Insights on the molecular mechanism of anti-inflammatory effect of formula from Islamic traditional medicine: An in-silico study

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ABSTRACT

Background and aim: Traditional medicine is an important source for drug discovery. However, many challenges face the scientific community to develop novel drugs from it. To investigate the rationale behind the medical legacy of centuries of precious knowledge from traditional medicine, we aimed at performing virtual screening to identify potential leads from the middle-age textbook, The Canon of Medicine.

Experimental procedure: A database of chemical constituents of plants mentioned within the book was built and docked against different molecular targets associated with inflammation such as phospholipase A2, p38 alpha mitogen activated protein kinase, cyclooxygenase-2 and leukotriene B4 dehydrogenase, after that literature survey was done to determine the consistency of traditional uses and molecular docking results with the current knowledge obtained from previous studies and reports.

Results and conclusion: The in-silico study revealed the ability of several chemical constituents, in the plants under investigation, to bind effectively to different targets associated with inflammation, which was consistent with previous reports, indicating that Islamic traditional medicine can be considered as a reliable promising source for developing new anti-inflammatory agents with low toxicity and minimal side effects.

1. Introduction

Inflammation is the body's attempt at removing harmful stress, including damaged cells, irritants or pathogens to start the healing process, which leads to release of pro-inflammatory mediators into the blood or affected tissues causing increased blood flow to the of injury or infection and may result in redness and warmth, also chemicals like prostaglandins cause a leak of fluid into the tissues resulting in swelling. This protective process may stimulate nerves and cause pain.[1,2] However, the continuous inflammatory state is believed to be responsible for the pathogenesis of several diseases such as metabolic disorders, several types of cancer and Alzheimer's disease. Consequently, targeting chemical mediators controlling inflammation should be a promising approach for preventing and management of several diseases.[2–4]

Chemical mediators of the inflammatory process include a variety of substances originating in the plasma and the cells of un-injured tissue and possibly from the damaged tissue. Moreover, a lot of enzymes like phospholipase A2, p38 mitogen activated protein kinase (p38 MAPK), cyclooxygenase-2 (Cox-2), Leukotriene B4 dehydrogenase (LTB4DHR) is associated with inflammation process.

Phospholipase A2 is a member of a family of esterases that are involved in a wide array of physiological and pathological processes. It catalyzes the hydrolysis of phospholipids leading to the production of free fatty acids and lysophospholipids, which is converted to arachidonic acid. Subsequently, arachidonic acid is converted to prostaglandin H_2 which is the main precursor for production of prostaglandins and pain producing substances. Since it plays an important role in the generation of pro-inflammatory lipid mediators, it's considered as one of the therapeutic targets of interest to develop new anti-inflammatory agents.[5–8]

During inflammation, multiple intracellular signaling cascades

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Abbreviations

PLA$_2$	Phospholipase A2	ADME	Absorption, Distribution, Metabolism, And Excretion
p38 MAPK	p38 Mitogen Activated Protein Kinase	iNOS	Inducible Nitric Oxide Synthase
Cox-2	Cyclooxygenase-2	LPS	Lipopolysaccharide
LTB4DHR	Leukotriene B4 Dehydrogenase	PGE2	Prostaglandin-2
AP-1	Activator Protein-1	TNF-alpha	Tumor Necrosis Alpha
ATF-2	Activating Transcription Factor 2	IL-1β	Interleukin-1 Beta
TM	Traditional Medicine	FCA	Freund's Complete Adjuvant
VS	Virtual Screening	IL-12	Interleukin-12
SBVS	Structure Based Virtual Screening	NO	Nitric Oxide.
PDB	Protein Data Bank	NF-KB	Nuclear Factor-Kappa B
DL	Human Drug-Likeness	JNK	C-Jun N-Terminal Kinase
OB	Oral Bioavailability	(ERK)1/2	Extracellular Signal-Regulated Kinase
		ADME	Absorption, Dissolution, Metabolism, Excretion

activate the (p38 MAPK) pathway, which control the recruitment of leukocytes to sites of inflammation.[9,10] Furthermore, it can regulate several inflammatory pathways by activation of several transcriptional factors such as activator protein-1 (AP-1), activating transcription factor-2 (ATF-2)[11] direct phosphorylation of Phospholipase A2 to initiate the arachidonic acid pathway[12] also it was found that expression of COX-2 and PGE2 is sensitive to p38 MAPK blockade[13,14] and recent work has shown that regulation of COX-2 activity may depend on MAPK activation of the Nuclear Factor-Kappa B (NF-κβ) pathways.[15]

The expression of COX-2 is induced selectively by pro-inflammatory cytokines at the site of inflammation. It is involved in the conversion of arachidonic acid to prostanoids. Since COX-2 has been localized primarily to inflammatory cells and tissues, many drugs were developed to inhibit this enzyme selectively and achieved excellent clinical outcomes.[16,17]

After the initiation of inflammatory response, there are intrinsic mechanisms to resolve the inflammatory action by production of anti-inflammatory Lipoxins which work as a stop signaling and stimulate the release of other resolving factors[18] however, the eicosanoid inactivating enzymes such as LTB4DHR could limit the anti-inflammatory effect of such mediators leading to extended inflammatory conditions. Interestingly, it was found that clinically used drugs such as indomethacin and diclofenac don't exert their actions by only inhibiting the cyclooxygenase enzyme but also by preventing the degradation of the anti-inflammatory eicosanoids by inhibiting LTB4DHR, which suggest the importance of the regulation of this target in treatment of inflammatory diseases.[19,20]

Traditional medicine (TM) is the total of the knowledge, skills, and practices, based on the theories, beliefs, and experiences indigenous to diverse cultures, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.[21,22] The Canon of Medicine written by the medieval physician Ibn-Sina (Avicenna), was the main medical reference till the 18th century[23,24] however, the advances in medical knowledge and the introduction of synthesized medication led to the neglection of such invaluable legacy.

While TM proved its efficacy over a long time of experimentation and observations, still the lack of the tools and technology for identification and standardization of components of TM poses a great challenge for the development of new therapeutics from traditional medicine. Moreover, TM is based on empirical philosophy that defines the health and disease state considering the body as a whole unit, and the balance between different forces controlling the physiological functions. This is contrary to the western medicine point of view which relies more on understanding the etiology of diseases by investigating molecular cascades that induce

and manage the pathogenesis of different ailments.[25,26]

Although the accumulation of huge amount of phytochemical information addressing the isolation of chemical constituents of medicinal plants; it will be almost impossible to evaluate their biological activities especially with the increasing number of therapeutic targets, this is where virtual screening (VS) can make an important contribution.[27–29]

VS is specialized discipline that uses computational methods to simulate drug-receptor interaction in a virtual way, which is known as structure based virtual screening (SBVS); using such tool could provide a chance to identify phytochemicals that are responsible for the effectiveness of traditional method.[30]

This work aims to employ SBVS to investigate the molecular mechanisms behind the anti-inflammatory effect of compounds derived from formula mentioned in The Canon of Medicine, by assessing their ability to bind with the beforementioned molecular targets; also we will shed the light on evidences from the literature that supports such claim.

2. Materials and methods

2.1. Choosing herbal formula

The formula was chosen from The Canon of Medicine, 1593 manuscript digitized by American University of Beirut (http://ddc.aub.edu.lb/projects/saab/avicenna/), under the treatise 3, on general management of bites (stings) and driving away (repelling) insects, and on signs of snake bites and their types, Avicenna claimed that these formulae were used for treatment of scorpion bites which are associated with incidence of inflammation.

To translate the plants from Arabic to Latin, the appendix accompanies the electronic version(http://ddc.aub.edu.lb/projects/saab/avicenna/appendix_1.html) in addition to other Arabic-Latin dictionaries were used.[31]

2.2. Retrieving chemical constituents of the plants

The active constituents of the plants under investigation were retrieved mainly from dictionary of natural products (http://dnp.chemnetbase.com), KNApSAcK Core System (http://kanaya.naist.jp/knapsack_jsp/top.html) metabolomics database, and reviewing available phytochemical literature.

2.3. Building database of the chemical constituents of the plants

The compounds were drawn using Chembiodraw, Cambridge soft corporation, (Version 14) as a neutral species with the correct

stereochemistry and then saved in SDF format, the files were exported to MOE software 2015, and converted to the 3D structure using quick prep module without changing the stereochemical aspects of the compounds, energy minimization was done using the MMFF94x force field using gradient of 0.1 RMS Kcal/Mol/A[232] The compounds exported to MONA software (http://www.biosolveit.de/Mona/), and add the compounds were saved to as one file in mol2 format.[33]

2.4. Applying Lipinski's rule of five

The database was subjected to filtration using MONA software Lipinski's rule of five filter which is a guideline for choosing compounds with a greater chance of yielding successful drugs that have a good bioavailability[34]

2.5. Choosing the molecular targets

Therapeutic target database (TTD) (http://bidd.nus.edu.sg/group/cjttd/) was consulted for target selection and target validation, 5 targets were chosen to represent different targets involved in inflammation) their X-ray crystal structure were retrieved from protein data bank(www.pdb.org), their PDB- ID was as following; phospholipase A2(PLA2): 1DB4, p38 α mitogen activated protein kinase (p38 MAPK): 1OUK, Leukotriene B4 dehydrogenase (LTB4DHR): 2DM6, and Cyclooxygenase-2 (Cox-2): 3NT1.

2.6. Preparation of receptor for virtual screening

Essential amino acids required for good binding affinity was determined from the Pose view generated interaction provided by the PDB, and the bounded reference ligand was exploited to determine the binding site using the default options in the receptor preparation wizard in LeadIT software (https://www.biosolveit.de/LeadIT/).The binding site was defined as 6.5 Å around the ligand in the active site, water molecules were removed if they don't have a role in the interaction of the ligand with the active site.

2.7. Molecular docking studies

The molecular docking was performed using FlexX docking engine in LeadIT software which uses the robust incremental construction algorithm. The validation of the software was assessed by redocking the co-crystalized ligand in the active site of the target; taking in consideration the root-mean-square deviation (RMSD) value should be less or equal to one, and the ability of the software to reproduce the same interactions observed experimentally using X-ray crystallography.

The database of the compounds was loaded and docked in the active site using the default options, the maximum number of solutions per iteration and fragmentation were set to 200 and the top 3 poses for each compound were kept for visualization. The best 10 compounds in term of binding affinity, and the ability to bind with essential amino acids in the active were selected for post-docking analysis, also ADME parameters namely, human drug-likeness (DL), oral bioavailability (OB) and Caco-2 permeability (Caco-2)

were employed to calculate their pharmacokinetic using TCMSP database.[35]

3. Results and discussion

To investigate the claims of anti-inflammatory effect of Islamic traditional medicine; we chose a formula from the Canon of Medicine, based on reports indicating that plants used for treatment of scorpion bites are usually possessing anti-inflammatory effects[28] the plants were translated to their corresponding Latin names (Supplementary.Table.1) to facilitate the access to phytochemical information found in the literature.

One hundred and fifty-seven compounds derived from the plants under investigation were collected from different phytochemicals database, and available literature. ADME filtration revealed that 153 compounds were obeying to Lipinski's rule of five.

The validation of SBVS was done by redocking the co-crystallized ligands, in all cases, the software could reproduce the experimental binding mode with RMSD equal or less than one, also HYDE assessment predicted binding affinity like those reported in protein data bank (Table 1).

The molecular docking of the database of the 3D structure of the compounds in the active site of the selected target was done, the best 10 compounds in terms of binding affinity and the ability to interact with essential amino acid in the binding site were selected for post docking analysis.

It was found that 22 compounds could bind effectively at least to single target only; eleven of them were able to bind with more than putative target which suggests that the formula exerts its action by targeting different steps in the pathway of inflammation. Remarkably, out of the nine plants under investigation, twelve compounds (Supplementary Fig.1) were found to be in 3 plants, *Mentha pulegium*, *Rumex patientia* and *Taraxacum officinale* (Table 2), their Pharmacokinetic properties can be found at Table 3.

In case of docking the compounds into the binding site of PLA2; it was noticed that the best 3 compounds in the term of binding energy (**5,8** and **12**) were able to interact with Gly29, Gly31, His47 or Asp48 amino acids (Fig. 1)which is consistent with other reports indicating the importance of these interactions to achieve inhibitory effect on this enzyme.[36,37]

For p38 MAPK, the post docking analysis of the 3 best compounds (**4,5** and **7**) in the binding energy showed the interaction with amino acids such as MET 109 and GLY 110 (Fig. 2), which is indicative for the ability to inhibit the activity of this type of kinases.[38]

Analyzing the interaction between the compounds (**7, 8** and **10**), with the active sites of the crystal structure of COX-2, revealed that they could interact with two essential amino acids Ala 120 and Tyr 355 by hydrogen bonding and hydrophobic interactions with the following amino acids Val349, Ala527, Leu352 which has been shown to be an important residues for the proper positioning of amino acids, required for enzyme activity(Fig. 3).[3,9]

Finally, the interaction between compounds (**8,9** and **10**) and the binding site of LTB4DHR demonstrate their ability to bind with Arg56, Tyr262, Val 272 (Fig. 4) which prevents the activation of the

Table 1
Validation of molecular docking software by redocking the co-crystallized ligands.

PDB code	Co-crystallize Ligands	FlexX SCORE	RMSD	Interaction with essential amino acid/co-factors
1db4	8In-200-A	−38.19	0.7598	Gly29, Gly31, His47,
1OUK	084−501-A	−34	0.9218	Met109, Asp168, Thr106
3NT1	Naproxen	−29.23	0.7902	Arg120, Tyr355
2DM6	Indomethacin	−19.18	0.8632	Arg56, Tyr262, Tyr273

Table 2
Post-docking analysis of compounds achieving best binding energy with the selected targets.

|		PLA$_2$		p38 alpha		COX-2		LTB4DHDH	
Plant	Compound	Binding energy ΔG (kcal/mol)	Interaction with amino acids	Binding energy ΔG (kcal/mol)	Interaction with amino acids	Binding energy ΔG (kcal/mol)	Interaction with amino acids	Binding energy ΔG (kcal/mol)	Interaction with amino acids
Mentha pulegium	Pedalitin	−22.75	Asp48, Lys62, Leu2, Gly29	−24.16	Asp168,Ala34,Met109, His107,Gly110	−18.20	Arg 120, Tyr 355, Val349, Ala527, Leu352	−	−
	Jaceosidin	−22.75	Asp48, Lys62, Leu2,Gly29	−25.8	Asp168,Ala34,Met109, Gly31,His107, Gly110	−	−	−17.96	Cys239,Tyr 245, Tyr262, Tyr273
	Thymonin	−	−	−25.04	Asp168,Ala34,Met109, His107,Gly110	−	−	−17.18	Cys239,Tyr 245, Tyr262, Val, 272, Tyr273
Rumex patientia	Emodin	−27.17	Asp48, Gly31, Glu55, Thr61	−27.1	Met109,Gly110, Ala111, His107	−	−	−18.14	Cys239, Val 272
	Physcion	−23.81	Gly31, Gly29, Thr61	−27.85	Met109,Gly110,His107, Ala111,Asp112	−	−	−	−
	Rhabarberone	−22.5	Lys52,Asp48,Gly31,Gly29	−	−	−20.04	Tyr 355, Ser 530 Val349, Ala527, Leu352	−	−
	Chrysophanol			−27.32	Met109,Gly110,Ala111, His107	−21.03	Tyr 355, Val349, Ala527, Leu352, Met 522	−	−
	Methylemodin	−28.19	Gly31, Gly29, Glu 55, Thr61, Asp48	−	−	−	−	−	−
Taraxacum officinale	Cis-Caffeoyl Tartaric Acid	−25.81	Lys52,Lys62,Asp48,His47, Gly29,Glu55	−	−	−23.71	Arg 120, Tyr 355, Val349, Ala527, Leu352	−19.27	Arg56, Tyr262, Val 272
	Caftaric Acid	−23.29	Glu55,Gly29, Gly31,Lys62, Lys52	−	−	−19.24	Arg 120, Met 522, Val349, Ala527, Leu352	−22.55	Arg56, Tyr262, Val 272
	Luteolin	−	−	−26.50	Met109,Gly110,His107, Gly31,Ala34,Asp168	−23.56	Arg 120, Tyr 355, Val349, Ala527, Leu352	−19.66	Cys239, Tyr262, Tyr273
	Hydroxycinnamic Acid	−	−	−	−	−20.77	Arg 120, Tyr 355, Val349, Ala527, Leu352	−18.06	Ala53, Arg56, Tyr262

Table 3
Pharmacokinetic properties of compounds achieving best binding energy with the selected targets.

NO.	Compound	OB (%)	Caco-2	DL
1	Pedalitin	34.02	0.38	0.31
2	Jaceosidin	2.14	0.42	0.34
3	Thymonin	1.97	0.65	0.41
4	Emodin	24.40	0.22	0.24
5	Physcion	22.29	0.52	0.27
6	Rhabarberone	83.38	−0.12	0.24
7	Chrysophanol	18.64	0.62	0.21
8	Methylemodin	20.44	0.43	0.27
9	Cis-Caffeoyl Tartaric Acid	11.33	−1.42	0.20
10	Caftaric Acid	N/A	N/A	N/A
11	Luteolin	36.16	0.19	0.25
12	Hydroxycinnamic Acid	53.60	0.48	0.04

* OB(%): oral bioavailability, CaCo-2: intestinal permeability, DL: Drug likeness.

enzyme by restricting the access of its normal substrate 15-oxo-PGE2.[40]

It was noticed that best three compounds in the binding affinity were almost superimposed in the active site of the 4 targets as presented in Fig. 5 which indicates that they possess the same binding mode. Collectively, our *in-silico* study showed that the active compounds in the formula could inhibit different molecular targets under investigation, the proposed mechanism of action summarized in Fig. 6 suggests that some of the compounds could inactivate PLA2 enzymes by direct inhibition or through preventing its phosphorylation by blocking the MAPK pathway which also would prevent the release of cytokines responsible for chemotaxis and further progress of inflammation, also the compounds might be able to stop the production of prostaglandins and the hydrolysis of anti-inflammatory lipoxin by inhibiting COX-2 and LTB4DHR
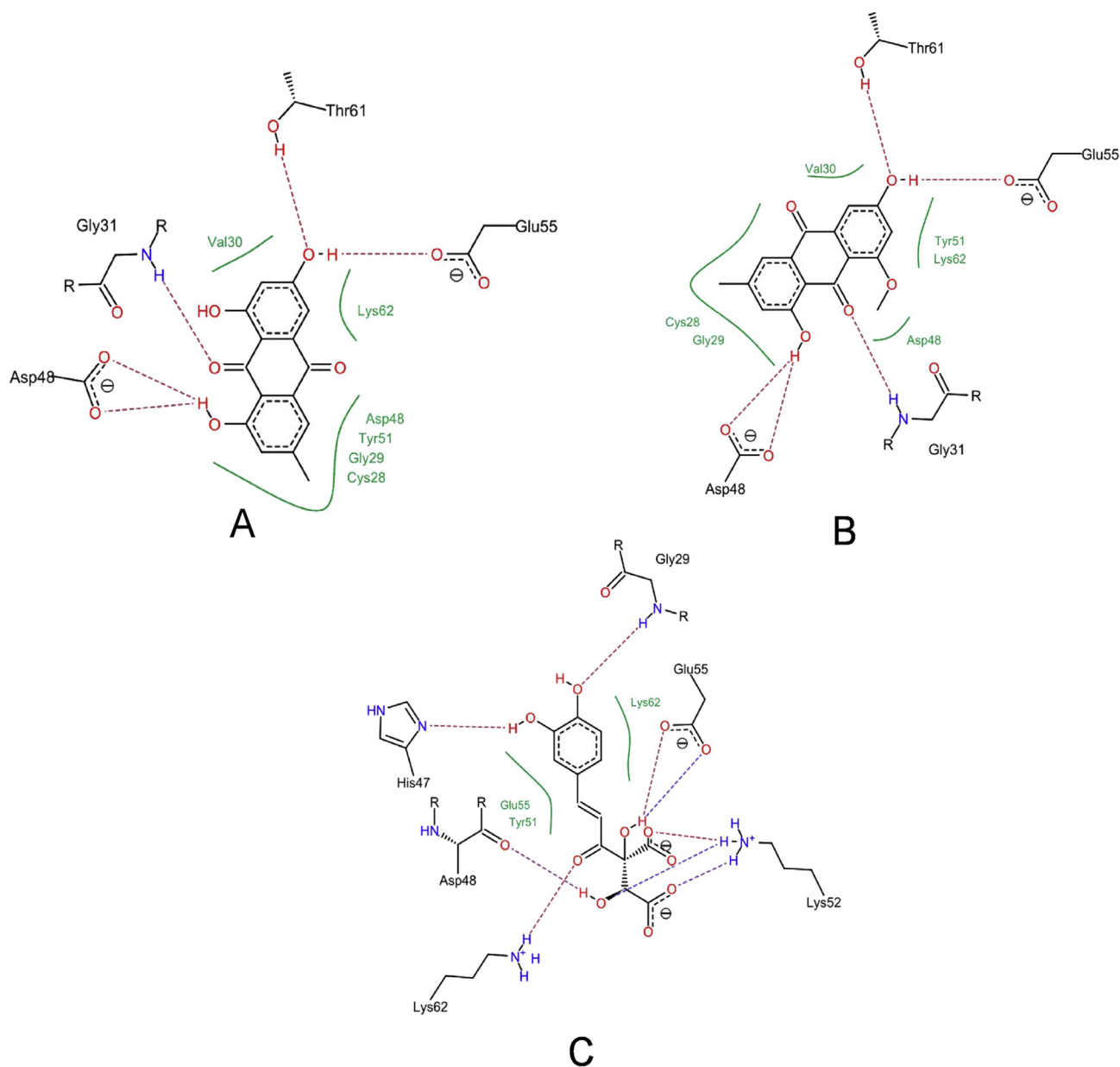
Fig. 1. Ligand interaction diagram of compounds with PLA2 (PDB ID: 1db4). A) PLA2/Methylemodin complex (B) PLA2/Emodin complex (C) PLA2/cis-caffeoyl tartaric acid complex.
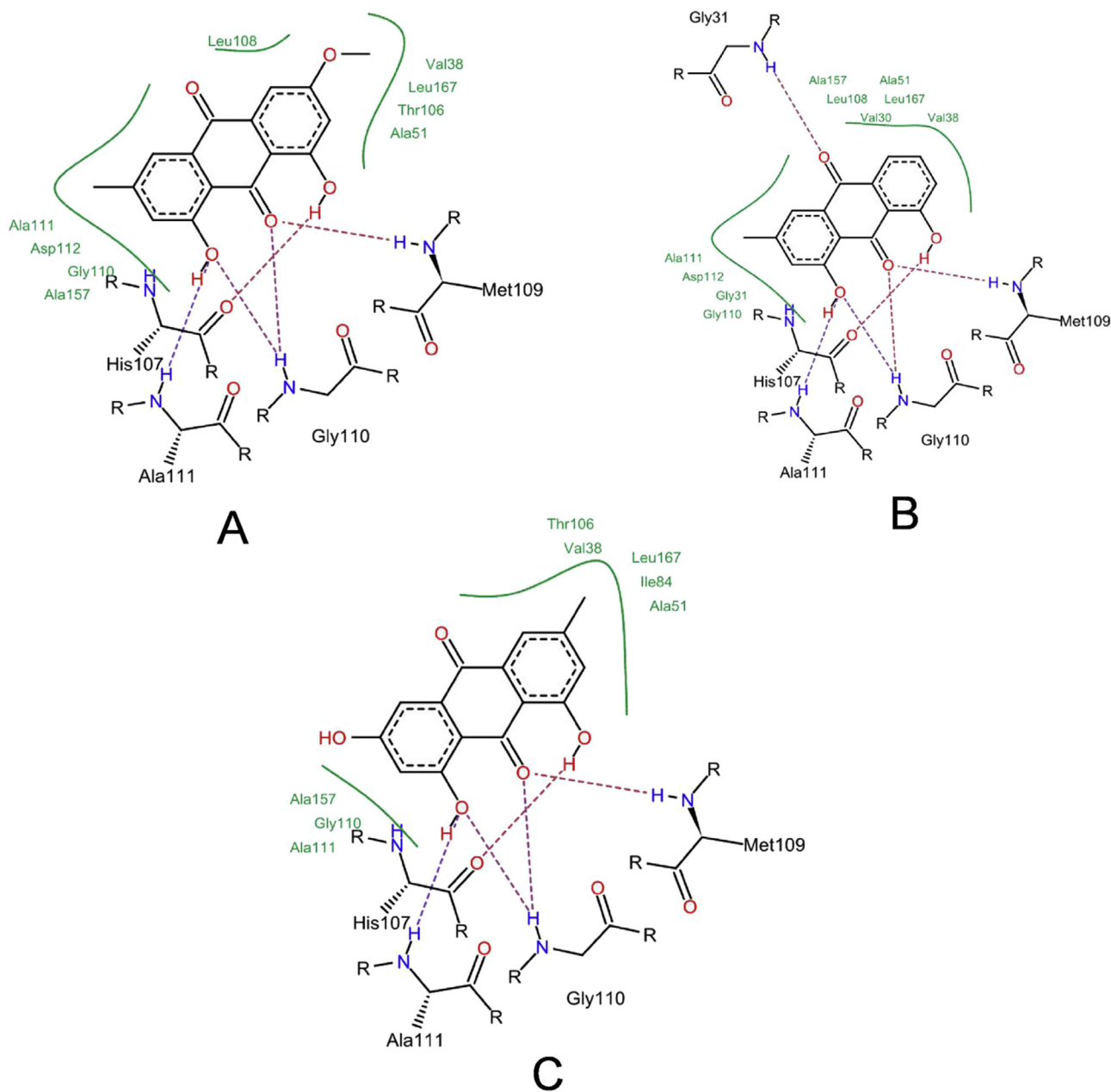
Fig. 2. Ligand interaction diagram of compounds with p38 α MAPK (PDB ID: 2QD9). A) P38 α/Physcion complex (b) P38α/Chrysophanol complex (c) P38 α/Emodin complex.

respectively.

It's worthy to note that reviewing the literature showed that there are many reports addressing the anti-inflammatory effect of the plants mentioned in the formula prescribed by Avicenna; *Ferula szowitsiana* and its sesquiterpene coumarins was found to decrease the inflammation in the carrageenan induced paw edema significantly[41,42] in addition, methyl galbanate an active compound found in the plant was able to inhibit nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression in Lipopolysaccharide (LPS) stimulated RAW264.7 cells at a concentration of 10 μM.[43,44] *Ferula asafoetida* a closely related species was reported to have analgesic activity by inhibiting the production of prostaglandins[45]

Rumex patientia aqueous and alcoholic extract is reported to possess anti-inflammatory effect against carrageenan induced paw edema[46] also it inhibited capillary permeability induced by xylol

and hyaluronidase, and found to be as effective as indomethacin.[47] The phytochemical investigation of this plant revealed the presence of anthraquinones such as Emodin and Chrysophanol, which are known to have anti-inflammatory effect.[48,49]

Taraxacum officinale was found to exert its anti-inflammatory effect through its inhibition of NO production, COX-2 expression and/or its antioxidative activity.[50] In agreement to our *in-silico* study, the extract of the leaves of *T.Officinale* was able to down-regulate nitric oxide, PGE2, and pro-inflammatory cytokines and reduced expressions of iNOS and COX-2 via inactivation of the MAPK signal pathway in LPS stimulated RAW264.7 cells.[51] Taraxasterol a pentacyclic triterpene isolated from *T.Officinale* significantly inhibited the overproduction of serum TNF-α, IL-1β and PGE2 in Freund's Complete Adjuvant (FCA) induced arthritis in rats.[52]

Mentha pulegium is commonly used traditionally for treating
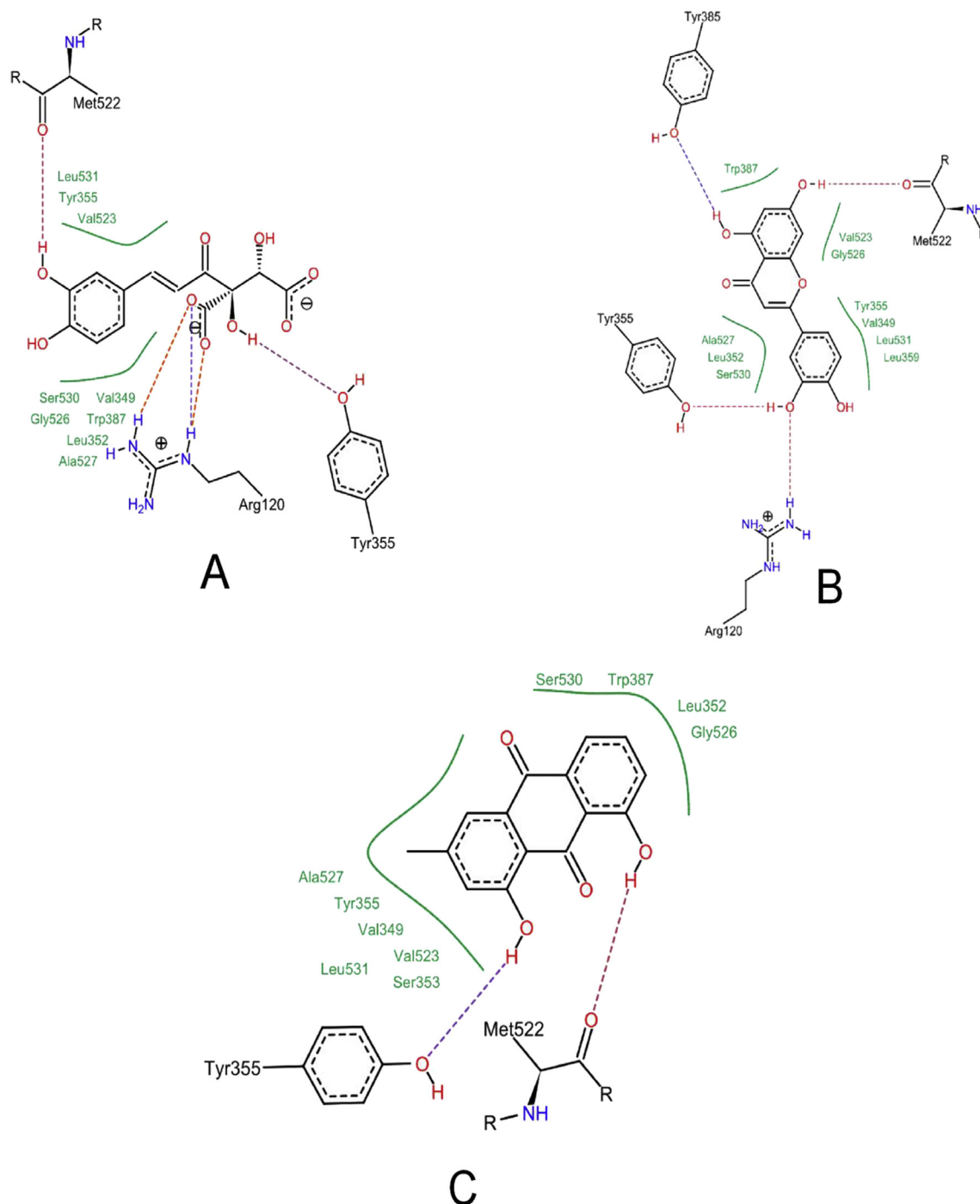
Fig. 3. Ligand interaction diagram of compounds with COX-2 (PDB ID: 1NT3). A) COX-2/Cis-caffeoyl tartaric acidcomplex (b) COX-2/Luteolin complex (c) COX-2/Chrysophanol complex.

snake bites, however, there're no reports that address the anti-inflammatory effect of this plant, also some studies proposed that its anti-inflammatory is due to its phenolic content which acts as strong anti-oxidants,.[53,54]

On the other hand, Pedalitin a flavonoid that is isolated from *M. Pulegium*[55] showed anti-inflammatory properties by decreasing the production of NO and pro-inflammatory cytokines such as TNF-α and IL-12[56]; again this is in concordance with our molecular docking investigating which showed the ability of Pedalitin to inhibit PLA2 and P38 MAPK, which play critical role on the production of inflammatory cytokines[57,58]

Cichorium intybus a variety of *C. endivia* roots demonstrated significant dose-dependent decrease in paw edema, which can be explained by the observed diminished the serum TNF-α, IL-6, and IL-1β levels in comparison to control group[59] also 8-deoxylactucin, a major sesquiterpene found in chicory extract was reported to be an inhibitor of COX-2 induction.[60]

Bryonia alba is a medicinal plant which is rich in Cucurbitacins and their glycosides,[61] while there're no studies discussing its anti-inflammatory effect, several studies indicated the anti-inflammatory effect of this class of compounds.[62–64]

The anti-inflammatory effect of *Myrtus communis* was
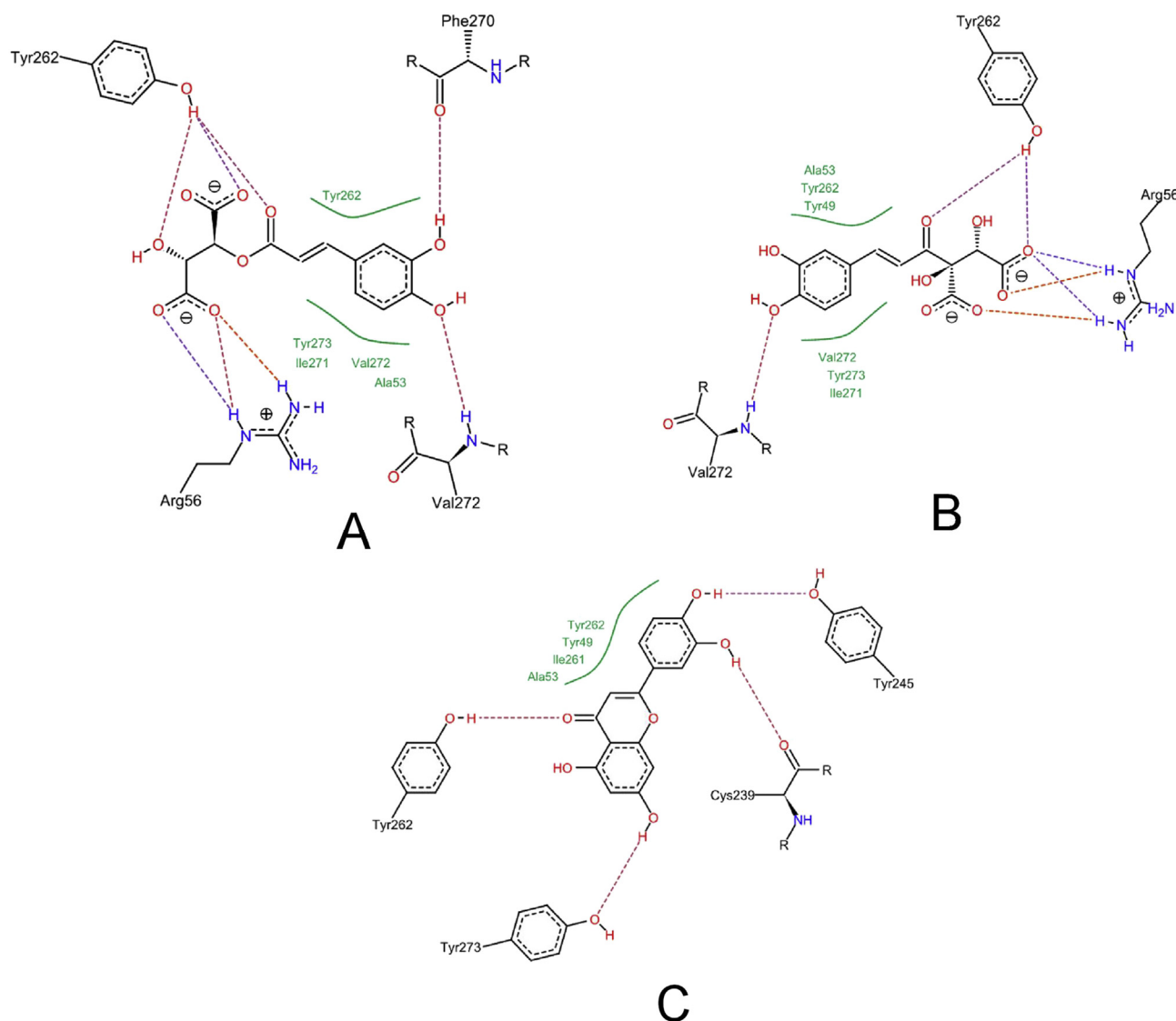
Fig. 4. Ligand interaction diagram of compounds with COX-2 (PDB ID: 1NT3). A) LTB4DHR/Cafteric acid complex (b) LTB4DHR/Luteolin complex (c) LTB4DHR/Cis-caffeoyl tartaric acid complex.

investigated in several reports; its extract exhibited strong inhibitory activity against IL-8 secretion[65] also the essential oil of *Myrtus communis* reduces leukocyte migration to the damaged tissue and exhibits anti-inflammatory activity.[66] Another study showed that the anti-inflammatory effect of the Oligomeric Nonprenylated Acylphloroglucinols isolated from *M.Communis* through inhibition of eicosanoid biosynthesis.[67]

Cyperus longus is one of the most common plants in traditional medicine, however there're no studies discussing the anti-inflammatory effect of it. On the other hand, the biological evaluation of the extract and compounds isolated from it showed that they possessed anti-oxidant, immunomodulatory and cytotoxic effect.[68–70]

On the other hand, the chemical classes of the hits suggested by the molecular docking study, were flavonoids, phenolic acids and anthraquinones. Available literature showed that Pedalitin, a known inhibitor for 5-lipoxygenase enzyme[71] was reported to decrease the nitrite production after treatment of RAW 264.7 cell line with LPS at concentration 10 μg/ml.[72]

Jaceosidin showed significant anti-inflammatory at 40 μg by

inhibiting the production of (NF-κβ) activity, (NO) production, and suppressed expression of inducible nitric oxide synthase (iNOS) in (LPS) induced RAW264.7 cell line[73] also it inhibited COX-2 expression and NF-κB activation, and markedly reduced TNF-α, IL-1β, and prostaglandin E2 (PGE2) levels in carrageenan induced model in mice.[74]

Luteolin is a common bio-flavonoid with prominent biological activities, several reports indicated that it exerted its anti-inflammatory effect through inhibition of MAPKS, COX-2, NF-κB, Leukotriene B4 and suppression of release other several cytokines[75]

While there's no reports addressing the anti-inflammatory effect of Thymonin, but the extract of *Zataria multiflora* seeds which contains this flavonoid was reported to decrease the level of serum levels of nitric oxide, nitrite, PLA2, and histamine in sensitized Guinea pigs.[76] There were no specific studies addressing the anti-inflammatory effect of caftaric acid, or Cis-Caffeoyl tartaric acid, however phenolic acids are well known to be a good inhibitors for COX and interfering with inflammasome pathways.[77]

Emodin and Rhabarberone are anthraquinones which were reported to possess anti-inflammatory effect by reducing the
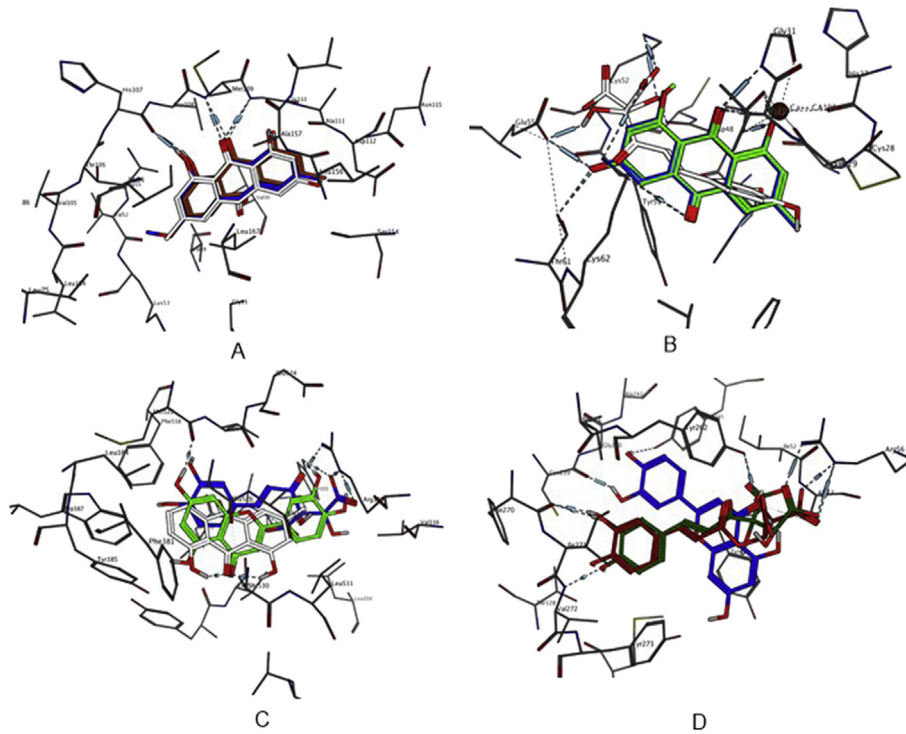
Fig. 5. Molecular interaction of the best ranking compounds (Superimposed) with the binding site of targets under investigation (A) Binding of Physcion (blue), Chrysophanol (Red) and Emodin (White) in the active site of p38α MAPK (B) Binding of Methyl Emodin (Green), Cis-caffeoyl tartaric acid (White), Emodin (Blue) in the active site of PLA2 (C) binding of Luteolin (Green), Chrysophanol (White) and Cis-caffeoyl tartaric acid (Blue) in the active site of COX-2 (D) Cis-caffeoyl tartaric acid (Green), Caftaric acid (RED), Luteolin (blue) in the active site of LTB4DHR.
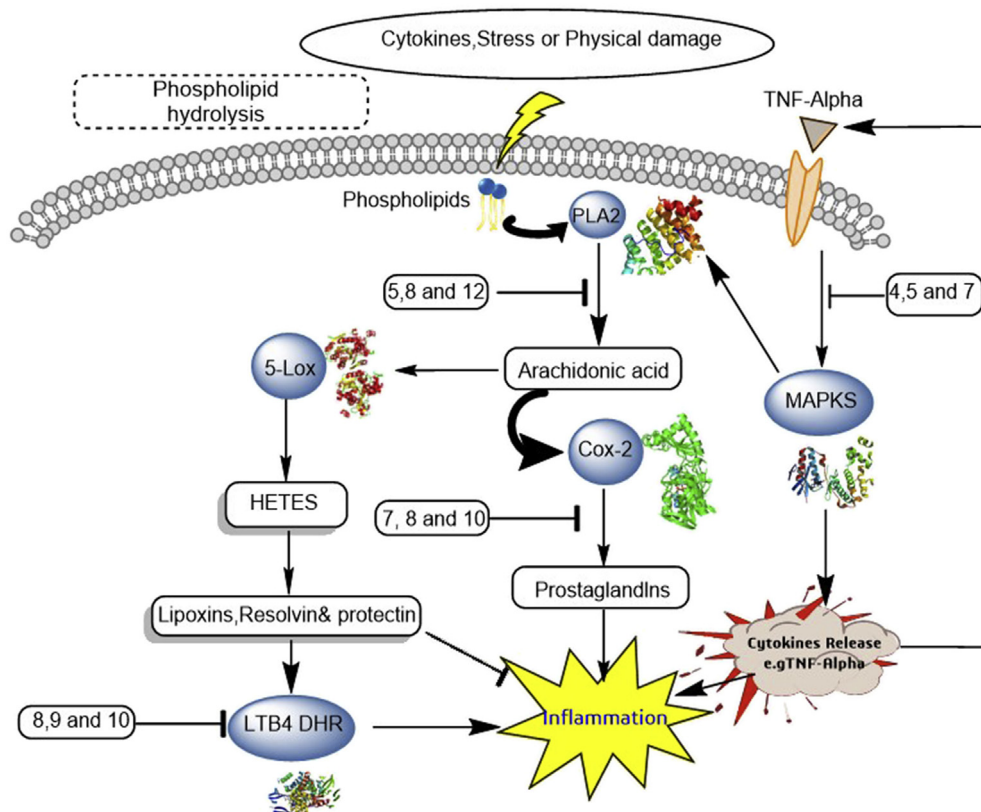
Fig. 6. Scheme of the proposed Anti-inflammatory mechanism for compounds derived from the formula. The compounds inhibit different steps in the inflammation pathway. MAPKS: Mitogen activated protein kinase, PLA2: Phospholipase A2, 5-LOX: 5-Lipoxygenase, Cox-2: Cyclooxygenase-2, HETES: Hydroxyeicosatetraenoic acid, LTB4DHR: Leukotriene B4 dehydrogenase, TNF-α: Tumor necrosis alpha 4) Emodin 5) Physcion 7) Chrysophanol 8) Cis-caffeoyl tartaric acid 9) Caftaric acid 10) Luteolin 12) Methylemodin.

expression of several cytokines such as TNF-α, Il-2, COX-2 and transcription of NF-κB.[78—80] In another study Physcion and emodin caused 65—68%reduction of edema volume at 40 mg/kg in Carrageenan-induced inflammation rat paw and decreased the production of iNOS in LPS stimulated macrophage in doses dependent manner[81]

The Glycoside of physcion (physcion 8-O-β-glucopyranoside) was able to inhibit wide array of inflammatory markers in different pathways such as c-Jun N-terminal kinase (JNK), P38 MAPK, extracellular signal-regulated kinase (ERK)1/2 in collagen-induced arthritis model.[82] In the same context Chrysophanol protected against proinflammatory cytokine expression and release in LPS induced inflammation in RAW264.7 and showed protective effect in dextran sulphate induced colitis in rats by modulation of the activity of NF-κB/Caspase-1.[83,84]

4. Conclusion

In this work, we investigated the ability of several compounds, derived from formula found the Canon of Medicine, to bind with distinctive molecular targets associated with inflammation; such as phospholipase A2, p38 alpha mitogen activated protein kinase, Cyclooxygenase-2 and Leukotriene B4 dehydrogenase, using structure based virtual screening.

Eleven compounds from three plants could interact with more than one target suggesting that the formula exerts its action through synergism, moreover, consulting the literature revealed the consistency of our *in-silico* results with several previous *in-vitro* and *in-vivo*; indicating that there's rationale behind choosing the plants to treat inflammatory conditions, however, more clinical trials, standardization and safety studies are required before their employing in clinical practice.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2018.09.004.

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