Journal of King Saud University – Science 32 (2020) 2540–2552

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Mining novel natural reactive oxygen species (ROS) inhibitors by targeting Rho Kinase for prevention of secondary spinal cord injury: An *in-silico* trial using traditional Chinese medicinal compounds

Lei Fang [1], Ziliang Shen [1], Shengming Xu, Yu Chen, Shuqiang Wang *

Department of Orthopaedic Surgery, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 15 November 2019
Revised 19 March 2020
Accepted 20 March 2020
Available online 29 March 2020

Keywords:
Secondary spinal cord injury
Reactive oxygen species
Traditional Chinese medicine
Molecular docking

ABSTRACT

Traditional Chinese Medicinal (TCM) compounds provide a plethora of natural chemiome for structure based novel drug discovery against unexplored targets of important diseases. One such disease is Secondary Spinal cord Injury (SSCI), a condition secondary to initial Spinal cord Injury (SCI) caused by a trauma. In SSCI oxidative stress and inflammation play a pivotal role in aggravating neural damage at the site of trauma. To look into it reactive oxygen species (ROS) inhibition is a good strategy. Our Study here focuses on finding novel ROS inhibitors from in-house TCM compound library using advanced structure based drug discovery methods. From Virtual screening, Molecular Docking, Molecular Dynamics Simulation and MM-PBSA calculations a single ROS inhibitor was proposed for targeting SSCI. Our study provides a platform for future structure based drug discoveries in the field of treating SCI by targeting SSCI pathways.

1. Introduction

Spinal cord injury (SCI) is an injury triggered event that is associated with permanent neurologic deficit. The deficit instigated by SCI leads to medical comorbidity, not only effecting sensory and motor capabilities, but also having impact on the physiological and economical condition of the patient (McDonald and Sadowsky, 2002). Edwin Smith papyrus, an Egyptian physician in 1700 BCE was the first one to document SCI as an "ailment not to be treated. Since then SCI has been recorded as one of the devastating conditions where most of the cases are exanimate before any patient care is given. The surviving SCI cases remain morbid and are more prone to mortality,in USA alone 10–40 people in a million in a year are effected by SCI. The total number of cases estimated in USA as reported in 2016 are at staggering 282,000, to which every year 11,000 new cases are added. Billions of dollars in USA alone are spend on this disease, making it one of the economically devastating diseases. (White and Black, 2016).

SCI is categorized into primary spinal cord injury(pSCI)and secondary spinal cord injury(sSCI), pSCI is defined as the injury inflicted at the time of trauma and sSCI is defined by the injury causedby the body's response to initial trauma (Cadotte and Fehlings, 2011). The consequences of the SCI are defined by the extent of secondary damage, which is initiated by a cascade of molecular cellular events triggered by pSCI. The pSCI triggers glutamatergic excitotoxicity, free radical damage, cytokine production and inflammation, all of them effecting the survival of neurons and glial cells, thus setting a base for onset of sSCI, leading to other patho-mechanisims that trigger neuropathic pain and autonomic dysfunction. Use of free-radical scavengers, anti-inflammatory drugs and anti-apoptotic drugs are suggested to be effective therapeutic strategies for the inhibition of sSCI (Zhou et al., 2014).

* Corresponding author at: Department of Orthopaedic Surgery, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, No 528 of zhangheng road pudong new area, Shanghai 2010203, China.
E-mail address: RonnyVaughanexo@yahoo.com (S. Wang).
[1] These two authors contribute to this work equally.

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2020.03.039
1018-3647/

In the pathogenesis of sSCI the role of reactive oxygen species (ROS) molecules in oxidative damage to spinal cord lipids, known as lipid peroxidation (LP) is well established Hall et al., 2016). The hydrogen peroxide and peroxinitrites are non-radical ROS reported to play important roles in post pSCI onset, among them OH, NO_2 and CO_3 peroxinitrites are more prominent to initiate LP.The expression of peroxinitrites is regulated by RhoA GTPase (RhoA) / Rho-associated kinase (ROCK) pathway, other than this ROCK is also associated with cytoskeletal rearrangement and cell movement function in a cell. This RhoA/ROCK pathway is implicated in disorders like cardiovascular disease (Budzyn et al., 2006) and central nervous system diseases (Yamamoto et al., 2014). Spinal cord injury is one of the prominent CNS disease among others like multiple sclerosis, Alzheimer's disease, glaucoma and stroke to be regulated by RhoA/ROCK pathway (Tokushige et al., 2011).

Computer aided drug designing (CADD) is a promising strategy in novel drug discovery for rare diseases. This powerful tool is an established standard for novel drug designing and discovery, novel leads have been reported as enzyme inhibitors as well as protein – protein interaction disruptors using this method (Amin et al., 2016). In this study we are trying to look into novel natural lead compound of traditional Chinese medicine (TCM) origin that will alter RhoA/ROCK pathway by inhibiting the ATP binding site of ROCK. The main aim will be to discover a compound that will alter ROS based sSSI. We have used TCM database of 672 compounds for multistep structure based CADD method, virtual screening technique, followed by docking and simulation was employed for identifying promising lead compound, the approach is very well used method for identifying novel leads.

2. Material and method

2.1. Protein and ligand preparation

For CADD the atomic coordinates for ROCK protein (PDB ID: 3V8S) was taken. The structure was checked for missing atoms and the complete structure was energy minimised using swiss PDB viewer (SPDBv) (Viewer et al., 2001). The RMSD (Root Mean Square Deviation) was monitored and using GROMOS96 43B1 force field (van Gunsteren et al., 1996). Six hundred and seventy two highly active TCM compounds from in-house database (Table 1) was used for targeting ATP binding site of ROCK protein.

3. Virtual screening drug likeliness prediction

A total of 128 TCM compounds were shortlisted after virtual screening based on their binding energy (ΔG) calculations (Trott and Olson, 2010). The selected compounds were further limited by subjecting them to rules set by lipiski (Lipinski, 2004). The Lipinki Rule of five (RO5) parameters gave us five compounds for further analysis.

3.1. Molecular docking analysis

AutoDock 4.2 tool was employed for molecular docking study to achieve structure based drug againstcPLA2 protein (Morris et al., 2009). The tool calculates energy values by classification of energies as; internal energy, and torsional free energy.

$$\Delta G = \Delta G_{vdw} + \Delta G_{hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv}$$

Table 1

Ingredient_name
Chrysophanic acid
4-hydroxybenzoic acid
Succinate
hexose
Aspartate
Glutamine
Hexadecenoic acid
Octadecenoic acid
Cardiolipin
glutamate
caffeic acid phenethyl ester
tretinoin
cytochalasin B
lovastatin
serine
gibberellic acid
Arabinose
benzoate
norethynodrel
7-methoxycoumarin
10-hydroxy-camptothecin
trans-p-Hydroxycinnamic acid
2,6-Di-*tert*-butyl-4-methylphenol
ammonium glycyrrhizinate
palmatine chloride
acacetin
artemisin
atropine
avicularin
baicalin
belladonnine
biflorin
bilirubin
biochanin a
biotin
brucine
budlein a
butein
caffeine
camptothecin
catharanthine
cephalomannine
cholesterol
chrysin
colchicine
cortisone
coumestrol
cryptopine
cucurbitacin e
cucurbitacin i
curcumin
daidzein
digoxin
dubinidine
ellipticine
enhydrin
epicatechin
erysovine
erythraline
estrone
eucalyptin
formononetin
fructose
galangin
galanthamine
genipin
genistein
ginkgolide a
grandisin
guanidine
guanosine
harmaline

(*continued on next page*)

Table 1 (*continued*)

Ingredient_name
harmine
harringtonine
hesperetin
homoeriodictyol
homoharringtonine
honokiol
humulone
hyoscyamine
isoquercitrin
isovitexin
kaempferol
khellin
kinetin
lapachol
alpha-lapachone
beta-lapachone
licarin a
luteolin
maltose
mangiferin
morin
naringenin
nobiletin
orientin
perfamine
phytosphingosine
piceid
picrotin
picrotoxinin
piplartine
podophyllotoxin
porphyrin
precocene ii
pregnenolone
procyanidin b2
tryptanthrin
gallic acid
epigallocatechin
salicylic acid
caffeic acid
ellagic acid
catechin
artemisinin
hyperoside
estradiol
pseudoephedrine
reynoutrin
asparagine
erythrinin
quinic acid
fluoxetine
nifedipine
methylprednisolone
galgravin
artesunate
artemether
melatonin
secoisolariciresinol
alternariol
velutin
vicenin-2
mannitol
apigetrin
cholic acid
lithocholic acid
physostigmine
riboflavin
ginkgolide
quercitrin
reserpine
ribalinine
rutin

Table 1 (*continued*)

Ingredient_name
sanguinarine
sophocarpine
sphingomyelin
sucrose
swertisin
tanshinone i
tanshinone iia
taxol
tetrandrine
thebaine
theophylline
tiliroside
tremulacin
7,3′,4′-trihydroxyflavone
3,5,3′-triiodothyronine
triptolide
tropine
tryptanthrine
valine
veraguensin
vincristine
vitexin
yohimbine
5-o-caffeoylquinic acid
ursodeoxycholic acid
taxifolin
sorbitol
icariin
rosmarinic acid
gallocatechin
(−)-epicatechin
(−)-noradrenaline
(+)-catechin
(+)-epicatechin
1,16-hexadecanediol
1,2-benzenediol
11-deoxojervine
15,16-dihydrotanshinone i
17-hydroxycryptotanshinone
1-hydroxyanthraquinone
1-kestose
1-ketoisocyptotanshinone
2,5-dihydroxy benzoic acid
2-acetamido-2-deoxy-d-glucose
2′-deoxythymidine
2-hydroxyanthraquinone
2-hydroxybenzoic acid
2-methoxycinnamic acid
2-methyl-1,4-naphthoquinone
3,3′,4′,5,5′,7-hexahydroxyflavone
3,4-dihydroxybenzoic acid
3,4-phenanthrenedione
3,7,11,15-tetramethyl-2-hexadecen-1-ol
3-hydroxycyptotanshinone
3-hydroxy-glabrol
3-hydroxykynurenine
3-hydroxymethylenetanshinquinone
3-hydroxytanshinone iib
3-methylquercetin
3′-o-acetylhamaudol
3-phenyl-2-propen-1-ol
4-coumaric acid
4-hydroxy-3-methoxybenzaldehyde
4-hydroxybenzoic acid
4-hydroxybenzoylcholine
4-hydroxyphenylacetic acid
4-methylpyrazole
5′-adenosine monophosphate
5-methyluracil
6,7-dihydroxycoumarin
6-aminopurine
6-methoxy-7-hydroxycoumarin

Table 1 (*continued*)

Ingredient_name
8-geranyloxy psoralen
abscisic acid
acacetin
acaciin
acetylcholine
acrylic acid
adenine
adenosine
adonitol
aesculetin
aesculin
afzelin
agmatine
albiflorin
allantoin
allocryptopine
aloin
alpha-bisabolol
alpha-copaene
alpha-tocotrienol
alpha-tocotrienol
amber acid
amentoflavone
aminoacetic acid
aminopyrine
anabasine
aniline
anserine
anthranilic acid
apigenin
apigenin 7-o-beta-d-glucopyranoside
apigenin-7-o-glucoside
apigenin-7-o-neohesperidoside
apigetrin
arachidonic acid
asparagine
aspartic acid
aspidocarpine
astragalin
atenolol
atrazine
atropine
baicalin
behenic acid
benzaldehyde
benzoate
benzoic acid
benzophenone
berberine
bergapten
beta-alanine
beta-carotene-5,6-epoxide
betaine
beta-thujaplicin
beta-tocopherol
betonicine
bicuculline
biochanin a
biotin
boldine
brassicasterol
brucine
butanedioic acid
butyrate
butyric acid
cadaverine
caffeic acid
caffeine
caffetannic acid
callistephin

Table 1 (*continued*)

Ingredient_name
calycosin-7-o-beta-d-glucoside
camphene
canavanine
canthaxanthin
caproic acid
capsaicin
carnitine
carnosine
catechin
catechol
chalcone
chelidonine
chenodeoxycholic acid
chlorogenic acid
cholalic acid
choline
chrysanthemin
cinaroside
cinchonine
cinnamic alcohol
cirsimarin
cis-4-hydroxyproline
cis-9-octadecenoic acid
cis-aconitic acid
citrin
citrulline
cocaine
codeine
coniferaldehyde
coniferyl aldehyde
cordycepic acid
cosmosiin
coumarin
creatine
creatinine
crithmene
cyanidin-3-glucoside
cyanin
cyclamin
cyclopamine
cystathionine
daidzein
daidzin
danshenxinkun a
daphnetin
decanedioic acid
delphinidin
delphinidin-3-glucoside
delta-tocotrienol
deoxycholic acid
d-glucuronic acid
diazinon
dichlorvos
dihydrocapsacine
dihydrocapsaicin
dihydrochelerythrine
dihydromelilotoside
dihydroquercetin
dimethyl malate
diosmin
dopamine
dulcitol
emetine
emetine
enanthic acid
ephedrine
epicatechin
epiprogoitrin
eriodictyol
esculetin

(*continued on next page*)

Table 1 (*continued*)

Ingredient_name
esculin
eserine
estrone
ethanolamine
ferulic acid
fipronil
fisetin
flavanone
flavanone
flavin mononucleotide
fluoxetine
foliosidine
formononetin
fortunellin
galactitol
galactosamine
gamma-aminobutyric acid
gamma-linolenic acid
gamma-nonalactone
gamma-terpinene
gamma-tocotrienol
genistein
gentiobiose
gentisic acid
gibberellic acid
ginsenoside rb1
ginsenoside rb2
ginsenoside rc
ginsenoside rd
ginsenoside re
ginsenoside rg1
glabrene
glabridin
glabrol
glucoerucin
gluconasturtiin
glucosamine
glucotropaeolin
glutamic acid
glutaric acid
glutathione
glycerin
glycerol
glycine
glycocholic acid
glycolic acid
glycyrrhetinic acid
glycyrrhizic acid
glycyrrhizin
glycyrrhizinate
glycyrrhizinic acid
gomisin e
gomisin f
gomisin g
gossypin
guanidine
guanosine
haplopine
harmaline
harman
harmane
heptadecane
heptanoic acid
hesperetin
hesperidin methyl chalcone
heteroauxin
hexanoic acid
hippuric acid
hirsutrin

Table 1 (*continued*)

Ingredient_name
hispaglabridin a
hispaglabridin b
homogentisic acid
homoorientin
homoserine
hymecromone
hyoscyamine
hyperin
hyperoside
hypoxanthine
hypoxanthine
icariin
indole
indole-3-acetonitrile
indole-3-carboxaldehyde
inosine
isoamylamine
isobetanin
isobutyric acid
isocitric acid
isoguvacine
isoliquiritin
isomaltose
isoorientin
isoquercetin
isorhamnetin
isorhamnetin-3-beta-d-galactopyranoside
isorhamnetin-3-o-glucoside
isorhamnetin-3-o-rutinoside
isosakuranetin
isovaleric acid
juniperic acid
kaempferide
kaempferitrin
kaempferol
kaempferol-3-o-glucoside
kaempferol-3-rhamnoside
kinetin
kynurenic acid
kynurenine
laudanosine
levodopa
l-homocysteine
l-homoserine
licochalcone b
lignoceric acid
linarin
linoleic acid
liquiritin
liquiritin apioside
lumichrome
lutein
luteolin
luteolin 7-beta-d-glucopyranoside
luteolin-4′-o-glucoside
luteolin-7-o-glucoside
luteoloside
lysine acid
malonic acid
malvin
m-coumaric acid
melatonin
meletin
mesaconic acid
methyl dihydrojasmonate
methyl octadecanoate
methyl salicylate
methyl stearate
methylprednisolone

Table 1 (*continued*)

Ingredient_name
metolachlor
miltirone
miscanthoside
monodydroxytanshinone i
morin
morphine
mucic acid
myo-inositol
myricetin
narcissin
naringenin
naringenin-7-o-glucoside
naringin
neoeriocitrin
neohesperidin
niacinamide
nicotiflorin
nicotinamide
nicotine
nicotinic acid
noradrenaline
norvaline
notoginsenoside r1
o-aminophenol
o-coumaric acid
oenin
oleic acid
ononin
o-phenylenediol
orientin
orotic acid
oxalacetic acid
paeoniflorin
paeonin
palmatine
palmitoleic acid
pantothenic acid
p-coumaric acid
pelargonidin
pentanoate
pentanoic acid
peonidin
peoniflorin
peonin
petunidin
phenethylamine
phloretic acid
phloretin
phloridzin
phlorizin
phosphoenolpyruvate
p-hydroxybenzoic acid
pipecolic acid
piperazine
piperidine
p-methoxycinnamic acid
polyprenol
poncirin
procyanidin b1
procyanidin b2
procyanidin b3
procyanidin b4
procyanidin c1
progoitrin
propranolol
prostaglandin e1
protocatechuic acid
protopine
prunin

Table 1 (*continued*)

Ingredient_name
puerarin
putrescine
pyridoxine
pyrocatechol
pyroglutamic acid
quercetin
quercetin-3-arabinoside
quercetin-3-o-alpha-l-rhamnopyranoside
quercetin-3-o-rutinoside
quercetin-3-rhamnoside
quercetin-4′-glucoside
quercetrin
quercitin
querciturone
quinone
quisqualic acid
raffinose
raphanin
reserpine
resveratrol
retinol
reynoutrin
rhamnetin
rhoifolin
ribitol
riboflavin
ricinine
robinin
rosmarinic acid
rotenone
rutin
sabinene
salicylic acid
salsolinol
sanguinarine
saponarin
sarcosine
sarsasapogenin
sativin
schisantherin a
schisantherin b
scopoletin
scopolin
scoulerine
sebacic acid
sennoside a
serotonin
sinalbin
sinapaldehyde
sinapic acid
sinapine
sinapyl alcohol
sinigrin
sinomenine
sissotrin
smilagenin
solasodine
sophoricoside
sparteine
spermidine
spermine
spiraeoside
styrone
suberic acid
succinic acid
sulfanilic acid
synephrine
syringaldehyde
syringic acid

(*continued on next page*)

Table 1 (*continued*)

Ingredient_name
syringic aldehyde
syringin
tamarixetin
tanshindiol b
tanshinone i
tanshinone iia
tanshinone iib
tanshinone vi
taurine
taurocholic acid
thebaine
theobromine
theophylline
thymol
tiliroside
trans-2-hexenal
trans-aconitic acid
trans-cinnamaldehyde
trans-cinnamic acid
triacanthine
tribuloside
trifolirhizin
trigonelline
trijuganone b
tropine
tropinone
tryptamine
tyramine
uridylic acid
urocanic acid
veratramine
vincetoxicoside b
vitexin
xanthine
xanthohumol
xanthotoxin
xylitol
zearalenone
zeatin
zeaxanthin

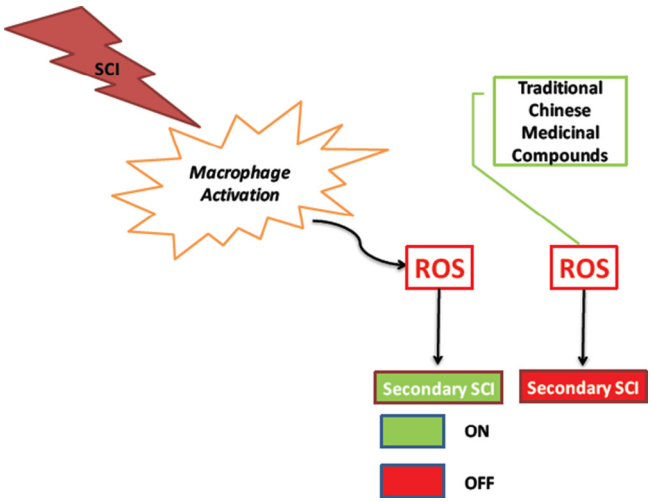

Fig. 1.

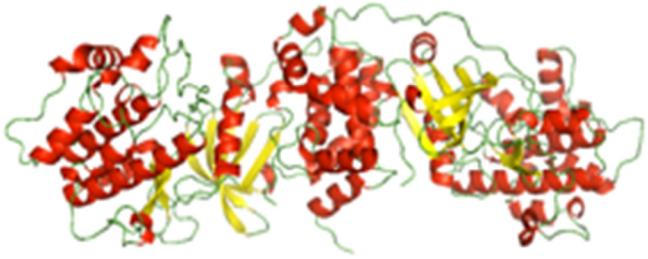

Fig. 2.

ΔG represents the overall binding energy. ΔG_{vdw}, ΔG_{hbond}, ΔG_{elec} represents Vander Waals, hydrogen bonding, and electrostatic energies respectively. ΔG_{tor} represents translation and rotation and the term ΔG_{desolv} indicates the desolvation on binding and hydrophobic effect. Lamarckian genetic algorithm (GA) default parameters were used for calculating ΔG of each shortlisted compound. Grid box ($60 \times 60 \times 60$ A°) was build around the active site. Energy values generated and the binding mode with cPLA2 protein site was used to limit the compound to single molecule.

3.2. Molecular visualization:

The cPLA2-Lead4 complex was studied using visualization tools Pymol (DeLano, 2002) and Discovery Studio (Studio, 2013).

4. Result and discussion

Virtual Screening: A database of in-house highly active TCM compounds were used to inhibit the ROCK protein by targeting its ATP binding site (Fig. 1). ROCK is composed of a ATP binding and a catalytic domain as where phosphorylation takes place, shown in Fig. 2. The ATP binding domain was used to generate inhibitors against ROCK protein. This inhibition has a role in reducing sSCI induced tissue damage via reduction of LP and decrease in oxidative stress. To come up with a novel ROCK protein inhibitor virtual screening, drug-likeliness, docking and molecular dynamics simulation methods were used. Virtual screening helped us to limit the number of compounds from the 672 natural productsto 128, based on their binding energy (ΔG Kcal/mol).

Drug likeliness: To limit the focus on compounds that could be promising for further development, we checked each compound for drug-likeliness. Drug-likeliness of shortlisted compounds was defined by mutagenic and carcinogenic property and rule of five (RO5) set by Lipinski RO5 properties include number of hydrogen bond donor (HBD), number of hydrogen bond acceptor (HBA) molecular weight (MW) and octanol/water partition coefficient (logP), the permissible range is HBD ≤ 5, HBA ≤ 10, MW ≤ 500 Dalton and clog p ≤ 5. Table 2 shows drug-likeliness properties, five compounds were shortlisted on their drug-likeliness values. All the compounds are accommodating the values expected from typical drugs.

Molecular Docking: The five final shortlisted natural compounds from IBS database were docked using AutoDock 4.2 tool into the optimized binding site of ROCK protein (Fig. 3). In Table 3 we have shown the results generated. Three of the Five natural compounds were found to form hydrogen bond with ROCK protein (Table 4). AutoDock tool was used for molecular docking simula-

Table 2
Top 124 compounds scrrened for further analysis.

Drug	Plant source	CID No.	CSID No.
Absinthin	*Artemisia absinthium* Linn	CID 442138	
Aescin	*Aesculus indica* colebr. & Camb. (Hippocastanaceae)		CSID 23089563
Aesculin	*Aesculus hippocastanum* Linn	CID 5281417	
Aglycone	*Eryngium coeruleum* Bieb.		CSID 16736194
Alantolactone	*Inula racemosa* HK. F.	CID 72724	
Amaroswerin	*Gentiana kurroo* Royle	CID 45359883	
Andromedotoxin (Acetyllandromedol)	*Rhododendron campanulatum* D. Don.		CSID 7827535
Apigenin	*Meconopsis horridula*	CID 5280443	
Apigravin	*Apium graveolens* L.		CSID 30776837
Apiumoside (Apiin)	*Apium graveolens* L.		CSID 4444321
Arnidiol	*Calendula officinalis* Linn.	CID 470259	
Artabsin	*Artemisia absinthium* L	CID 442146	
Artemisinin	*Artemisia drancunculus* L.	CID 68827	
Asarone	*Acorus calamus* Linn		CSID 552532
Ascaridol	*Chenopodium ambrosioides* L.	CID 10545	
Astragalin	*Aesculus indica* colebr. & Camb. (Hippocastanaceae)	CID 5282102	
Atisine	*Aconitum heterophyllum* Wallich ex Royle	CID 9548630	
Atropine	*Atropa acuminata*	CID 174174	
Avicularin	*Polygonum aviculare* Linn.	CID 5490064	
Azulene	*Achillea millefolium* L.	CID 9231	
Barrigenol A1	*Eryngium coeruleum* Bieb.	CID 177603	
Barringenol R1	*Eryngium coeruleum* Bieb.	CID 44202129	
β-Dihydrofucosterol (Azuprostat)	*Euphorbia helioscopia* Linn.	CID 457801	
Berberine	*Berberis aristata* DC	CID 2353	
Bergapten	*Apium graveolens* L.	CID 2355	
Bergenin	*Bergenia stracheyi* Hook	CID 2356	
Bikhaconitine	*Aconitum violaceum* Jacq.	CID 441713	
Borneol	*Prangos pabularia Lindl.*	CID 64685	
Camphene	*Prangos pabularia* Lindl.	CID 6616	
Cannabinin	*Cannabis sativus* Linn.		CSID 8372337
Cannabinol	*Cannabis sativus* Linn.	CID 2543	
Capillarin	*Artemisia drancunculus* L.		CSID 2340963
Carpesterol	*Solanum xanthocarpum*	CID 21155918	
Carvacrol	*Carum carvi* Linn.	CID 10364	
Carvone	*Carum carvi* Linn.		CSID 21106424
Celerin	*Apium graveolens* L.		CSID 137753
Choline	*Dictamnus albus* Linn.	CID 305	
Chrysophanic Acid (Chrysophanol)	*Rheum emodi* Wall.	CID 10208	
Citronellol	*Mentha arvensis* Linn.	CID 8842	
Colchicine	*Colchicum leteum* Baker	CID 6167	
Convolvulin (Convolvin)	*Convolvulus arvensis* L.		CSID 245689
Coriandrol	*Coriandum sativum* Linn.	CID 67179	
Coumarin	*Angelica glauca* Edgew.	CID 323	
Cryptopine	*Fumaria indica* L.	CID 72616	
Cyanidin	*Asparagus racemosus* Willd.	CID 68247	
Diosgenin	*Dioscorea deltoidea* Wall	CID 99474	
Ecdysterone	*Achyranthes aspera* L.	CID 5459840	
Emodin	*Rheum emodii*	CID 3220	
Ephedrine	*Ephedra gerardiana*	CID 5032	
Esculetin	*Koelpinia linearis* Pall.	CID 5281416	
Etoposide	*Podophyllum hexandrum* Royle	CID 36462	
Faradiel	*Calendula officinalis* Linn.	CID 122856	
Filicin	*Dryopteris filixmas* L.	CID 197044	
Fumaramine	*Fumaria indica* L.	CID 6450006	
Gentianine	*Gentiana kurroo* Royle	CID 354616	
Gentiopicrin	*Gentiana kurroo* Royle		CSID 32697064
Harmaline	*Peganum harmala* Linn.	CID 5280951	
Harmalol	*Peganum harmala* Linn.	CID 5353656	
Harmine	*Peganum harmala* Linn.	CID 5280953	
Hetisine	*Aconitum heterophyllum* Wallich ex Royle		CSID 10226875
Hetisinone	*Aconitum heterophyllum* Wallich ex Royle		CSID 10226887
Hexacosane	*Anagallis arvensis* L.		CSID 11901
Hyoscine	*Datura stramonium* Linn	CID 3000322	
Hyoscyamine	*Datura stramonium* Linn	CID 64692	
Hyperoside	*Asparagus racemosus* Willd.	CID 5281643	
Imperialine (Kashmirine)	*Fritillaria imperialis* Linn.	CID 442977	
Indaconitine	*Aconitum violaceum* Jacq.	CID 441740	
Inokosterone	*Achyranthes aspera* L.	CID 441828	
Intybin	*Cichorium intybus* L.	CID 174863	
Irigenin	*Iris kashmiriana*	CID 5464170	

(*continued on next page*)

Table 2 (*continued*)

Drug	Plant source	CID No.	CSID No.
Isoalantolactone	*Inula racemosa* HK. F.	CID 73285	
Isoatisine	*Aconitum heterophyllum* Wallich ex Royle	CID 245006	
Isoimperatorin	*Anthriscus nemorosa* Spreng	CID 68081	
Isopimpinellin	*Apium graveolens* L.	CID 68079	
Kaempferol	*Anagallis arvensis* L.	CID 5280863	
Lactucin	*Cichorium intybus* L.	CID 3756497	
Lactucopicrin	*Lactuca serriola Linn.*		CSID 2723771
Laureline	*Skimmia laureola* Hk. f.	CID 821373	
Lignans	*Daphne oleoides*	CID 9917980	
Luteolin	*Meconopsis horridula*	CID 5280445	
Malvalic Acid	*Althaea officinalis* L.	CID 10416	
Marrubin	*Marrubium vulgare* L.		CSID 66118
Maslinic Acid	*Epilobium angustifolium* Linn.	CID 73659	
Mezerein	*Daphne oleoides*	CID 9549167	
Myrcene	*Prangos pabularia* Lindl.	CID 31253	
Nepetalactone	*Nepeta cataria*	CID 161367	
Obaculactone (Dictamnolactone)	*Dictamnus albus* Linn.	CID 65071	
Obtusilobin (Obtusifolin)	*Anemone obtusiloba* D. Don	CID 3083575	
Oleanolic Acid	*Epilobium angustifolium* Linn.	CID 10494	
Osthenol	*Apium graveolens* L.	CID 5320318	
p-Cymene	*Thymus serpyllum* Linn.	CID 7463	
Peganine	*Peganum harmala* Linn.	CID 72610	
Pinoresinol	*Daphne oleoides*	CID 234817	
Podophyllotoxin	*Podophyllum hexandrum* Royle	CID 10607	
Prangolarin	*Anthriscus nemorosa* Spreng	CID 17536	
Protopine	*Argemone mexicana* L.	CID 4970	
Quercetin	*Aesculus indica* colebr. & Camb. (Hippocastanaceae)	CID 5280343	
Rutin	*Aesculus indica* colebr. & Camb. (Hippocastanaceae)	CID 5280805	
Sabinen	*Nepeta cataria*	CID 18818	
Safranal	*Crocus sativus* L.	CID 61041	
Sanguinarine	*Fumaria indica* L.	CID 5154	
Santonin	*Artemisia maritima* Linn	CID 221071	
scopoletin	*Artemisia drancunculus* L.	CID 5280460	
Sesamin	*Daphne oleoides*	CID 72307	
Seselin	*Apium graveolens* L.	CID 68229	
Sesquiterpene	*Acorus calamus* L.		CSID 19953446
shikonin	*Arnebia guttata* Bunge	CID 479503	
Sitosterol	*Adonis aestivalis* L.	CID 222284	
Spathulenol	*Nepeta cataria*	CID 522266	
Stigmasterol	*Asparagus racemosus* Willd.	CID 5280794	
Taraxacin	*Taraxacum officinale*	CID 5241825	
Taraxasterol	*Taraxacum officinale*	CID 5270604	
Tectoreginin	*Iris kashmiriana*	CID 5281811	
Trigonelline	*Achillea millefolium* L.	CID 5570	
Tropane	*Atropa acuminata*	CID 637986	
Umbelliferone	*Skimmia laureola* Hk. f.	CID 5281426	
Ursolic Acid	*Epilobium angustifolium* Linn.	CID 64945	
Valepotriate	*Valeriana jatamansi* Jones	CID 442436	
Xylopinine (Govanine)	*Corydalis govaniana*	CID 226520	
1-Hentriacontanol	*Aesculus indica* colebr. & Camb. (Hippocastanaceae)		CSID 61640
1,4-Cineole (Natural)	Artemisia maritima L.	CID 10106	
7-Methoxycoumarin (herniarin)	*Artemisia drancunculus* L.	CID 10748	
16-Hentriacontanone (palmitone)	*Aesculus indica* colebr. & Camb. (Hippocastanaceae)		CSID 85480

tions, the top binding pose based on ΔG were taken for further analysis. Each binding pose was studied using discovery studio, the default parameters were used to calculate all the possible interactions. The interactions studied are van der waals, conventional hydrogen bond, carbon hydrogen bond, pi-cation, pi-donor hydrogen bond, alkyl and pi- alkyl interaction. The lead2-ROCK complex has binding energy of −7.34 Kcal/mol andis forming two conventional hydrogen bonds with TYR96 and HIS62 of cPLA2′s C2 domain. The binding pocket of lead2 (1-cyclohexyl-5-(4-methoxybenzyl)-5-(((1R)-8-oxo-5,6-dihydro-1H-1,5-methano pyrido[1,2-a][1,5]diazocin-3(2H,4H,8H)-yl) methyl)pyrimidine-2, 4,6(1H,3H,5H)-trione) comprises following amino acids TYR96, VAL97, ASP40, LYS32, THR41, PRO42, ASP43, HIS62, ASN64, ASN65, ASP93, ALA94, and ASN95. The O_{22} atomic site of lead2 shows hydrogen bond interaction with TYR96 and HIS62, with a distance between the lead and ROCK of 1.88 Å and 1.67 Å respectively. Lead 4 (3-(furan-2-yl)-N-(furan-2-ylmethyl)-3-(p-tolyl)propan-1-amine) shows three conventional hydrogen bond interactions with the ATP binding domain of ROCK. Three atoms of lead4, $N_{15,}$ O_2 andO_5 are forming the bond with TYR96, HIS62 and ASN95 with a bond length of 1.82 Å, 2.14 Å and 1.97 Å respectively. The binding pocket of lead 4 comprises of nine amino acids: ASP40, THR41, ASN65, ASP43, ASN64, HIS62, ASN95, TYR96, and VAL97. Lead4 is having ΔG of −10.09 Kcal/mol, the best reported among the top ten compounds. The third compound showing interaction is Lead6 ((12bS)-7-(2-ethoxy-3-methoxyphenyl)-2-(3-isopropoxy

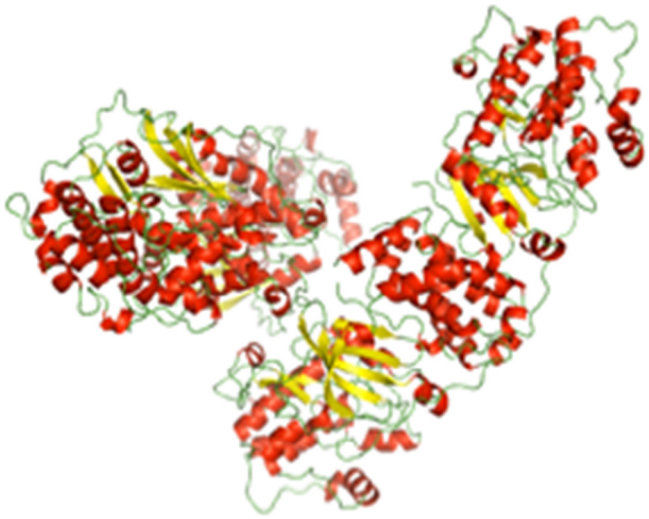

Fig. 3.

propyl)-12b-methyl-2,3,6,7-tetrahydropyrazino [1′,2′:1,2] pyrido [3,4-b]indole-1,4(12H,12bH)-dione),the binding pocket of the lead 6 molecule with lipid binding C2 domain of CPLA2 comprises of following amino acids viz. TYR96, ALA94, ASN95, LEU39, LYS32, ASP40, ASP43, ASN65, ASN64 and HIS62. Out of them lead6 forms hydrogen bond with TYR96 and HIS62, the interaction of our interest here is formed by lead6 O_{16} and O_{23} position with TYR96 and HIS62 at bond length 1.31 Å and 2.01 Å respectively. Based on ΔG and the number of interactions lead 7 (1-((S)-2-amino-4-methylpentanoyl)-N-((S)-1-((4-fluorobenzyl)amino)-3-methyl-1-oxobutan-2-yl)piperidine-4-carboxamide hydrochloride) is the least ranked among the top ten natural compounds inhibiting ATP binding domain of ROCK. Its binding pocket comprises of eleven amino acids; ASN64, ASP43, ASN65, LYS32, MET38, GLY36, LEU39, ASP37, THR41, ASP93, ASN95, TYR96, and ALA94. Lead7 shows ΔG of −6.07 Kcal/mol and has single hydrogen bond interaction between lead4′s O_{13} position and CPLA2′s TYR96 with bond length of 1.52.

Novel ROCK Inhibitor: The top natural compound inhibiting ATP binding domain of ROCK. The compound has the best binding energy and forms maximum number of hydrogen bonds, thus jamming the important ATP binding site in ROCK protein. To look into

Table 3
Gibbs free energy score, Physico-Chemical and biological properties of bioactive compounds.

Compound	Gibbs Free Energy (kcal/mol)	Drug-likeness						Absorbtion Distribution Metabolisim Excretion				
		Mutagenicity	Carcinogenicity	HBA	HBD	TPSA	MW	BBB	Caco2	HIA	MDCK	PPB
Carpesterol	−13.3787	yes	No									
Peganine	−11.0601	Yes	No									
Cyanidin	−10.4529	Yes	No									
Isoimperatorin	−10.3158	Yes	No									
Capillarin	−10.2923	Yes	Yes									
Atropine	−10.195	No	Yes									
Taraxacin	−10.0508	Yes	Yes									
Aescin	−10.0481											
Irigenin	−10.0414	No	No	Q	Q	Q	Q	0.043263	9.21056	86.80184	1.77078	79.69777
Hyoscyamine	−10.0339	Yes	No									
Osthenol	−9.58138	Yes	No									
Sabinen	−9.50068	Yes	No									
p-cymene	−9.42523	Yes	Yes									
Sanguinarine	−9.35777	Yes	Yes									
Coumarin	−9.33665	Yes	Yes									
Safranal	−9.29266	No	No	Q	Q	Q	Q	1.06267	23.0033	100	249.139	15.17273
Cannabinol	−9.18551	No	Yes									
Myrcene	−9.10893	Yes	No									
Ephedrine	−9.01662	Yes	No									
Carvone	−8.96438	Yes	No									
Intybin	−8.92803	No	Yes									
Azulene	−8.92402	Yes	Yes									
Carvacrol	−8.89384	Yes	No									
Faradiol	−8.89069	No	Yes									
Bergenin	−8.86525	Yes	No									
Hyperoside	−8.78649	No	No	V	V	Q	Q					
Avicularin	−8.67923	Yes	No									
Taraxasterol	−8.49042	Yes	No									
Emodin	−8.43599	No	No	Q	Q	Q	Q	0.668094	20.2745	90.42972	44.9367	100
Maslinic Acid	−8.40496	No	Yes									
Kaempferol	−8.36988	Yes	No									
Asarone	−8.34362	Yes	Yes									
Ecdysterone	−8.33694	No	Yes									
Apigenin	−8.32192	Yes	No									
Obaculactone	−8.29701	Yes	No									
Umbelliferone	−8.281	Yes	Yes									
Amaroswerin	−8.23858	No	Yes									
Tectorigenin	−8.23005	No	No	Q	Q	Q	Q	0.126227	5.59415	88.18405	16.8164	87.63624
Oleanolic Acid	−8.21107	No	Yes									
Celerin	−8.15443	Yes	No									
Barringenol A1	−8.02007	No	Yes									

(*continued on next page*)

Table 3 (*continued*)

Compound	Gibbs Free Energy (kcal/mol)	Drug-likeness						Absorbtion Distribution Metabolisim Excretion				
		Mutagenicity	Carcinogenicity	HBA	HBD	TPSA	MW	BBB	Caco2	HIA	MDCK	PPB
Obtusifolin	−8.00917	Yes	No									
Esculetin	−7.94481											
7-Methoxycoumarin	−7.94033											
Arnidiol	−7.92807											
Chrysophanic Acid	−7.90567											
Astragalin	−7.90475											
Fumaramine	−7.8825											
Ursolic Acid	−7.83721											
Sitosterol	−7.78748											
Marrubin	−7.76988											
Aesculin	−7.76358											
Shikonin	−7.70705											
Hetisine	−7.62995											
Seselin	−7.57116											
Ascaridol	−7.54342											
Hyoscine	−7.5294											
Malvalic Acid	−7.48294											
Quercetin	−7.4617											
Inokosterone	−7.41245											
Luteolin	−7.39778											
Imperialine	−7.38899											
Santonin	−7.25135											
Camphene	−7.22408											
Stigmasterol	−7.2086											
Diosgenin	−7.20553											
Sesquiterpene	−7.19878											
Isopimpinellin	−7.10912											
Absinthin	−7.08183											
Filicin	−7.06472											
Colchicine	−7.05574											
Isoatisine	−6.99133											
Laureline	−6.98638											
Lactucopicrin	−6.96841											
ß-Dihydrofucosterol	−6.95752											
Scopoletin	−6.88906											
Andromedotoxin	−6.86834											
Gentiopicrin	−6.84314											
Hetisinone	−6.83401											
Harmalol	−6.82384											
Podophyllotoxin	−6.82185											
Harmaline	−6.81269											
Govanine	−6.78949											
Bergapten	−6.75754											
Lactucin	−6.75577											
Sesamin	−6.74654											
Harmine	−6.72931											
Protopine	−6.71054											
Apiin	−6.68698											
Artabsin	−6.64119											
Berberine	−6.62831											
Valepotriate	−6.59665											
Barringenol A1	−6.59396											
Apigravin	−6.52009											
Artemisinin	−6.51894											
Spathulenol	−6.4596											
Alantolactone	−6.43428											
Isoalantolactone	−6.42729											
Convolvin	−6.33586											
Rutin	−6.23839											
Pinoresinol	−6.23388											
Citronellol	−6.21042											
Nepetalactone	−6.19349											
Atisine	−6.18617											
1,4-Cineole	−6.02481											
Tropane	−5.85134											
Borneol	−5.81539											
Prangolarin	−5.73041											
Cryptopine	−5.59433											
Coriandrol	−5.59364											
Trigonelline	−5.55835											

Table 3 (*continued*)

Compound	Gibbs Free Energy (kcal/mol)	Drug-likeness						Absorbtion Distribution Metabolisim Excretion				
		Mutagenicity	Carcinogenicity	HBA	HBD	TPSA	MW	BBB	Caco2	HIA	MDCK	PPB
16-Hentriacontanone	−5.45843											
Lignans	−5.4099											
Hexacosane	−5.35611											
Cannabinin	−5.15507											
Mezerein	−4.89756											
Choline	−4.69109											
Etoposide	−4.47279											
1-Hentriacontanol	−1.20476											
Aglycone	24.0676											

Q: *Qualified;* **V**: *Violated.*

Table 4
Auto Dock analysis of four compounds. The ligand binding pocket and the hydrogen bond formation was calculated using Discovery Studio 3.5 software. The bold amino acids represent the one which are involved in forming hydrogen bond with the ligand.

NAME	Chem ID	ΔG Kcal/mol	Ligand binding pocket	H-bonds
Irigenin	5,464,170	−10.04	GLY42, **ILE43**,PHE47,GLU45,**HIS44**	IRIGENIN:H31 -:GLU45:O(2.082 Å). GLU45:H – : IRIGENIN:O3(1.81 Å). GLU45:H – : IRIGENIN:O4(2.29 Å). **HIS44:HD1 – : IRIGENIN:O3(2.19 Å).** **HIS44:HD1 – IRIGENIN:O4(1.89 Å).** **IRIGENIN:H33 – ILE43:O (2.19 Å).** **ILE43:H – : IRIGENIN:O6(2.43 Å).**
Safranal	61,041	−9.29	**HIS44**, TYR68, LEU46, GLY61, TYR36, LEU62, LEU59	TYR36:HH – :SAFRANAL:O1(1.99 Å).
Emodin	3220	−8.43	ASP41, GLY42, **ILE43**, GLU45, **HIS44**	EMODIN:H30 – GLU45:OE1 (2.03 Å). **ILE43**:H – : EMODIN:O2. (2.37 Å). EMODIN:H28 – A:ASP41:O(1.84 Å).
Tectorigenin	5,281,811	−8.23	GLU45,**ILE43**,GLY42,ASP41,**HIS44**	Tect:H34 – GLU45:OE1(1.91 Å). **Tect:H29 – :ILE43:O(2.12 Å).** **ILE43:H – :TECT:O3(2.05 Å).**

Table 5
MM-PBSA calculations.

Summary	Values
Van der Waal energy	−160.104 ± 23.737 kJ/mol
Electrostatic energy	−8.257 ± 8.986 kJ/mol
Polar solvation energy	39.374 ± 14.616 kJ/mol
SAV energy	−92.616 ± 17.234 kJ/mol
Binding energy	−221.602 ± 35.657 kJ/mol

Lead 4 drug-ability, its Absorption, Distribution, Metabolism and Excretion (ADME) properties were calculated using in-silico ADME/Tox server (https://preadmet.bmdrc.kr/). In this calculation features like Caco-2 cell permeability (Caco-2p), MDCK cell permeability (MDCKp), Human intestinal absorption, Plasma Protein Binding and Blood Brain Barrier values of Lead 4 were calculated. The results generated are shown in Table 5. For drug absorption Caco-2 cell model and MDCK cell model were used and the value ranges in permissible range, the human intestinal absorbance (HIA) of 92.59% shows that Lead 4 can be well absorbed and can reach the target site easily. The plasma protein binding of lead 4 is 17.71% and shows its availability to reach the target protein is high. Evaluation of cell cytotoxicity revealed the IC50 for lead4 at 134.2 ± 6.8 μg/ml.

Conflict of interest

The authors declared no conflict in this manuscript and publications.

References

Amin, A. et al., 2016. Irigenin, a novel lead from Western Himalayan chemiome inhibits Fibronectin-Extra Domain A induced metastasis in Lung cancer cells. Sci. Rep., 6

Budzyn, K., Marley, P.D., Sobey, C.G., 2006. Targeting Rho and Rho-kinase in the treatment of cardiovascular disease. Trends Pharmacol. Sci. 27 (2), 97–104.

Cadotte, D.W., Fehlings, M.G., 2011. Spinal cord injury: a systematic review of current treatment options. Clin. Orthopaedics Related Res.® 469 (3), 732–741.

DeLano, W.L., 2002. The PyMOL molecular graphics system.

Hall, E.D. et al., 2016. Lipid peroxidation in brain or spinal cord mitochondria after injury. J. Bioenerg. Biomembr. 48 (2), 169–174.

Lipinski, C.A., 2004. Lead-and drug-like compounds: the rule-of-five revolution. Drug Discov. Today: Technol. 1 (4), 337–341.

McDonald, J.W., Sadowsky, C., 2002. Spinal-cord injury. Lancet 359 (9304), 417–425.

Morris, G.M. et al., 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 30 (16), 2785–2791.

Studio, D., 2013. Accelrys Inc. San Diego, CA, USA.

Tokushige, H. et al., 2011. Effects of Y-39983, a selective Rho-associated protein kinase inhibitor, on blood flow in optic nerve head in rabbits and axonal regeneration of retinal ganglion cells in rats. Curr. Eye Res. 36 (10), 964–970.

van Gunsteren, W.F. et al., 1996. Biomolecular simulation: the {GROMOS96} manual and user guide.

Viewer, S.-P., Kaplan, W., Littlejohn, T.G., 2001. Briefings Bioinf. 2 (2), 195–197.
Trott, Oleg, Olson, Arthur J., 2010. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 31 (2), 455–461.
White, N.-H., Black, N.-H., 2016. Spinal cord injury (SCI) facts and figures at a glance.
Yamamoto, K. et al., 2014. The Novel Rho Kinase (ROCK) Inhibitor K-115: a new candidate drug for neuroprotective treatment in glaucomanovel rho kinase inhibitor. Invest. Ophthalmol. Vis. Sci. 55 (11), 7126–7136.
Zhou, X., He, X., Ren, Y., 2014. Function of microglia and macrophages in secondary damage after spinal cord injury. Neural Regener. Res. 9 (20), 1787.