BIOINFORMATION
Discovery at the interface of physical and biological sciences

open access www.bioinformation.net Hypothesis

Volume 10(12)

Alkyloxy carbonyl modified hexapeptides as a high affinity compounds for Wnt5A protein in the treatment of psoriasis

Ankit Kelotra[1]*, Sadashiv M Gokhale[1], Seema Kelotra[2], Vaidehi Mukadam[4], Komal Nagwanshi[4], Srinivas Bandaru[3], Anuraj Nayarisseri[4] & Anil Bidwai[5]

[1]School of Biochemistry, Devi Ahilya University, Takshashila Campus, Khandwa Road, Indore (M.P.) – 452 017, India; [2]M.B Khalsa College, Near Gangwal Bus Stand, Indore (M.P.)-452011, India; [3]Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad – 500 016, India; [4]In silico Research Laboratory, Eminent Biosciences, Indore – 452 010, India; [5]Index medical College, Indore (M.P)-452010, India; Ankit kelotra – Email: ankitkelotra22@gmail.com; *Corresponding author

Received December 18, 2014; Accepted December 24, 2014; Published December 31, 2014

Abstract:
Psoriasis is one of the most prevalent chronic inflammatory diseases of the skin. The Wnt pathways have been documented to play essential role in stem cell self-renewal and keratinocyte differentiation in the skin. Antagonizing the Wnt5a protein would emerge as a novel therapeutics in psoriasis treatment. In this view, we have developed and characterized series of compounds by attaching varied tertiary alkyloxy carbonyl groups at the N-terminal end of the hexapeptide (Met-Asp-Gly-Cys-Glu-Leu) bestowed to inhibit Wnt/Ca2+ signaling in psoriasis. Hexapeptide compound with 1,1-diphenylethoxy carbonyl group attached to N-terminal end of hexapeptide demonstrated highest binding affinity amongst all the evaluated compounds. The compound identified in the study can be subjected further for *in vitro* and *in vivo* studies for ADMET properties.

Keywords: Psoriasis, Wnt5A, Protein Modeling, Alkyloxy carbonyl modified hexapeptides, Molecular docking

Background:
Psoriasis is an autoimmune and non-infectious dermatological disease well characterized by defined red, slightly raised plaques and papules with silvery scales on skin. In psoriasis, hyperproliferative keratinocytes lack terminal differentiation **[1]** as seen by the formation of hyperparakeratotic stratum corneum **[2, 3].** The universal occurrence varies from 0.1% to 3 % in different parts of the world **[4]** and about 5% population suffers in the Indian subcontinent **[5, 6].** According to the National Psoriasis Foundation, 20,000 children under 10 years of age are diagnosed with psoriasis annually **[7].** Till date, the precise cause of psoriasis is unknown; however the genetic component is now believed to play an important role in pathogenesis.

In the recent years, a range of signaling pathways were found to be modulated in psoriatic plaques. In particular, the Wnt pathways **[8, 9]** have an essential role in stem cell self-renewal and keratinocyte differentiation in the skin **[10, 11].** The involvement of Wnt pathways in psoriasis has been evidenced from the pioneering studies by Joachim Reischl et al., 2007 **[12]** wherein differentially expressed genes in canonical Wnt/ β-catenin or the non-canonical Wnt/Ca2+ pathways were involved in the pathophysiology of psoriasis. Functionally, Wnt5a lowers the concentration of IFN required to induce target genes, and increases the magnitude of IFN target gene induction, suggesting a molecular mechanism underlying IFN hypersensitivity in psoriasis **[13].** The marked over expression of Wnt5a and Fzd5 in psoriasis suggests that this ligand receptor pair may actively derive the chronic inflammatory and hyper- proliferative nature of this phenotype. The broad implications of Wnt/β-catenin signaling in psoriasis skin render the pathway a prime target for pharmacological research and therapeutic interventions. Therefore, in the view

of above, we have developed and characterized a Wnt5a specific antagonist peptide with the capacity to inhibit Wnt/Ca2+ signaling in psoriasis. Peptides inhibiting Wnt pathway has been well established in melanoma cells, however the condition of psoriasis has been still untouched. Since the Wnt pathway operates the similar way in malanoma as well as in psoriasis we anticipate the compounds proposed in the present study can come with novel therapeutic strategy in controlling the pathological condition.

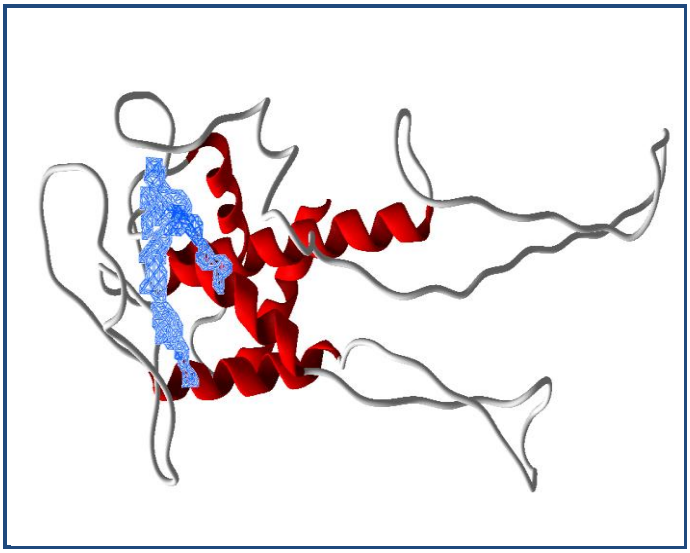


Figure 1: Modeled structure of the Wnt5a protein. Binding site (blue mesh) in the protein was detected using Gaussian Filter enabled DoGSite server

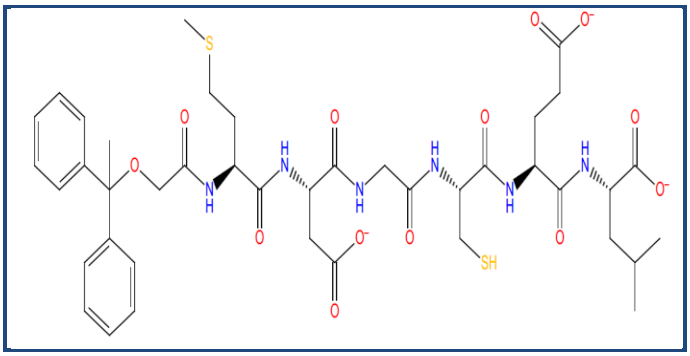


Figure 2: Compound 11. 1,1-diphenylethoxy group attached to N-terminal of hexapeptide (Met-Asp-Gly-Cys-Glu-Leu)

Interestingly, it has previously been shown that modification of a formylated bacterially derived chemotactic peptide (formyl-Met-Leu-Phe), converted the molecule from an agonist to an antagonist analogue. Specifically, the modification involved substitution of the N-terminal formyl group for a t-butoxycarbonyl (t-boc) group. As hypothesized by Jenei *et al.* **[14]** such a modification could also change its Wnt5a agonist functions to that of an antagonist. Therefore in the pioneering study, Jenei et al. synthesized and purified such a t-boc-Met-Asp-Gly-Cys-Glu-Leu peptide which acts as a potential antagonist for Wnt5a protein. However, in the further endeavors, we have proposed series of compounds by attaching tertiary alkyloxy carbonyl groups at the N-terminal end of the hexapeptide. Given the distinct lack of therapeutics available for psoriasis progression and the potency of Wnt5a to increase the pathological condition we believe that inhibition of Wnt5a expression and signaling would be a promising therapeutic approach for this disease.

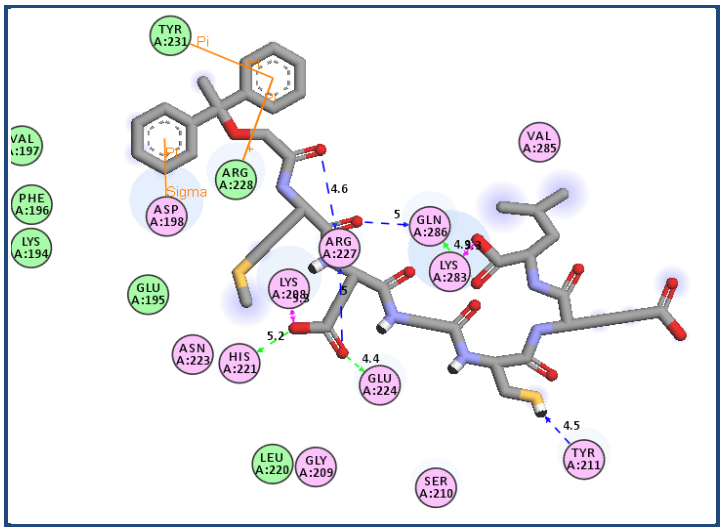


Figure 3: Interactions of compound 11 with Wnt5 a protein. Residues circled in green participate in van der waals interaction with the ligand while residues in pink forms electrostatic interactions. Hydrogen bonds with bond lengths are shown as blue arrows between ligand and residues

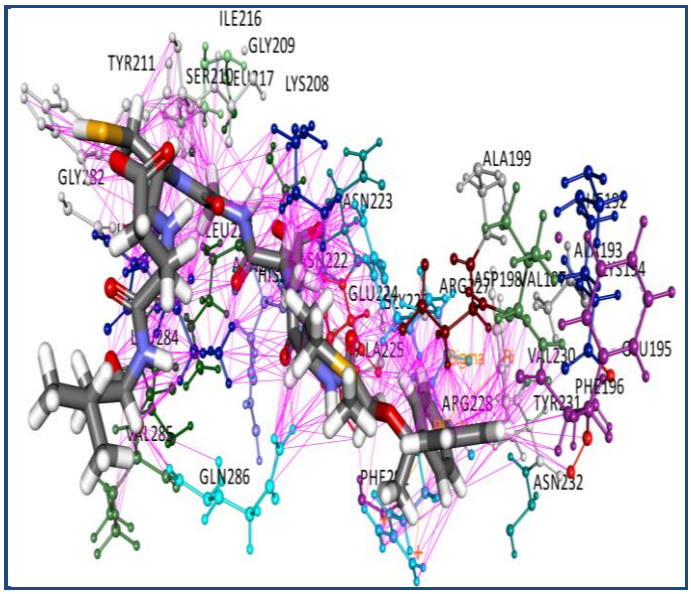


Figure 4: Binding pattern of compound 11 with Wnt5A. The pink lines represent various interactions like electrostatic, van der Waals, stearic, hydrogen bonding and hydrophobic interactions that enable energetically favourable binding of the ligand in the cavity.

Methodology:

Ligand Design and Core Optimization

The sequence of hexapeptide (Met-Asp-Gly-Cys-Glu-Leu) was designed and optimized using Accelrys Discovery Studio 3.5 DS Visualizer. The alkyloxy carbonyl groups **Table 1 (see supplementary material)** were attached to the N′ terminal of the hexapeptide at methionine residue by Marvin Sketch 5.6.0.2 **[15].** The series of structures were further optimized and

energy minimized using through Ligprep module of Schrodinger Suite 2013.

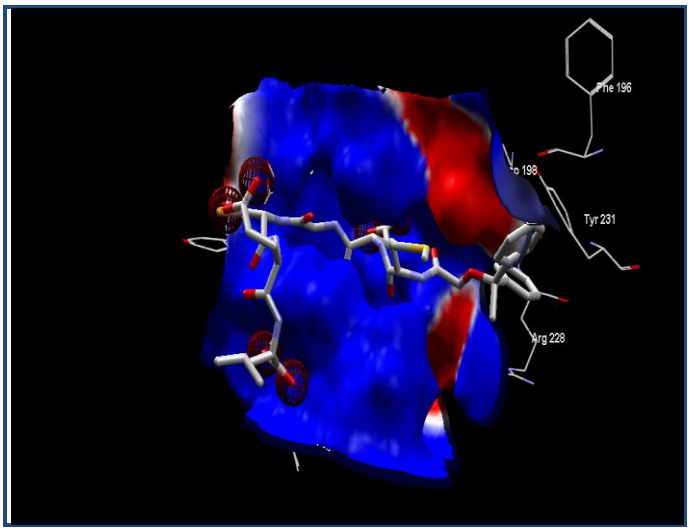


Figure 5: Electrostatic surface of Wnt5a receptor bound with compound 11

Protein Modeling and Validation

Protein sequence of Wnt 5a retrieved from NCBI using accession num: AAH74783.2. 3D structure of Wnt 5a is not available in Protein Data Bank; hence 3D structure prediction of Vif has been performed using Swiss model server. The model was further validated using Procheck **[16, 17, 18, 19]**.

Prediction of Binding Site

The inhibitory site of the modeled structure was identified using Q site finder **[20].** Q site finder is a method of ligand binding site prediction which works by hydrophobic (CH_3) probes to the protein and finding clusters of probes with most favourable binding energy. These clusters are placed in rank order of the likelihood of binding site according to the sum total binding energies of each cluster.

Molecular Docking of Compounds

Molecular docking program - Molegro Virtual Docker 2010.4.0. provide a flexible platform for docking compounds. The structure based virtual screening of compounds was based on rerank score which is the mathematical representation for ligand-protein affinity. Rerank score is based on MolDock scoring function (MolDock Score) derived from the Piecewise Linear Potential (PLP) scoring functions **[21, 22].** Docking parameters were set to 0.20 Å as grid resolution, maximum iteration of 1500 and maximum population size of 50. Energy minimization and hydrogen bonds were optimized after the docking. Simplex evolution was set at maximum steps of 300 with neighborhood distance factor of 1. After the ligand was docked the total energy was minimized using Nelder Mead Simplex Minimization **[22]** (using non-grid force field and H bond directionality). Binding affinity and interactions of the compound with receptor was evaluated on the basis of the internal electrostatic, hydrogen bond interactions and sp2-sp2 torsions. On the basis of rerank score, best compound with optimal binding affinity was selected against Wnt 5a protein.

Pharmacophoric Mapping

Pharmacophric mapping which involves ligand interaction patterns, hydrogen bond interaction, hydrophobic interactions and solvent accessible surface area upon ligand binding was evaluated using Accelrys Discovery Studio 3.5 DS Visualizer **[21].**

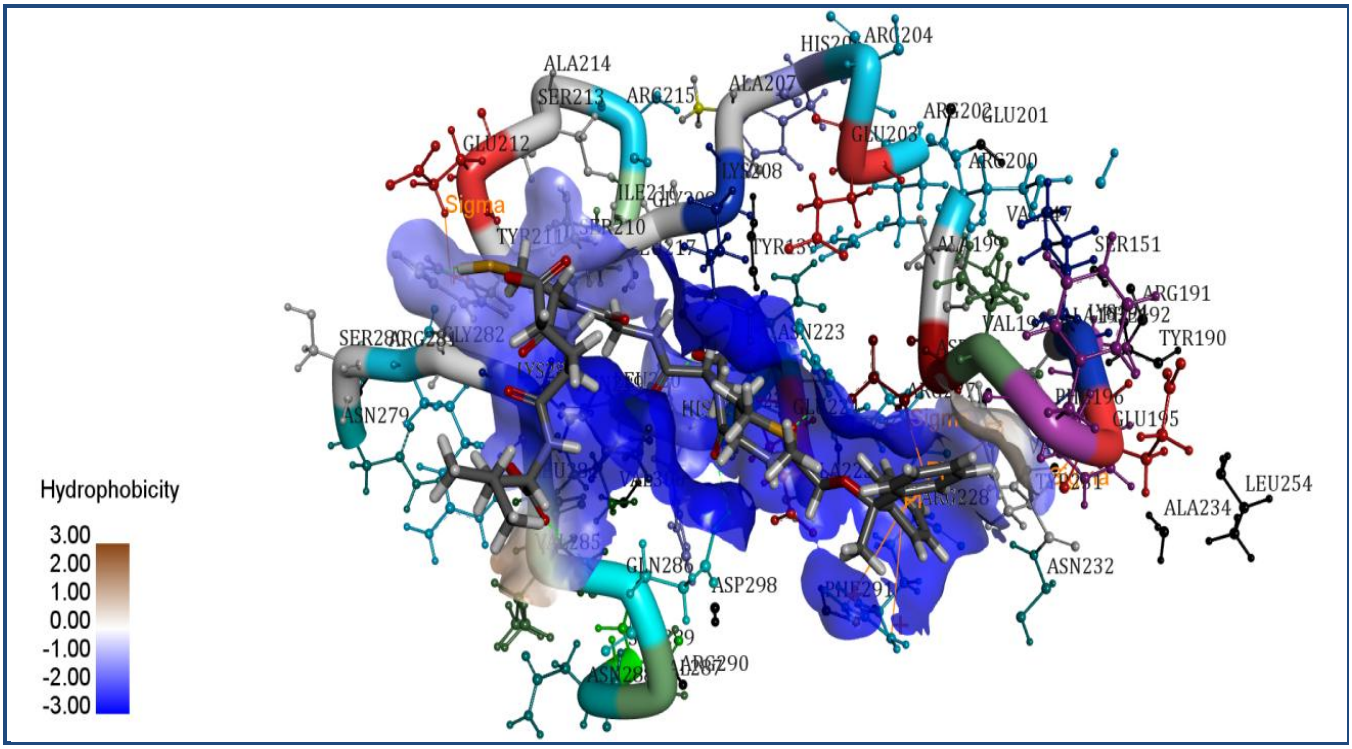


Figure 6: The active site of receptor is shown with hydrophobic intensities. The hydrophobic intensities of the binding site ranges from -3.00 (least hydrophobic area - blue shade) to 3.00 (highly hydrophobic area –brown shade).

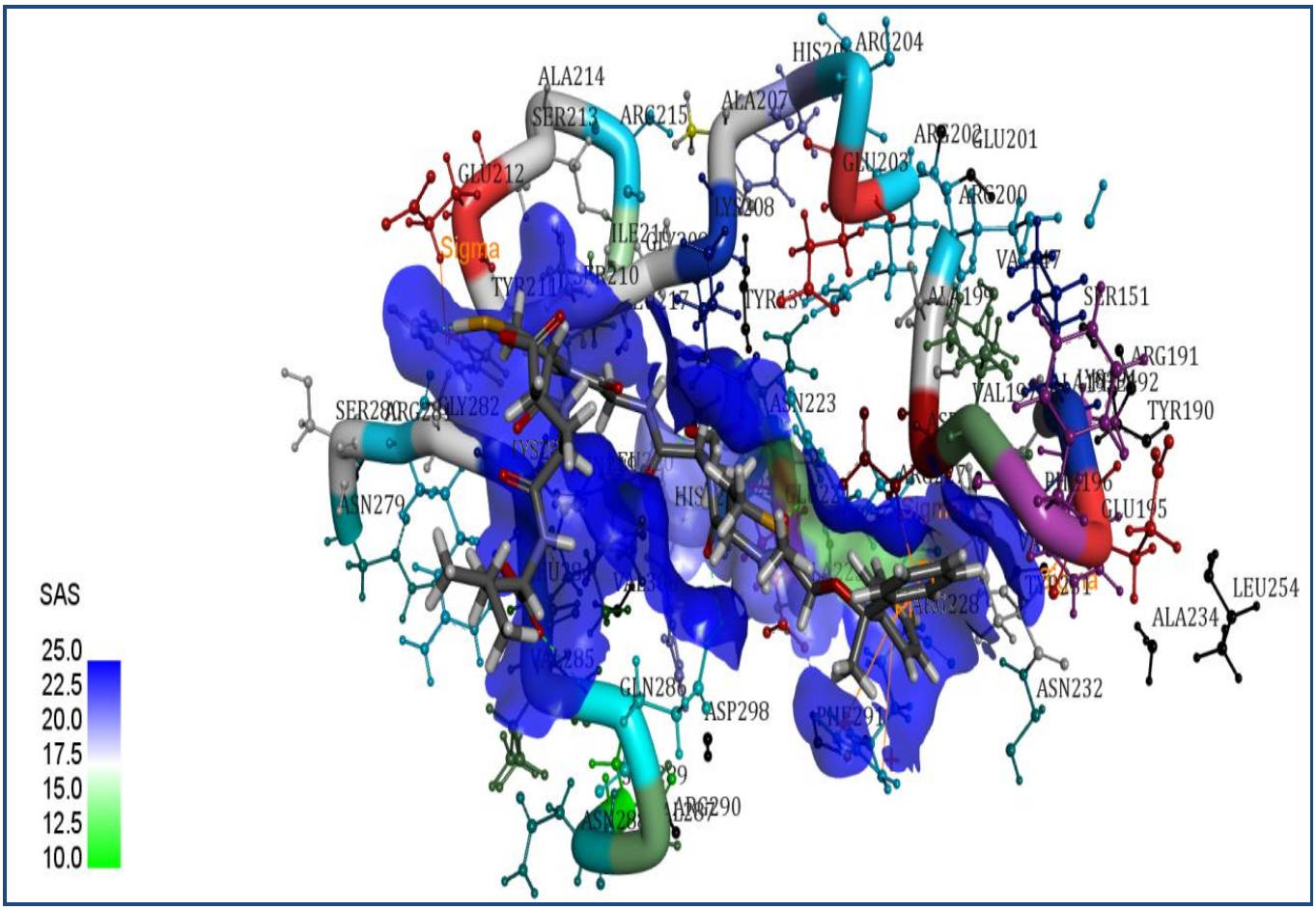


Figure 7: The active site of receptor is shown with solvent accessible surface areas. The accessibility to solvent of the binding site ranges from 10.00 (least solvent accessibility - green shade) to 25.00 (highly solvent accessible –blue shade).

Results & Discussion:

The procheck results revealed the modeled Wnt5a protein to be bonafide **(Figure 1).** Three cavities with different volume were detected by Qsite finder in Wnt5a protein. As a convention, the volume with highest volume was considered to be an active (inhibitory) site. The inhibitory site had a volume of 841 Cubic Angstroms with minimum coordinates of (-21, -15, -10) and maximum coordinates of (-1, 14, 14). The inhibitory site is shown in **Figure 1**. Evident from rerank score, out of 13 compounds, compound 10 showed highest and optimal binding affinity. The overall ligand binding affinity of all the compounds against Wnt 5a is shown in **Table 2 (see supplementary material)**.

Since compound 11 (**Figure 2**) showed highest binding affinity against Wnt5a protein it was further subjected to pharmacophoric identification. In the figure 3, in the binding site of Wnt 5 A compound 11 demonstrates van der waals interaction with Tyr 231, Val 197, Arg 228 Glu 195, Leu 220 Lys 194 and electrostatic interactions with Asp 198, Asn 223, His 221, Gly 209, Arg 227. Residues like Lys 283 Tyr 211, acts as hydrogen bond donors while, Glu 224, His 221, lys 283, Arg 227, Gln 286 acts as an acceptor. The hydrogen bonding profile is shown is shown in **Table 3 (see supplementary material)**. The ligand binding pattern is shown in **Figure 4**. Further the electrostatic, hydrophobic interactions and solvent accessible area upon ligand binding of compound 11 with Wnt receptor is shown in **Figures 5, 6 & 7** respectively.

Conclusion:

In spite of major advances in medical science, the treatment of psoriasis is still in infancy and there is a need for better Wnt 5a antagonists. Hence, we have proposed alkyloxy modified hexapeptides and evaluated its binding affinity against Wnt5a receptor. In the present study, out of 13 proposed compounds, in particular compound 11 with 1,1-diphenylethoxy group attached to N-terminal of the hexapeptide (Met-Asp-Gly-Cys-Glu-Leu) found to high affinity compound against Wnt 5a protein, however further consideration are required using *in vitro* analysis.

References:

[1] Watt FM *et al. EMBO J.* 2002 **21**: 3919 [PMID: 12145193]
[2] Djemadji- Oudjiel N *et al. Arch Dermatol Res.* 1996 **288:** 757 [PMID: 8950456]
[3] Gammal EL *et al. Acta Derm Venereol Suppl (Stockh).* 1994 **186:** 173 [PMID: 8073827]
[4] Raychaudhuri SP & Farber EM, *J Eur Acad Dermatol Venereol.* 2001 **15**: 16 [PMID: 11451313]
[5] Dogra S & Yadav S, *Indian J Dermatol Venereol Leprol.* 2010 **76**: 595 [PMID: 21079301]
[6] Azfar RS & Gelfand JM, *Curr Opin Rheumatol.* 2008 **20**: 416 [PMID: 18525354]

[7] Christophers E, *Clin Exp Dermatol*. 2001 **26**: 314 [PMID: 11422182]
[8] Reya T & Clevers H, *Nature.* 2005 **434**: 843 [PMID: 15829953]
[9] Li M *et al. Nature.* 2000 **407**: 633 [PMID: 11034212]
[10] Blanpain C *et al. Cell*. 2004 **118 :** 635 [PMID: 15339667]
[11] Hong J *et al. ACS Chem Biol*. 2007 **20**: 171 [PMID: 17348628]
[12] Gudjonsson JE *et al. J Invest Dermatol.* 2010 **130**: 1849 [PMID: 20376066]
[13] Romanowska M *et al. PLoS One.* 2009 **4**: e5354 [PMID: 19399181]
[14] Jenei V *et al. Proc Natl Acad Sci U S A*. 2009 **106**: 19473 [PMID: 19901340]
[15] Csizmadia F, *J Chem Inf Comput Sci.* 2000 **40**: 323 [PMID: 10761134]
[16] Laskowski RA *et al. J Biomol NMR*. 1996 **8**: 477 [PMID: 9008363]
[17] Morris AL *et al. Proteins*. 1992 **12:** 345 **[**PMID: 1579569**]**
[18] Tabassum A *et al. Interdiscip Sci.* 2014 **6**: 32 [PMID: 24464702]
[19] Nayarisseri A *et al. Interdiscip Sci.* 2013 **5**: 274 [PMID: 24402820]
[20] Sinha C *et al. Bioinformation*. 2014 **10**: 611 [PMID: 25489169]
[21] Bandaru S *et al. Bioinformation.* 2014 **10**: 652 [PMID: 25489175]
[22] Bandaru S *et al. Curr Top Med Chem.* 2013 **13**: 1650 [PMID: 23889054]

Edited by P Kangueane
Citation: **kelotra *et al.*** Bioinformation 10(12): 743-749 (2014)

BIOINFORMATION

open access

Supplementary material:

Table 1: Proposed compounds in the study. The hexapeptides (Met-Asp-Gly-Cys-Glu-Leu) have been modified at the N-terminal with various Alkyloxy groups

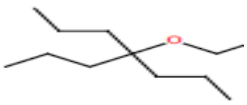
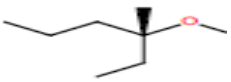
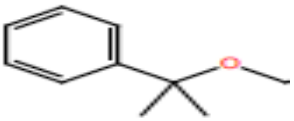
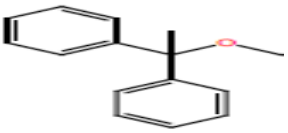
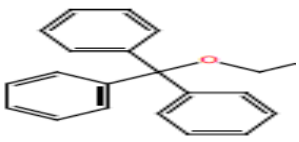
Srl No.	Hexapeptide	N-terminal Alkyloxy Group	Structure
1	Met-Asp-Gly-Cys-Glu-Leu	(3-ethylpentan-3-yl)oxy	
2	Met-Asp-Gly-Cys-Glu-Leu	(3-ethylpentan-3yl)oxy	
3	Met-Asp-Gly-Cys-Glu-Leu	(2,3-dimethylbutan-2-yl)oxy	
4	Met-Asp-Gly-Cys-Glu-Leu	(2,3,4-trimethylpentan-3-yl) oxy	
5	Met-Asp-Gly-Cys-Glu-Leu	(2-methylpentan-2-yl)oxy	
6	Met-Asp-Gly-Cys-Glu-Leu	(4-methylheptan-4-yl)oxy	
7	Met-Asp-Gly-Cys-Glu-Leu	(4-propylheptan-4-yl)oxy	
8	Met-Asp-Gly-Cys-Glu-Leu	(4-ethylheptan-4-yl)oxy	
9	Met-Asp-Gly-Cys-Glu-Leu	(3-methylhexan-3-yl)oxy	
10	Met-Asp-Gly-Cys-Glu-Leu	(2-phenylpropan-2-yl)oxy	
11	Met-Asp-Gly-Cys-Glu-Leu	1,1-diphenylethoxy	
12	Met-Asp-Gly-Cys-Glu-Leu	triphenyl methoxy	
13	Met-Asp-Gly-Cys-Glu-Leu	t-botoxy	

Table 2: Binding affinity score (Rerank score) of hexapeptide compounds belonging to each series. The compounds have been arranged to their descending order of their affinity

Ligand	MolDock Score	Re-rank Score
Compound 11	-233.572	-150.967
Compound 9	-197.889	-138.036
Compound 8	-219.397	-137.988
Compound 5	-212.187	-130.637
Compound 7	-247.833	-122.887
Compound 10	-228.073	-119.464
Compound 2	-215.247	-116.761
Compound t-boc	-186.442	-115.457
Compound 4	-188.042	-111.814
Compound 12	-172.592	-102.846
Compound 3	-199.724	-99.6207
Compound 1	-197.941	-98.6507
Compound 6	-193.195	-94.7702

Table 3: Hydrogen bonding profile of compound 11 with Wnt5a

Participating Residue	Energy
Arg 227	-2.09664
Gln 286	-2.5
Lys 283	-0.46511
Tyr 211	-0.59265
Glu 224	-0.10557
His 221	-1.29006
Lys 288	-0.5182
His 221	-2.34436