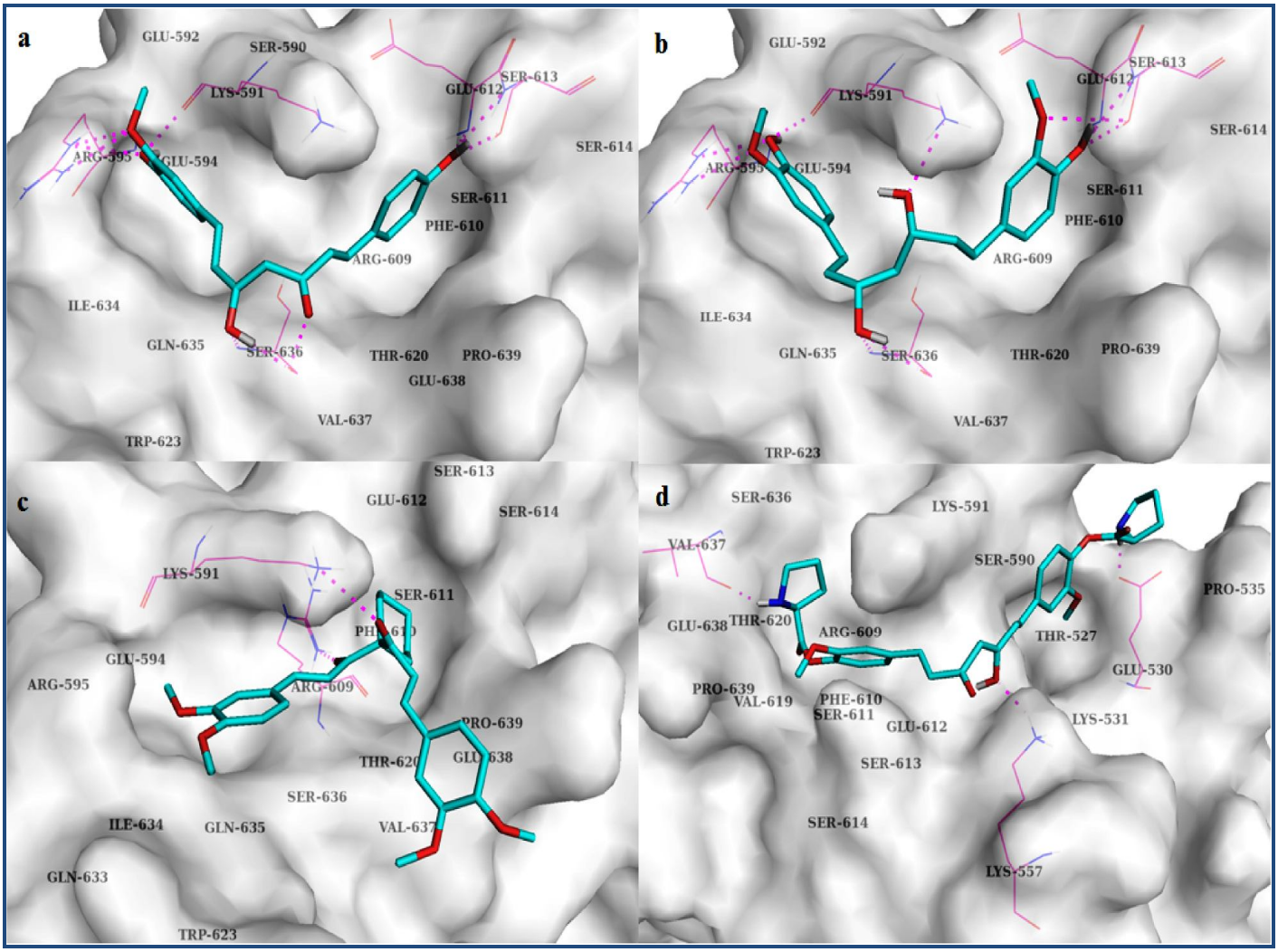


Figure 2: Binding modes of **(a)** demethoxycurcumin **(b)** hexahydrocurcuminol **(c)** FLLL32 **(d)** curcumin-proline conjugate (1,7-Bis (4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) with Stat3 SH2 domain of a Stat3 monomer.

Discussion:

Stat3 monomer contains four domains, a protein interaction domain which helps in cooperative DNA binding, all-alpha domain comprises of a bundle of four antiparallel helices connected by short loops, DNA binding domain comprises of eight-stranded β-barrel and SH-2 dimerization domain comprises of a central three-stranded β-pleated sheet flanked by a helix and two strands. To predict the inhibition of Stat3 dimerization by curcumin natural derivatives and its amino acid conjugates, these small molecules were docked over SH2 domain of a Stat3 monomer and their binding modes were analyzed **Table 2 & 3 (see supplementary material).**

Demethoxycurcumin bound to SH2 domain with ΔG of -7.80 kcal/mol and KI of 1.93 μM **(Figure 2a)**. Methoxy group of demethoxycurcumin was found to form polar interaction with side chain of ARG-595 while the neighboring hydroxyl group was in polar interaction range with main chain of LYS-591 and side chain of ARG-595. Hydroxyl group present at the other side of the molecule (methoxy group lacking) formed polar interactions with SER-613. Both keto and hydroxyl group present in the linker region were in polar interaction range with SER-636.

It was found that hexahydrocurcuminol bound to SH2 domain with ΔG of -7.69 kcal/mol and KI of 2.31 μM **(Figure 2b)**. One methoxy group of hexahydrocurcuminol was found to form polar interaction with side chain of ARG-595 while the neighboring hydroxyl group was in polar interaction range with LYS-591 and ARG-595. At the other side of the molecule methoxy group formed polar interactions with SER-613 while hydroxyl group was in polar interaction range with GLU-612 and SER-613. In the linker region, one of the hydroxyl group formed polar interaction with SER-636 while other interacted with side chain of LYS-591. Amongst known inhibitors FLLL32, static and sta21, FLLL32 bound to SH2 domain with lowest ΔG of -6.69 kcal/mol and KI of 12.54 μM **(Figure 2c).** Keto groups present in the linker region were found to form polar interactions with LYS-591 and ARG-609 respectively.

Amongst the curcumin-amino acid conjugates curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) docked with lowest ΔG of -6.29 kcal/mol and KI of 24.55 μM **(Figure 2d).** Prolinoyl group at one side of the molecule was in polar interaction range with GLU-530 while at other side it interacted with VAL-637. The hydroxyl group present in linker region of the conjugate formed

polar interaction with LYS-557. The peptide based known inhibitor (Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn) docked with ΔG of -5.50 kcal/mol and KI of 93.10 μM and formed polar interactions with LYS-591, ARG-595 and ARG-609.

Curcumin natural derivatives and its amino acid conjugates bound to SH2 domain through polar interactions with LYS-591, ARG-609, SER-611, GLU-612, SER-613, SER-636 and VAL-637 among which LYS-591, ARG-609, SER-611 and SER-613 are the amino acid residues which remain highly conserved in SH2 domain and play an important role in Stat3 dimerization by forming polar interaction with pTYR-705 residue of other monomer.

Conclusion:

The present computational study provides insights into the inhibition of Stat3 dimerization by curcumin natural derivatives and its conjugates with amino acids. The involvement of residues like LYS-591, ARG-609, SER-611, GLU-612, SER-613, SER-636 and VAL-637 play an important role in binding of curcumin natural derivatives and its amino acid conjugates with SH2 domain. Demethoxycurcumin followed by Hexahydrocurcuminol were predicted to be the most potent inhibitors amongst all the curcumin natural derivatives and known inhibitors (FLLL32, Sta21 and Stattic) docked. Amongst the curcumin-amino acid conjugates curcumin-proline conjugate (1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one) was predicted to be the most potent inhibitor of Stat3 dimerization.

Acknowledgement:

Anil Kumar thank IITG and MHRD (Government of India) for the fellowship to pursue Ph.D. We thank developers of the free softwares used for this study and DBT, Govt of India for providing funds for establishment of Institutional Biotech Hub (BT/04/NE/2009) through which this work was carried out.

References:

[1] Bromberg J, *Breast Cancer Res.* 2000 **2**: 86 [PMID: 11250696]
[2] Yu H & Jove R, *Nat Rev Cancer.* 2004 **4**: 97 [PMID: 14964307]
[3] Bowman T *et al. Oncogene.* 2000 **19**: 2474 [PMID: 10851046]
[4] Turkson J & Jove R, *Oncogene.* 2000 **19**: 6613 [PMID: 11426647]
[5] Buettner R *et al. Clin Cancer Res.* 2002 **8**: 945 [PMID: 11948098]
[6] Turkson J, *Expert Opin Ther Targets.* 2004 **8**: 409 [PMID: 15469392]
[7] Darnell JE, *Nat Med.* 2005 **11**: 595 [PMID: 15937466]
[8] Turkson J *et al. J Biol Chem.* 2001 **276**: 45443 [PMID: 11579100]
[9] Turkson J *et al. Mol Cancer Ther.* 2004 **3**: 261 [PMID: 15026546]
[10] McMurray JS, *Chem Biol.* 2006 **13**: 1123 [PMID: 17113993]
[11] Schust J *et al. Chem Biol.* 2006 **13**: 1235 [PMID: 17114005]
[12] Goel A *et al. Biochem Pharmacol.* 2008 **75**: 787 [PMID: 17900536]
[13] Alexandrow MG *et al. Eur J Cancer Prev.* 2012 **21**: 407 [PMID: 22156994]
[14] Bill MA *et al. Mol Cancer.* 2010 **9**: 165 [PMID: 20576164]
[15] Anand P *et al. Mol Pharmacol.* 2007 **4**: 807 [PMID: 17999464]
[16] Anand P *et al. Biochem Pharmacol.* 2008 **76**: 1590 [PMID: 18775680]
[17] Parvathy KS *et al. Food Chemistry*. 2010 **120**: 523
[18] Dubey SK *et al. Eur J Med Chem.* 2008 **43**: 1837 [PMID: 18201805]
[19] Kumar S *et al. Nucleic Acids Symp Ser.* 2000 **44**: 75 [PMID: 12903275]
[20] Singh RK *et al. Eur J Med Chem.* 2010 **45**: 1078 [PMID: 20034711]

Edited by P Kangueane

Citation: Kumar & Bora, Bioinformation 8(20): 988-993 (2012)

BIOINFORMATION

open access

Supplementary material:

Table 1: Curcumin-amino acid conjugates used in the study

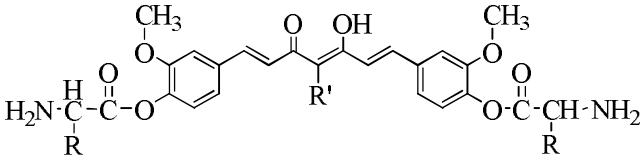
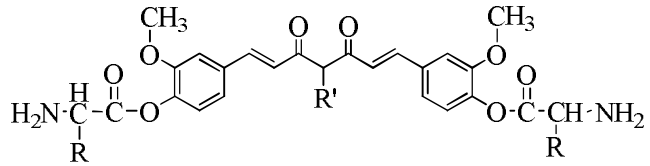
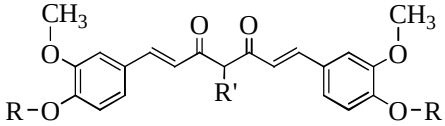
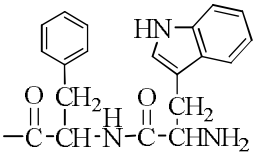
Compound name	R=
Scheme –I (R'=H) [17] 	
1,7-Bis(4-O-L-leucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2CH(CH_3)CH_3$
1,7-Bis(4-O-L-phenylalaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2C_6H_5$
1,7-Bis(4-O-L-alaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_3
1,7-Bis(4-O-L-isoleucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH(CH_3)CH_2CH_3$
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH(CH_3)CH_3$
1,7-Bis(4-O-L-serinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_2OH
1,7-Bis(4-O-L-cysteinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—CH_2SH
1,7-Bis(4-O-L-phenylglycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—C_6H_5
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—H
1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	—$CH_2CH_2CH_2$— (part of pyrrolidine ring)
Scheme –II (R'=H) [18] 	
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—H
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—$CH(CH_3)CH_3$
1,7-Bis(4-O-L-glutamatoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	—CH_2CH_2COOH
Scheme –II (R'=$COCH_2NH_2$) [19]	
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-4-glycyl-1,6-heptadiene-3,5-dione	—H
Scheme –III (R'=H) [20] 	
1,7-Bis(4-O-L-tryptophanylphenylalaninoyl-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione	

Table 2: Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interactions of curcumin natural derivatives with Stat3 monomer

Compound Name	ΔG (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
Demethoxycurcumin	-7.80	1.93	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637
Hexahydrocurcuminol	-7.69	2.31	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637
Hexahydrocurcumin	-7.32	4.39	LYS- 591, ARG-595, GLU-612,SER-613, SER-636	PHE-610, ILE-634,VAL-637
Bisdemethoxycurcumin	-7.19	5.39	LYS- 591, ARG-595,SER-611,GLU-612,SER-613,SER-636	PHE-610, ILE-634,VAL-637
Curcumin sulphate	-7.11	6.16	LYS-557,ILE-634	PHE-610, VAL-619,VAL-637
Curcumin (keto)	-7.09	6.31	LYS- 591, ARG-595,ARG-609,GLU-612,SER-613,SER-636	PHE-610, ILE-634, VAL-637

Curcumin (enol)	-6.94	8.16	LYS- 591, ARG-595, ARG-609, GLU-612	PHE-610, ILE-634,VAL-637
FIII32*	-6.69	12.54	LYS- 591,ARG-609	PHE-610, ILE-634,VAL-637
Sta21*	-6.61	14.25	ARG-609, SER-636	ILE-634,VAL-637
Tetrahydrocurcumin	-6.49	17.56	LYS- 591, ARG-595, ARG-609, SER-613,SER-636	PHE-610, ILE-634,VAL-637
Stattic*	-6.45	18.79	LYS- 591, ARG-595	ILE-634
Cyclocurcumin	-6.42	19.82	LYS- 591, ARG-609,GLN-635	PHE-610, ILE-634,VAL-637
Curcumin glucuronide	-5.97	42.40	LYS- 591, ARG-595, ARG-609,ILE-634	PHE-610,VAL-637
β-Turmerone	-5.39	112.11	LYS- 591	ILE-634,VAL-637
α-Turmerone	-5.31	127.61	LYS- 591	ILE-634,VAL-637

*Known inhibitor of Stat3

Table 3: Free energy of binding (ΔG) and predicted inhibition constant (KI) estimated with AutoDock 4.2.1 and interactions of curcumin-amino acid conjugates with Stat3 monomer

Compound name	ΔG (kcal/mol)	KI (μM)	Polar interactions	Hydrophobic residue in 5 Å region
1,7-Bis(4-O-L-prolinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-6.29	24.55	GLU-530, LYS-557,VAL-637	PHE-610,VAL-619
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-5.96	42.93	GLU-530,LYS-591,ARG-595,SER-636	ILE-634,VAL-637
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-5.51	91.60	LYS-591,ARG-609,ILE-634, GLU-638	PHE-610,VAL-637
Phpr-pTYR-LEU-cis-3,4-methanoPRO-GLN-NHBn*	-5.50	93.10	LYS-591,ARG-595, ARG-609	PHE-610, ILE-634,VAL-637,TYR-657
1,7-Bis(4-O-L-isoleucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-5.32	125.58	GLU-530,LYS-557,SER-590,SER-636	VAL-637
1,7-Bis(4-O-L-tryptophanylphenylalaninoyl-3-methoxyphenyl)- 1,6-heptadiene-3,5-dione	-5.17	162.04	LYS-557,LYS-591,GLU-592,SER-613,GLU-638	PHE-610, ILE-634,VAL-637,TYR-640
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-4-glycyl-1,6-heptadiene-3,5-dione	-5.00	216.91	LYS-591,ARG-609,SER-613,GLU-638	PHE-610,ILE-634,VAL-637
1,7-Bis(4-O-L-phenylalaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.97	226.37	GLU-530,LYS-557,SER-636,VAL-637	PHE-610,ILE-634
1,7-Bis(4-O-L-valinoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-4.89	261.36	GLU-592,ARG-595,SER-636	ILE-634,VAL-637
1,7-Bis(4-O-L-leucinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.79	307.46	GLU-530,ARG-595,SER-636	ILE-589,ILE-634,VAL-637
1,7-Bis(4-O-L-alaninoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.77	317.43	LYS-557,GLU-612,GLU-638	PHE-610,VAL-637
1,7-Bis(4-O-L-serinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.62	407.78	GLU-530,GLY-558,SER-590,GLU-592,ARG-593,GLU-612	PHE-559
1,7-Bis(4-O-L-glutamatoyl-3-methoxyphenyl)-1,6-heptadiene-3,5-dione	-4.57	448.31	LYS-557,LYS-591,ARG-609,GLU-638	PHE-610,VAL-637
1,7-Bis(4-O-L-glycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-4.17	871.38	GLU-530,SER-636	LEU-528,ILE-634,VAL-637
1,7-Bis(4-O-L-phenylglycinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-3.75	1790.00	LYS-591,GLN-635,SER-636	LEU-532,ILE-634,VAL-637
1,7-Bis(4-O-L-cysteinoyl-3-methoxyphenyl)-1,4,6-heptatriene-5-ol-3-one	-3.62	2210.00	SER-613,LYS-615,GLU-616,SER-636	VAL-619,ILE-634,VAL-637,TYR-640

*Known inhibitor of Stat3