Int J Pharm Bio Sci 2015 Oct; 6(4): (P) 70 - 82

Research Article **Pharmaco Informatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EFFECTIVE *IN SILICO* SCREENING OF NATURAL AND SYNTHETIC COMPOUNDS FOR INHIBITION OF P-38 MAPK AS A THERAPEUTIC APPROACH IN PROGRESSIVE END-STAGE RENAL FAILURE

RAMESH KUMAR G* AVINASH RAMANI AND MANONANTHINI THANGAM

Bioinformatics Lab, AU-KBC Research Centre, MIT, Anna University, Chennai 600044, INDIA

ABSTRACT

Apoptosis and Epithelial to Mesenchymal Trans-differentiation (EMT) are considered to be the primary cellular pathophysiological mechanism that are mediated by response-specific p-38 signaling networks in various forms of inflammatory fibrogenic diseases. Growth factors such as Transforming Growth Factor-β (TGF – β),Mitogen Activated Protein Kinases (MAPKs) including p-38 MAPK and others are important for the signal transduction of response-specific signaling networks in various forms of renal tissue injury leading to fibrosis. p-38 MAPK is an important target for the complex pro-inflammatory cytokine pathway and play a major role in the pathophysiology of various diseases like cancers, liver cirrhosis, renal failure, or cystic fibrosis. Over-expression of p-38 MAPK can induce renal fibrosis, causing kidney tubule damage, and ultimately End-Stage Renal Disease (ESRD). It has been recently found that, using various types of antagonists against these kinases, one can halt or even reverse renal fibrosis and even partially cure renal failure. Thus, targeting p-38 MAPK will play a major role in the novel targeted drug development for the prevention of complete damage of the kidney. Structurally diverse natural anti-inflammatory compounds such as flavones, indoles and stilbenes and their semi-synthetic derivatives were screened for the evaluation of inhibitory potential of p-38 MAPK by Virtual High Throughput Screening (vHTS). The p-38 MAPK protein was remodeled and the compounds were docked using an efficient docking algorithm to find out the good inhibitor with better pharmacological action. Quantitative Structural Activity Relationship (QSAR) studies were performed for the evaluation of molecular descriptors for the prediction of drug likeliness and druggability properties. It helped in the identification of potential leads that could be used as an antagonist for p-38 MAPK.

KEYWORDS: p-38 MAPK, Virtual High Throughput Screening, End-Stage Renal Disease, anti-inflammatory compounds, druggability.

DR. G. RAMESH KUMAR
Bioinformatics Lab, AU-KBC Research Centre, MIT,
Anna University, Chennai 600044, INDIA.

INTRODUCTION

Apoptosis and Epithelial to Mesenchymal Transdifferentiation (EMT) are considered to be the primary cellular pathophysiological mechanism, mediated by response-specific p-38 signaling networks in various forms of inflammatory fibrogenic diseases [1]. Growth factors such as Transforming Growth Factor-β (TGF – β),Fibroblasts Growth Factors, Hepatocyte Growth Factors, Cytokines and Mitogen Activated Protein Kinases (MAPKs) that includes p-38 MAPK, c-Jun kinases or Stress-activated Protein Kinase (JNK / SAPK), Extracellualar signal-regulated kinases (ERKs) among others are essential signal transduction enzymes that regulates cellular processes and maintains homeostasis[1-4]. The p-38 MAPK are a class of MAPK which are responsive to stress stimuli such as cytokines, ultraviolet irradiation, heat shock, osmotic shock etc., and are involved in cell differentiation and apoptosis. There are four isoforms of p38-MAP Kinase, namely, the p38-α (MAPK14), p38-β (MAPK11), p38-γ (MAPK12 or ERK6) and p38–δ (MAPK13 or SAPK4). Activation of p38MAPK is an important signal transduction step in several acute and chronic inflammatory disorders, including liver cirrhosis, renal failure, cardiomyopathy, cancers, osteoporosis [5, 6]. The expressions, activation of each isoforms of p-38 MAPK are differentially regulated. Specific inhibitors for p38-α MAPK and p38-β MAPK are being developed and studied for various chronic inflammatory diseases [6, 7]. Several evidences regarding the pro-inflammatory cytokine pathway with EMT has been established in recent years.Interstitial infiltration of inflammatory macrophages in association with tubular atrophy, EMT and fibrosis characterizes End Stage Renal Disease [8]. EMT is considered to be the primary cellular pathophysiological mechanism, mainly mediated by response-specific p-38 signaling networks in various forms of renal tissue injury leading to fibrosis. Any small scar in renal tissue stimulates the proximal tubules of nephrons to produce growth-factors like TGF–β –1, chemokines and cell adhesion molecules such as MCP-1. The chemoattractant proteins like MCP–1 attract monocytes from systemic blood circulation and allow macrophages infiltration into the renal tissues, which are activated by cytokines, growth factors and nitric oxide and subsequently produce several profibrotic factors by the action with TGF–β – 1 to stimulate fibroblast cells leading to EMT [9,10]. The activation of both p38 MAPK and JAK/STAT signaling pathways plays an important role in the plasmin-mediated monocytes chemotaxis and subsequent induction of gene expression of MCP-1 and CD40, both of which are important effectors of inflammation and apoptosis [11]. It is also known that the binding of tumour necrosis factor (TNF-α) and interleukin (IL-1β) to their respective receptors are the results of activation of various kinases, including p38MAPK. Upon activation, these kinases lead to the activation of nuclear factor-$_K$B (NF_KB), which in turn translocates the nucleus and bind to promoters of target genes encoding cytokines, COX-II, iNOS, and proteases in a sequence-specific manner. Thus, the activation of p-38 MAPK plays an important role in the production of pro-inflammatory cytokines (TNF-α, IL-1β) and induction of enzymes such as COX-II and iNOS [12, 13]. Several experimental data demonstrates the competitive inhibition of p-38 MAPK activity by the administration of pyrazole and imidazole derivatives SB239063, SB203580, and SB202190. These compounds inhibit the endotoxins-induced CXC chemokine formation, leukocyte recruitment and cellular apoptosis in the kidneys, suggesting that p-38 MAPK may be a useful target to treat pathological information in kidney injury [3]. Blocking both the p38 MAPK and TGF-β1/Smad signaling pathways by administration of a p38 MAPK inhibitor (SB203580 and SB202190) and a TGF-β receptor 1 inhibitor (ALK5I) to ADR-injected mice rescued BM-derived PTC endothelial cells from apoptosis, reduced the loss of Peritubular Capillary (PTC), and restored kidney function [14, 15]. A few synthetic compounds with proven inhibitory properties for pro-inflammatory signaling enzymes such as the p-38MAPK, ERK or TGF- β inhibitors were taken as basic datasets. Many natural phytochemical compounds such as flavones, quinones, anthraquinones, polyphenol, stillbenes, terpenes, and alkaloids have been found to be capable of possessing anti-inflammatory properties [16 - 22]. The inhibitory potential of these new natural scaffolds was evaluated using a combination of *in silico* studies. This study helped in identification of new sets of natural compounds which could further be considered for novel drug discovery for chronic inflammatory diseases such as cancers, renal failure or liver cirrhosis.

MATERIALS AND METHODS

Datasets

The crystal structure of Human p-38 – MAPK protein in complex with 1-{3-[(6-aminoquinazolin-4-yl) amino] phenyl} - 3-[3-tert-butyl-1-(3-methylphenyl) -1H-pyrazol- 5-yl] urea (PDB ID: 3GCV) was taken from

Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/home/) [23]. Energy Minimization and structure refining was carried out using Swiss PDB Viewer(http://spdbv.vital-it.ch) and Accelrys Discovery Studio Visualizer (http://accelrys.com/products/discovery-studio/) to obtain a refined structure for performing docking studies [24].The ligand data set was collected by selectively choosing natural phytochemical compounds based on their immuno-modulatory, anti-inflammatory, diuretic, kinase inhibitory and anti-neoplastic properties. These natural compounds were then screened out for their satisfaction of the Lipinski's Rule of Five. A total of 64 alkaloids, 67 Quinones and 53 other natural compounds including terpenes, stillbenes and flavanoids along with their structural derivatives accounting for Three Hundred and Ninety Six structural analogues were drawn and optimized using ChemSketch(http://www.acdlabs.com/resources/freeware/chemsketch/) and Accelrys Discovery Studio Visualizer. These tools offers platform for functions like structure refining and optimization. This allows the structures to be in the ground-state with minimal energy value.Sixteen synthetic compounds, chiefly the proven inhibitors of key proteins of pro-inflammatory cytokine pathway like TGF-β, ERK, CXCR2, and JNK were taken as basic dataset for this comparative study and were screened for the inhibitory potential of the protein p-38 MAPK and compared with SB202190, the proven inhibitor of the same. The Table 1 below gives an account of the binding energies of these sixteen synthetic and semi-synthetic compounds.

Table 1
Docking Energy values of selected synthetic compounds

Sl. No.	Compound Name	Docking Energy
1	SB-431542	-11.37
2	a96	-11.06
3	Reparixin	-10.56
4	SB 203580	-10.48
5	a55f	-10.24
6	Gleevac	-9.98
7	FR180204	-9.77
8	SB 202190	-9.69
9	PD98059	-9.59
10	SB 225002	-9.50
11	U0126	-9.09
12	PG-892579	-9.05
13	VX-745	-8.88
14	2-aminoimidazole	-8.33
15	AG1295	-8.16
16	SB 239063	-7.61

Virtual High Throughput Screening

Molecular Docking and Virtual Screening is an integral part of modern drug discovery. The screening of all compounds for the calculations of binding energy at the active site was performed through molecular docking studies using AutoDock 4.2.6(http://autodock.scripps.edu/). A total of 412 structures, including the sixteen synthetic compounds were docked and ranked upon on the basis of their binding energy values. The active site for the docking was found using Q-site Finder. The grid box size was set at 1.829 x -7.317 x 14.072 points around the active site, with a spacing of 0.375. The active site for the docking was defined with the amino acids - Val-30, Ser-32, Tyr35, Ala-51, Glu-71, Cys-72, Lys-53, Met-109 His-148, Leu-167, Leu-171, Asp-168 and Phe-169. Default docking protocol was applied. Genetic Algorithm search was applied with an initial population of 150 randomly placed individual structures with a mutation rate of 0.02 and a crossover rate of 0.8 for a maximum of 2.7 x 10^4 generation and 2.5 x 10^6 energy evaluations. Only the best fifty-one natural compounds with lowest docking energy were taken for interactions analysis at the active–site. Among them, only the top fifteen which possessed good interactions with maximum residues at the active-site were further evaluated for the study of molecular properties such as drug-likeliness and ADME / Toxicity profiling. The table in the results section gives the summary of the

interactions of fifteen best- ranked natural compounds.

Molecular Descriptor Calculations

1) Quantitative Structure-Activity Relationships (QSARs) models are used to describe the molecular properties of compounds to the biologically significant activity. Basic Molecular electronic property descriptors such as solubility, logP, Hydrogen bond donors /acceptors count, number of rotatable bonds, Total Polar Surface Area, Molecular weight, Volume and Drug Likeliness properties, along with kinase inhibitory potential and ligand potential for G-Protein Coupled Receptors are predicted using Molinspiration (http://www.molinspiration.com/)

ADME/T Prediction

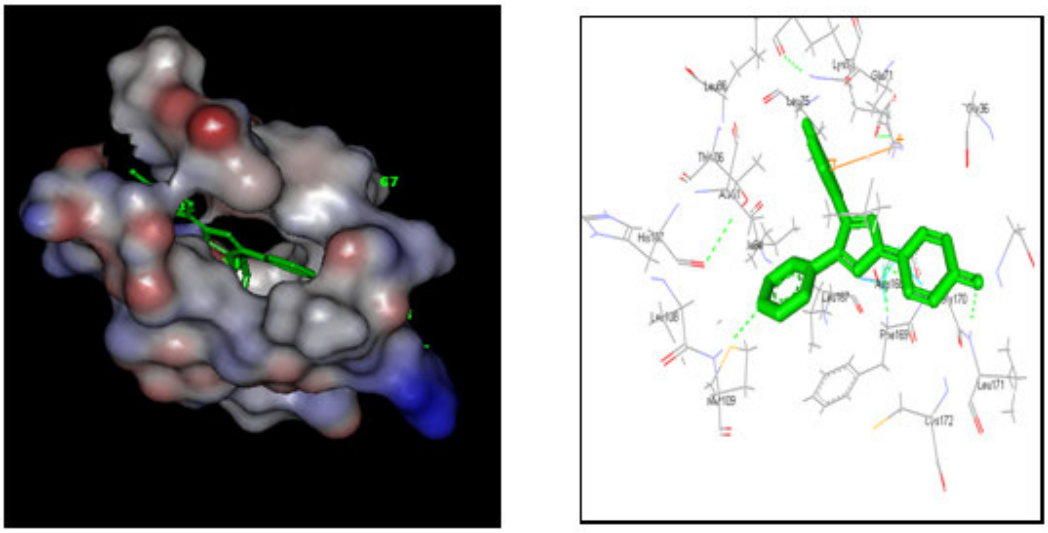
Absorption, Distribution, Metabolism and Excretion (ADME) describes the disposition of a pharmaceutical or natural compound within the body of an animal or human. Basic ADMET properties were predicted to find out the safety and effectiveness of natural phytochemical compounds. ADME tox-web server was used to predict the ADME properties of compounds with satisfactory physical properties [25].

RESULTS AND DISCUSSION

Synthetic Compounds: From the interaction analysis and binding models of the selective p-38 MAPK inhibitor – SB-202190, it was found that Asp – 168, Lys – 53, Met-109 and Leu-171 were critical in the inhibition of the p-38 MAPK at the active site and a pharmacophore model was derived for SB202190. The molecular interactions of SB – 202190 were compared with all other molecules, for all their respective interactions. The results for other drug possible "leads" for p-38 MAPK inhibitory activity shows SB– 225002, Reparixin, A96 and PD-98059, with least binding energies, good affinity and less toxic in ADMET profiling. Among them, A-96 was the overall best synthetic compound, satisfying all parameters. The Table 2(a) below shows the basic 2D QSAR and molecular properties for the top four synthetic compounds, while Figure-1 shows the structural visuals at the active site for SB-202190.The Tables 2(b) and 2(c) show the toxicity and ADME profile of the four synthetic compounds respectively. Figure-2 gives the visuals of interactions at the active-site for A-96, the best synthetic compound.

FIGURE 1

Structural Visualization at the active-site for SB-202190 denoting the interactions.



SYNTHETIC COMPOUNDS

Table 2(a)
2D Molecular properties for selected top synthetic compounds.

NATURAL COMPS.	LogP	TPSA	MW	N. rot bonds	H Donor	H Acceptors	volume	GPCR ligand	Ion-Channel	Kinase inhibitor	Nuclear receptor
SB-202190	3.586	61.545	331.35	2	2	4	287.999	-0.31	-0.23	0.43	-1.03
SB-225002	3.571	107.177	352.144	3	3	7	248.478	-0.19	-0.19	-0.28	-1.13
A-96	4.409	92.941	433.777	5	2	7	328.573	0.16	0.14	0.20	-0.32
PD-98059	3.181	65.468	267.284	2	2	4	236.83	-0.23	-0.19	0.60	-0.83

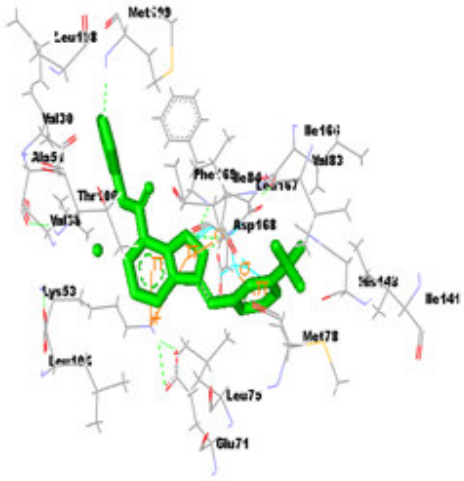
Table 2(b)
Toxicity properties of best synthetic compounds.

TOXICITY PARAMETERS	SB-202190	SB 225002	A-96	PD-98059
Probability of positive Ames test:	0.376	0.004	0.003	0.0169
Health Effects				
Blood:	0.27	0.79	0.67	0.56
Cardiovascular:	0.69	0.95	0.84	0.62
Gastrointestinal system:	0.83	0.71	0.75	0.77
Kidney:	0.31	0.66	0.55	0.54
Liver:	0.16	0.32	0.40	0.18
Lungs:	0.31	0.61	0.76	0.32
LD50 (Mouse)				
a) Intrperitoneal:				
Ld50(mg/kg):	450	280	100	810
pLD50:	-0.02	0.10	0.62	-0.48
b)Oral:				
Ld50(mg/kg):	670	930	410	1500
pLD50:	-0.19	-0.42	0.03	-0.75
c) Intravenous:				
Ld50(mg/kg):	75	62	53	87
pLD50:	0.76	0.76	0.91	0.49
d) Subcutaneous:				
Ld50(mg/kg):	240	370	100	1000
pLD50:	0.25	-0.02	0.62	-0.58
LD50 – (Rat)				
Intrperitoneal:				
Ld50(mg/kg):	320	240	110	740
pLD50:	0.14	0.17	0.59	-0.44
Oral:				
Ld50(mg/kg):	610	600	230	1500
pLD50:	-0.15	-023	0.27	-0.74

Table 2(c)
ADME properties of best synthetic compounds.

ADME PARAMETERS	SB-202190	SB 225002	A-96	PD-98059
Bio Availability (Oral Bioavailability)	Between 30% and 70%	Between 30% and 70%	Between 30% and 70%	More than 70%
ABSORPTION				
LogP	2.68	3.72	3.75	2.51
pKa(acid)	No pKa	8.00	No pKa	No pKa
pKa(base)	8.00	No pKa	2.60	3.30
Max. passive absorptn.	100%	100%	100%	100%
Trancellular route	99%	100%	100%	100%
Paracellular route	1%	0%	0%	0%
PERMEABILITY				
Human Jejunum scale (pH=6.5):	Pe, Jejunum = $0.94x10^{-4}$ cm/s	Pe, Jejunum = $4.09x10^{-4}$ cm/s	Pe, Jejunum = $4.60x10^{-4}$ cm/s	Pe, Jejunum = $2.89x10^{-4}$ cm/s
Caco-2 scale (pH=7.4, 500 rpm):	P_e,Caco-2 = $52.33x10^{-6}$ cm/s	P_e, Caco-2 = $223.85x10^{-6}$ cm/s	P_e, Caco-2 = $268.56x10^{-6}$ cm/s	P_e, Caco-2 = $133.25x10^{-6}$ cm/s
Absorption rate:	Ka = 0.027 min-1	Ka = 0.097min-1	Ka = 0.098min-1	K_a = 0.089 min^{-1}
Active Transport PepT1 and ASBT:	Not transported	Not transported	Not transported	Not transported
DISTRIBUTION				
%PPB:	92.19%	97.82%	98.67%	85.78%
LogKa HSA:	4.22	4.71	4.93	3.96
Volume of Distribution (L/Kg)	1.98	0.67	2.01	1.78
SOLUBILITY				
(In Water) [LogSw]	-0.83	-4.03	-3.67	-4.20
SW(mg/ml):	52.1	0.0416	0.0751	0.056
IONIZATION (LogD)				
pH =1.7 (Stomach):	-0.39	3.72	2.79	0.91
pH = 4.6 (Duodenum):	2.36	3.72	3.74	2.49
pH = 6.5 (Jejunum & Ileum):	2.89	3.70	3.74	2.51
pH = 7.4 (Blood):			3.75	2.51
pH = 8.0 (Colon):	2.90	3.62	3.75	2.51
	2.91	3.42		
Strongest pKa (Acid):	No Acid pKa	No Acid pKa	No Acid pKa	No Acid pKa
Strongest pKa (Base):	5.00=/-1.90	5.00+/-1.90	2.60+/-1.90	3.30=/-1.80
Number of ionizable groups:	3	1	3	1

FIGURE 2
Structural Visualization at the active-site for A-96 denoting the interactions.



Natural phytochemical compounds – Ventinone-A, Emodin, Emodin-8-glucoside and Aniflorine, were all structurally similar to each other, and also showed good interactions at active site. All of them showed more or less common interactions with Asp168 and Lys53. But there was a common interaction with Cys172 and Val38 in all the four of them. Emodin, possessed better interactions with minimal docking binding energy and satisfactory ADMET profile among the above four. Emodin was ranked finally as the fifth best in overall screening, while Ventinone-A was ranked fourth.

Table 3
Interactions of best fifteen natural compounds

COMPD. NAME	ATOM NAME	RESIDUE NAME	DISTANCE (A°)
Physcion-8 Glucoside	O25	Leu104 Ala51 Lys53	2.82 3.06 3.24
	O31	Met109	3.01
	O8	Val30	3.20
Emodin-8-glucoside	O23	Lys53	2.75
	O25	Glu71	2.84
	O26	Asp168	3.25, 3.09
	O28	Thr106	3.27
	O30	Met109	3.04
Diacerhein / Diacetylrhein	O19	Lys53	2.94, 3.11
	O25	Tyr35	3.20
	O11	Asp168	3.03, 2.97
	O23	Val-38	3.71
	O21	Leu171	3.17
	O22	Ser32	3.04.
Apigetrin	O25	Ile166	3.40
	O28	Glu71 Asp168	2.59 3.46, 2.99.
	O19	Lys53	3.13
	O30	Leu171	3.28
Dehydrocyclo-guanandine	O11	Asp168	3.40, 3.55
	O19	Asp168 Lys53	2.85 2.86
	O20	Leu171	3.02

Berberine	O1	Tyr35	3.06
	O14	Asp168	2.89, 3.26
	O20	Met109	2.95
	O23	Lys53	2.96
Terrestribisamide	O19	Tyr35 Arg67	2.86 2.51
	O31	Asp168	3.26, 3.18, 3.03.
	N12	Met109	2.73
	O23	Lys53	2.76
Genistein	O16	Asp168 Lys53	3.47, 3.30. 3.49
	O18	Cys172 Leu171	3.19 3.05
	O19	Val38	2.79
Emodin	O8	Lys53 Asp168	2.96 2.97, 3.04
	O16	Phe169	3.41
	O17	Leu171	3.14
	O18	Tyr35	2.80
	O19	Cys172	2.94
Tribulusterine	O16	Glu71 Asp168	3.09 3.12, 3.26
	O21	Phe169 Lys53	3.32 3.77
Aniflorine	O18	Phe169	3.51
	O19	Lys53	2.98
	O23	Met109	3.08
	O25	Asp168	2.28, 2.70
Pseudo-purpurin	O7	Asp168	3.00, 3.15
	O11	Cys172	3.38
	O16	Lys53	2.63
	O18	Tyr35	2.73
	O19	Lys53	3.08
	O20	Gly36	3.13
	O21	Cys172	3.29
Ventinone A	O18	Asp168	2.71
	O19	Tyr35	2.85
	O21	Val38	3.58
	O22	Lys53	3.21
Serpentine	O24	Phe169	2.77
	O27	Tyr35	2.78
	O9	Leu171	2.98
	N16	Asp168	3.00, 3.12
	O19	Lys53	3.13
Chrysophanol 8-glucoside	O27	Asp168	3.14
	O28	Asp168 Asp168	2.61 2.67
	O17	Asp168	3.37
	O12	Asp168	2.98
	O29	Ile166	3.12

Terrestribisamide from *Tribulus terrestris* not only had good interactions, but also resembled the “lead” A-96 structurally. It made interactions with Lys-53 and Met109, though Leu-171 was absent, showing the resemblance with A-96. It also was the third best in the overall screening, although its docking energy score is comparatively lower than others. On the other hand, Serpentine from *Rouwolfia Serpentina* did not resemble any other natural compound structurally, but showed a very good interaction profile and the best ADMET profile. It was ranked the second best. Diacetylrhein, a

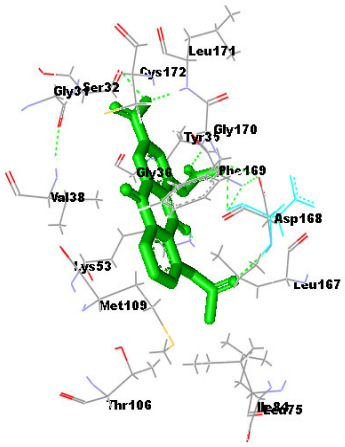
natural compound from Rhubarb, was found to have the best interaction at the least binding energy value of –10.74 kcal/mol, and reasonably good ADME profile, except for poor bioavailability in its crude form, which may be improve if adjuvants are added during drug development. Most of these compounds showed lesser docking energies than the synthetic inhibitors, suggesting the potential of phytochemical compounds for utilization in multi-target drug delivery systems. The Table 3 below shows the interaction profile of top fifteen natural while Table 4 highlights the molecular properties of selected eight phytochemicals. Tables 5(a) and (b) indicate ADME and toxicity properties of best natural compounds respectively.

Table 4
2D Molecular properties for selected top natural compounds

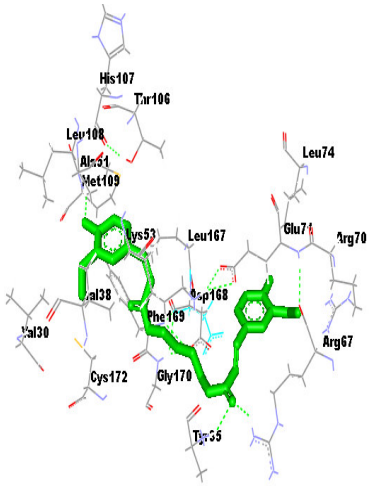
NATURAL COMPS.	Binding Score	LogP	TPSA	MW	N. rot bonds	H Donor	H Acceptors	volume	GPCR ligand	Ion-Channel	Kinase inhibitor	Nuclear Receptor
Emodin-8-Glucoside	-11.41	1.18	173.98	432.38	3	5	10	355.313	-0.68	-0.78	-0.41	-0.96
Diacerhein / Diacetylrhein	-10.74	3.88	124.04	368.29	5	1	8	298.627	-0.42	-0.46	-0.23	-0.69
Serpentine	-10.39	4.62	51.13	348.39	2	0	5	313.13	-0.29	-0.42	-0.07	-0.82
Ventinone A	-9.61	4.51	104.06	314.29	1	3	6	265.298	-0.32	-0.46	-0.12	-0.39
Terrestribisamide	-9.39	2.22	96.457	362.36	3	2	7	308.652	-0.14	-0.21	0.79	-0.65
Tribulusterine	-9.27	5.05	120.31	327.42	6	0	2	319.649	-0.01	-0.07	-0.45	-0.07
Aniflorine	-9.22	2.44	68.37	351.40	3	1	6	299.298	-0.25	-0.12	0.02	0.20
Emodin	-9.05	3.4	94.826	270.24	0	3	5	223.433	-0.23	-0.04	0.12	-0.31

FIGURE 3
Structural Visualization at active-site for top five natural phytochemical compounds

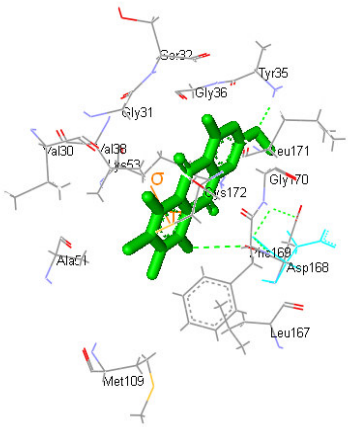
Diacerhein / Diacetylrhein



Terretribisamide

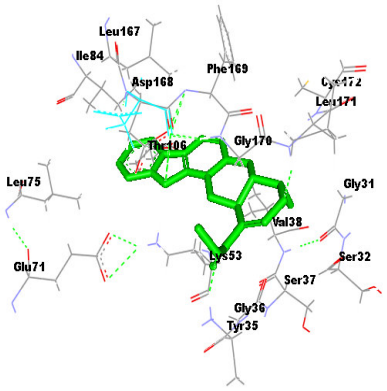


Ventinone A



Serpentine

Emodin



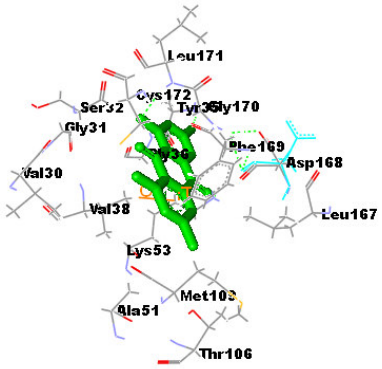


Table 5(a)
ADME properties of best natural compounds.

ADME PARAMETERS	Serpentine	Terrestribisamide	Aniflorine	Ventinone A	Emodin	Emodin-8-Glucoside	Tribulusterine	Diacetylrhein/ Diacerhein
Bio Availability (Oral Bioavailability)	Between 30% and 70%	Between 30% and 70%	Between 30% and 70%	Between 30% and 70%	Between 30% and 70%	Less than 30% - oral	Between 30% and 70%	Less than 30% - oral
Absorption								
LogP	4.62	2.22	2.44	4.51	3.79	1.18	5.05	3.88
pKa(acid)	No pKa	8.70	No pKa	8.00	8.00	8.00	No pKa	3.00
pKa(base)	8.00	No pKa	3.30	No pKa	No pKa	No pKa	No pKa	No pKa
Max. passive absorptn.	100%	100%	100%	100%	100%	90%	100%	100%
Trancellular route	100%	100%	100%	100%	100%	99%	100%	100%
Paracellular route	0%	0%	0%	0%	0%	1%	0%	0%
Permeability								
Human Jejunum scale (pH=6.5):	Pe, Jejunum = $5.52x10^{-4}$ cm/s	Pe, Jejunum = $1.71x10^{-4}$ cm/s	Pe, Jejunum = $3.26x10^{-4}$ cm/s	Pe, Jejunum = $5.08x10^{-4}$ cm/s	Pe, Jejunum = $4.18x10^{-4}$ cm/s	Pe, Jejunum = $0.52x10^{-4}$ cm/s	Pe, Jejunum = $6.32x10^{-4}$ cm/s	Pe, Jejunum = $1.44x10^{-4}$ cm/s
Caco-2 scale (pH=7.4, 500 rpm):	Pe,Caco-2 = $308.69x10^{-6}$ cm/s	Pe, Caco-2 = $41.05x10^{-6}$ cm/s	Pe, Caco-2 = $166.91x10^{-6}$ cm/s	Caco-2 = $285.56x10^{-6}$ cm/s	Caco-2 = $231.24x10^{-6}$ cm/s	Pe, Caco-2 = $2.78x10^{-6}$ cm/s	Pe, Caco-2 = $317.45x10^{-6}$ cm/s	Pe, Caco-2 = $27.84x10^{-6}$ cm/s
Absorption rate:	Ka = 0.100 min-1	Ka = 0.062 min-1	Ka = 0.092 min-1	Ka =0.099 min-1	Ka = 0.097 min-1	K_a =0.009 min^{-1}	K_a =0.100 min^{-1}	K_a = 0.051 min^{-1}
Active Transport PepT1 and ASBT:	Not transported	Not transported	Not transported	Not transported	Not transported	Not transported	Not transported	Not transported
DISTRIBUTION								
%PPB:	93.57%	98.86%	88.47%	95.74%	93.90%	97.96%	99.09%	99.29%
LogKa HSA:	5.84	3.87	4.06	4.23	4.34	3.63	4.91	5.17
Volume of Distribution (L/Kg)	1.29	0.77	1.83	1.02	0.92	0.89	3.06	0.23
SOLUBILITY								
(In Water) [LogSw]	-0.83	-4.03	-3.67	-3.46	-3.54	-3.50	-4.27	-4.11
SW(mg/ml):	52.1	0.0416	0.0751	0.109	0.0778	0.137	0.0174	0.0287

Table 5(b)
Toxicity properties of best natural compounds

TOXICITY PARAMETERS	Serpentine	Terrestribisamide	Aniflorine	Ventinon-A	Emodin	Emodin-8-Glucoside	Tribulusterine	Diacetylrhein/ Diacerhein
Probability of positive Ames test:	0.112	0.054	0.495	1.000	0.991	0.941	0.022	0.859
Health Effects								
Blood:	1.00	0.89	0.93	0.05	0.19	0.88	0.53	0.16
Cardiovascul:	1.00	0.91	0.88	0.49	0.57	0.74	0.63	0.28
Gastrointestinal system:	0.98	0.02	0.96	0.92	0.56	0.99	0.86	0.13
Kidney:								
Liver:	0.97	0.41	0.44	0.19	0.21	0.54	0.47	0.22
Lungs:	0.88	0.73	0.44	0.62	0.33	0.43	0.27	0.14
	0.94	0.71	0.45	0.62	0.68	0.90	0.70	0.46
LD50 (Mouse)								
a) Intrperitoneal:								
Ld50(mg/kg):	100	390	270	330	180	3200	410	440
pLD50:	0.54	0.05	0.11	-0.02	0.18	-0.87	-0.10	-0.08
b) Oral:								
Ld50(mg/kg):	420	1900	520	840	810	6400	1100	1200
pLD50:	-0.08	-0.62	-0.17	-0.42	-0.48	-1.17	-0.51	-0.51
c) Intravenous:								
Ld50(mg/kg):	24	52	79	110	53	360	37	180
pLD50:	1.16	0.93	0.65	0.45	0.71	0.08	0.95	0.32
d) Subcutaneous:								
Ld50(mg/kg):	160	570	360	460	300	4800	590	710
pLD50:	0.35	-0.11	-0.01	-0.16	-0.04	-1.04	-0.26	-0.29
LD50 – (Rat) Intrperitoneal:								
Ld50(mg/kg):	73	740	240	620	170	6100	360	1100
pLD50:	0.68	-0.22	0.17	-0.29	0.21	-1.15	-0.04	-0.48
Oral:								
Ld50(mg/kg):	300	3900	330	2100	1300	9200	1100	2000
pLD50:	0.06	-0.95	-0.03	-0.82	-0.70	-1.33	-0.53	-0.74

CONCLUSION

End stage renal failure is an outcome of a variety of reasons like genetic polymorphisms, stress, toxicity or tissue scarring. Growth factors, cytokines and p-38 MAPK signaling play important role in progression of the renal failure. TGF-β is the initiator of a series of kinase signaling that leads to EMT, a key pathophysiological process for fibrosis and apoptosis. Several literature points to the role of p-38 MAPK signaling in renal diseases. Targeting p-38 MAPK could prevent down-stream signaling process for leukocyte recruitment and fibrogenesis. Natural plant compounds possess a wide range of structural variations and physiochemical properties for exploration. Several phytochemical compounds such as curcumin are traditionally known for anti-inflammatory and antibiotic usages. Screening of phytochemical compounds threw light on their prospective usage for p-38 MAPK inhibitory properties. A comparative study between synthetic drug molecules and natural compounds for the inhibitory potential of p-38 MAPK activity was performed. Natural compounds such as Diacerrhein, Terrestribisamide, Serpentine and Ventinone-A, were found to be much more effective with lesser toxicity compared to their synthetic counterparts. Among natural compounds "Diacerrhein" was found to be the best compound and showed better effects than that of semi-synthetic Indole-based drug molecules such as SB-202190, SB-225002, PD-98059, etc. thereby highlighting the fact that these natural phytochemicals could be used for the development of novel drug with different structural derivatives from these basic structures. Also addition of adjuvants and hydrophilic ions could improve the bioavailability of these natural phytochemical compounds.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Tian W, Zhang Z, Cohen DM., MAPK signaling and the kidney, Am J Physiol Renal Physiol 279:F593-F604, (2000).
2. Pearson G, Robinson F, Beers Gibson T, Xu BE, Karandikar M, Berman K and Cobb MH, Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions, Endocr Rev, 22 (2): 153–83, (2001).
3. Saulo Klahr and Jeremiah J Morrissey, The role of vasoactive compounds, growth factors and cytokines in the progression of renal disease, Kidney Int Suppl, 75:S7-14, (2000).
4. Bottinger EP and Bitzer M, TGF-beta signaling in renal disease, J Am Soc Nephrol, 13:2600- 2610, (2002).
5. Stambe C, Atkins RC, Tesch GH, Masaki T, Schreiner GF and Nikolic-Paterson DJ, The role of p38alpha mitogen-activated protein kinase activation in renal fibrosis, J Am Soc Nephrol, 15:370-379, (2004).
6. Dominguez C, Powers DA and Tamayo N, p-38 MAP kinase inhibitors: many are made, but few are chosen, Curr Opin Drug Discov Devel, 8:421-430, (2005).
7. Lee JC, Kumar S, Griswold DE, Underwood DC, Votta BJ, Adams JL, Inhibition of p38 MAP kinase as a therapeutic strategy, Immunopharmacology, 47:185-201, (2000).
8. S. Patel, Ken-ichro Takagi, Jun Suzuki, A Imaizumi, T Kimura, RM Mason, T Kamimura and Zhi Zhang., RhoGTPase Activation Is a Key Step in Renal Epithelial Mesenchymal Transdifferentiation, J Am Soc Nephrol, 16, 1977-1984, (2005).
9. Vlahopoulos S, Zoumpourlis VC, JNK: a key modulator of intracellular signaling, Biochemistry (Mosc).;69(8):844-54, (2008).
10. Rossini M, Fogo AB, Mechanisms leading to progression of chronic renal injury: the interstitium, Drug Discovery Today: Disease Mechanisms, 1:65-72, (2005).
11. Burysek L, Syrovets T, Simmet T., The serine protease plasmin triggers expression of MCP-1 and CD40 in human primary monocytes via activation of p38 MAPK and janus kinase (JAK)/STAT signaling pathways, J Biol Chem. 277(36):33509-17, (2002).
12. Meldrum KK, Meldrum DR, Hile KL, Yerkes EB, Ayala A, Cain MP, Rink RC, Casale AJ, Kaefer MA, p38 MAPK mediates renal tubular cell TNF-alpha production and TNF-alpha-dependent apoptosis during simulated ischemia, Am J Physiol Cell Physiol, 281:C563-C570, (2001).
13. Walter Manucha, Biochemical-molecular markers in unilateral ureteral obstruction, Bio cell, 31(1): 1-12, (2007).
14. Gagliardini E and Benigni A, Therapeutic potential of TGF-beta inhibition in chronic renal failure, Expert Opin Biol Ther, 7(3):293-304, (2007).
15. Li J, Campanale NV, Liang RJ, Deane JA, Bertram JF, & Ricardo SD, Inhibition of p38 Mitogen-Activated Protein Kinase and Transforming Growth Factor-b1/Smad Signaling Pathways Modulates the Development of Fibrosis in Adriamycin-Induced Nephropathy, Am J Pathology, 169[5]:1527-1540, (2006).
16. B de las Heras, Rodríguez B, Boscá L, Villar AM, Terpenoids: sources, structure elucidation and therapeutic potential in inflammation, Curr Top Med Chem, 3(2):171-85, (2003).
17. B de las Heras, S Hortelano, N Girón, P Bermejo, B Rodríguez, and L Boscá., Kaurane diterpenes protect against apoptosis and inhibition of phagocytosis in activated macrophages, Br J Pharmacol, 152(2): 249–255, (2007).
18. Balogun E, Hoque M, Gong P, Killeen E, Green CJ, Foresti R, Alam J, Motterlini R., Curcumin activates the haem oxygenase-1 gene via regulation of Nrf2 and the antioxidant-responsive element, Biochem J, 371(Pt 3):887-95, (2003).
19. Zhang WJ, Hufnagl P, Binder BR, Wojta J., Antiinflammatory activity of astragaloside - IV is mediated by inhibition

of NF-kappaB activation and adhesion molecule expression, Thromb Haemost, 90(5):904-14, (2003).
20. Marie-Hélène Teiten, Serge Eifes, Mario Dicato and Marc Diederich., Curcumin—The Paradigm of a Multi-Target Natural Compound with Applications in Cancer Prevention and Treatment, Toxins, 2, 128-162, (2010).
21. Peng A, Y Gu, Lin SY., Herbal Treatment for Renal Diseases, Ann Acad Med, Singapore, 34:44-51, (2005).
22. Kim HP, Son KH, Chang HW, Kang SS., Anti-inflammatory plant flavonoids and cellular action mechanisms, J Pharmacol Sci., 96(3):229-45, (2004).
23. Pav, S., White, D. M., Rogers, S., Crystallization and preliminary crystallographic analysis of recombinant human p38 MAP kinase, Protein Sci, 6, 242–245, (1997).
24. Balani SK, Miwa GT, Gan LS, Wu JT, Lee FW. Strategy of utilizing in vitro and in vivo ADME tools for lead optimization and drug candidate selection, Curr Top Med Chem, 5(11):1033-38, (2005).