Check for updates

NPC Natural Product Communications

2014
Vol. 9
No. 12
1761 - 1764

Selective COX-2 Inhibitory Properties of Dihydrostilbenes from Liquorice Leaves–*In Vitro* Assays and Structure/Activity Relationship Study

Domenico Trombetta[a], Salvatore V. Giofrè[a,*], Antonio Tomaino[a], Roberto Raciti[a], Antonella Saija[a], Mariateresa Cristani[a], Roberto Romeo[a], Laura Siracusa[b] and Giuseppe Ruberto[b,*]

[a]*Dipartimento Scienze del Farmaco e dei Prodotti per la Salute,Università di Messina, Contrada Annunziata, I-98168 Messina, Italy*

[b]*Istituto del C.N.R. di Chimica Biomolecolare, Via Paolo Gaifami, 18 I-95126 Catania, Italy*

sgiofre@unime.it, giuseppe.ruberto@icb.cnr.it

Received: August 4th, 2014; Accepted: October 22nd, 2014

Three dihydrostilbenes belonging to the polyphenol pool characterized in the leaves of Sicilian liquorice (*Glycyrrhiza glabra* L.) have been tested for their antioxidant and anti-inflammatory activity. The three dihydrostilbenes (PA-82, GA-23, DO-07) were *in vitro* tested to evaluate their capability to scavenge the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), and to decrease thromboxane B2 (TxB2) and prostaglandin E2 (PGE2) release in human whole blood samples. On the basis of the observed capability of these compounds to affect the cell COX-1/COX-2 pathway, a molecular docking study was carried out in order to understand in detail the ability of these compounds to bind to COX-1 and COX-2. The results show that the liquorice dihydrostilbenes are preferred ligands for COX-2 rather than for COX-1, providing a good rational for the observed selectivity in *ex vivo* experiments. Therefore, they appear to be good candidates for employment in human therapy against inflammation-related pathological conditions.

Keywords: *Glycyrrhiza glabra*, Liquorice, Dihydrostilbenes, Anti-inflammatory activity, Cyclooxygenase (COX), Molecular docking.

Glycyrrhiza glabra L. (liquorice) is one of the most popular medicinal plants, the roots being the part most used. It possesses well known therapeutic properties, which are documented since the Egyptian age. In Traditional Chinese Medicine (TCM) liquorice, known as Gan-Cao, is included in a large number of herbal formulations, and is said to "harmonize" the entire ingredient in an herbal formula; for these reasons, liquorice root is definitely the second herb prescribed after ginseng [1-3]. Conversely, the aerial parts of the plant are scarcely used and considered an agrochemical waste. Nevertheless, the few phytochemical investigations of *G. glabra* leaves have shown the presence of some phenolic compounds which are either not present in the roots, or in small traces [4,5]. Previous investigations of the lipid extract of Sicilian *G. glabra* leaves allowed the isolation of various known flavonoids and nine novel dihydrostilbenes, which have been considered responsible for the antioxidant, antigenotoxic and anti-inflammatory activities of the extract [6-8]. Dihydrostilbenes, also known as bibenzyls, are structurally characterized by a 1,2-diphenylethane scaffold; although biosynthetically strictly related to flavonoids, their presence in the plant kingdom is much more restricted. Concerning the biological function of dihydrostilbenes in plants, an increasing accumulation of these components has been observed in infected samples, thus suggesting for them a phytoalexin-like role [9].

The main aim of the present study was to ameliorate our knowledge about the bio-properties of the dihydrostilbenes found in Sicilian *G. glabra* leaves. In particular, three compounds have been selected, namely α,α'-dihydro-3,4',5-trihydroxy-5'-isopentenylstilbene (**PA-82**), α,α'-dihydro-3,3',5-trihydroxy-4'-methoxy-5'-isopentenyl stilbene (**GA-23**) and α,α'-dihydro-3,3'-dihydroxy-5-β-D-*O*-glucopyranosyloxy-4'-methoxystilbene (**DO-07**) (Figure 1), and tested for their antioxidant and anti-inflammatory activities. In fact, there is great interest towards new, naturally-occurring compounds

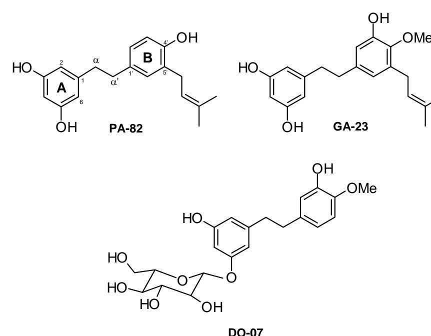

Figure 1: Dihydrostilbenes from *Glycyrrhiza glabra* leaves.

endowed with anti-inflammatory effectiveness and able to inhibit the cyclooxygenase (or prostaglandin G/H synthase, COX) isoforms COX-1 and COX-2 [10] The two COXs catalyze the first two steps in the synthesis of prostaglandins (PGs) [11].

Anti-inflammatory drugs are generally applied for the relief of pain, fever and inflammation. However, extensive research within the last two decades revealed that most chronic illnesses, including cancer, neurological, autoimmune and cardiovascular diseases, are mediated through chronic inflammation. In fact, the discovery of the COX isoforms led to establishing their importance in many non-arthritic or non-pain states where there is an inflammatory component to pathogenesis [12]. Unfortunately, a major limitation to the use of COX inhibitors is the adverse reaction they cause to the gastrointestinal (GI) tract, including the formation of gastric lesions, the potentiation of ulcerogenic responses to stress, and the impairment of gastric ulcer healing. On this basis, major efforts are currently underway to discover new compounds able to selectively inhibit either COX-1 or COX-2. In fact, while selective COX-2/COX-1 inhibitors are more efficacious than nonselective NSAIDs

for the therapy of different diseases, selective inhibitors show the advantage of reduced gastrointestinal toxicity associated with the administration of non-selective NSAIDs [13].

Furthermore, interest in natural products has increased due to the multiple mechanisms involved in the development of several pathologies (including those related to a chronic inflammatory condition), whereas mono-targeted therapies developed in the last decades have proven to be often ineffective and expensive in terms of costs and health. Conversely, naturally occurring compounds are generally able to act as multifactorial biological modifiers and thus might be more efficacious.

In the present study the aforementioned three dihydrostilbenes were tested *in vitro* to evaluate their capability to scavenge the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), and to decrease thromboxane B2 (TxB2) and prostaglandin E2 (PGE2) release in human whole blood samples. On the basis of the observed capability of these compounds to affect the COX-1/COX-2 pathway, a molecular docking study was carried out in order to understand in detail the ability of these compounds to bind to COX-1 and COX-2. In fact *in vitro* experiments can demonstrate if a compound is able to modulate either the COX-1 or COX-2 pathway, but they do not clarify if such a compound is able to directly interact with the gene product or its modulating transcription factors.

Figure 1 shows the structure of the three dihydrostilbenes, **GA-23**, **PA-82** and **D0-07**, selected from the pool of liquorice polyphenols. From a biosynthetic point of view stilbenoids come from the combination of three malonyl-CoA units and one cinnamic acid unit, producing two aromatic rings, A and B, of different biogenetic origin with a different substitution. In particular, ring A usually shows a 3,5 di-substitution pattern, as is the case for all the dihydrostilbenes isolated from *G. glabra* leaves.

Regarding the DPPH assay, the radical-scavenging activities of the dihydrostilbenes **DO-07**, **GA-23** and **PA-82** against DPPH are shown in Table 1. While **DO-07** showed good DPPH antiradical activities (IC_{50}: 48.8 μM, 95% CL 36.9-64.5 μM), **GA-23** and **PA-82** exhibited really weak radical-scavenging activity, having IC_{50} values > 450.00 μM. Despite the higher number of hydroxyl groups, the presence of the isopentenyl chain on the B ring leads to the loss of the radical scavenger power, very likely avoiding the interaction with the free radicals in the experimental environment.

Table 1: Free radical effect of liquorice leaf stilbenoids as evaluated against the DPPH radical.

	DPPH assay[a] **SC_{50} μM**	**CL 95 μM**
GA 23	469.7	366.4
DO 07	48.8	36.9
PA 82	472.6	191.6

[a]Experiments were carried out in triplicate and the results are expressed as mean scavenging concentration (SC_{50}) and 95% confidence limits (C.L.).

While COX-1 is homeostatic and is expressed in many tissues, including the gastrointestinal tract, kidney, and platelets, COX-2 is expressed at sites of inflammation, the hippocampus, female reproductive tissue, and in many cancers [14]. In particular COX-1 produces PGs and TxA2 that regulate gastrointestinal, renal, vascular and other physiological functions, and COX-2 regulates production of PGE2 involved in inflammation, pain and fever.

We assessed the ability of the dihydrostilbenes under study to act as selective inhibitors of COX-1 and COX-2 pathways [15], by decreasing release of TxB2 and PGE2 in whole blood. In fact, constitutive COX-1 and inducible COX-2 convert arachidonic acid released from the cell membrane in response to stimulation to PGH2, which is then further metabolized by other enzymes to various prostaglandins, prostacyclin and thromboxanes. Under these experimental conditions indomethacin and nimesulide (30 μM) were able to almost completely suppress TxB2 release (% inhibition in comparison with controls: 92.6±2.4) and PGE2 release (% inhibition in comparison with controls: 89.7±3.5), respectively. All three stilbenoids under study appeared lacking of a significant capability to inhibit TxB2 release at the highest doses tested (300 μM). Conversely, they showed a good dose dependent capability to inhibit PGE2 release, so suggesting a selective action of these molecules on the COX-2 pathway. However, the inhibitory effect of **GA-23** on PGE2 release (IC_{50}: 66.4 μM, 95% CL 35.3-125.0 μM) was very much higher than that observed for **PA-82** (IC_{50}: 275.0 μM, 95% CL 236.9-319.3 μM) and especially for **DO-07** (IC_{50}: 468.1 μM, 95% CL 366.5-597.8 μM) (Table 2B).

Therefore, the order of activities seems to be related to the presence of the isopentenyl chain on the B ring that is crucial for the stilbenoid to be endowed with the capability to inhibit COX-2; furthermore, this capability is improved by the presence of a 3'-OH group on the ring B.

Table 2: Effect of liquorice leaf stilbenoids on release of TxB_2 and of PGE_2 in human whole blood samples.

	TxB_2 release					
	GA 23		**DO 07**		**PA 82**	
	μM	% inhibition	μM	% inhibition	μM	% inhibition
	100	22.4±3.5	100	18.5±2.6	100	14.9±1.2
	200	25.9±2.5	200	14.6±2.0	200	18.7±2.2
	300	29.7±2.0	300	13.2±1.8	300	20.2±2.5
	400	34.6±2.7	400	18.3±2.3	400	23.8±1.3
	800	47.1±3.1	800	16.6±2.3	800	35.6±4.1
A	**PGE_2 release**					
	GA 23		**DO 07**		**PA 82**	
	μM	% inhibition	μM	% inhibition	μM	% inhibition
	20	35.1±2.3	50	11.9±1.8	50	6.7±0.9
	50	48.9±2.6	100	17.3±2.3	100	16.4±1.4
	75	53.9±3.2	200	28.0±1.9	200	35.7±2.8
	100	58.5±3.3	450	54.9±4.3	250	46.3±4.0
	200	77.8±4.6	600	67.5±5.0	350	65.0±5.8
	300	98.1±6.4	800	92.8±6.6	500	91.4±6.1

B		**TxB_2 release**			**PGE_2 release**	
		IC_{50} (μM)	CL (μM)		IC_{50} (μM)	CL (μM)
	GA 23	> 800.00	--	--	66.4	35.3-125.0
	DO 07	ND	--	--	468.1	366.5-597.8
	PA 82	> 800.00	--	--	275.0	236.9-319.3

Experiments were carried out in triplicate and the results are expressed as: A) per cent inhibition with respect to control values, reported as mean ±S.D; B) mean inhibitory concentration (IC_{50}) and 95% confidence limits (CL).

Unlike tests performed on purified enzymes that only show a purely drug–enzyme interaction, it should be considered that other interactions can exist in an *ex vivo* test where the inhibitory potency is measured at a cellular level. For example, binding of the investigated compounds to plasma proteins, enzymatic degradation of the drug into active metabolites or interaction between the drug and mediators of inflammation that can induce COX-2 can drastically influence the inhibitor potency measured at a cellular level. In particular, the rate of PGE2 production is related to COX-2 expression elicited by LPS, while the production of TxB2 is limited by the content of the constitutive enzyme COX-1. Thus, docking studies have been carried out to investigate at the molecular level the action mechanisms of these dihydrostilbenes (see Supplementary Information). In fact computational chemistry offers the possibility to explore protein–ligand interactions through docking procedures [16]. COX-1 and COX-2 are approximately 60% identical in terms of amino acid composition, and their

catalytic regions are widely conserved [17,18]. Furthermore, the two active sites of these isoforms differ by only two amino acids, at positions 523 (Ile for COX-1 and Val for COX-2) and 513 (His for COX-1 and Arg for COX-2) [17,19]. The differences between the active sites of COX-2 and COX-1 enzymes, which are described as hydrophobic channels, are the basis for the design of COX-2 selective inhibitors.

Table 3: Estimated binding free energy (ΔG), calculated inhibition constants (K_i) and calculated selectivity indices of compound GA-23, PA-82 and DO-07..

Drugs	IC_{50} (μM)		Exp. SI[a]	Δ*G*(Kcal/mol)		Calculated *K*i (μM)		Calcd SI[b]
	COX-2	COX-1		COX-2	COX-1	COX-2	COX-1	
GA-23	66.4	>800.0	≥ 12.0	- 8.94	-8.01	0.28	1.35	4.8214
PA-82	275.0	>800.0	≥ 2.9	- 8.31	- 8.04	0.8	1.27	1.5875
DO-07	468.1	ND	---	-7.91	- 7.84	1.3	1.5	1.1138

[a]Experimental Selectivity Index, [b] Calculated Selectivity Index.

Docking studies (results are summarized in Table 3) have demonstrated that the binding mode for the three compounds examined is slightly different due to the active site volume differences. In the complex formation with COX-1, the hydrogen bonding interaction with Ser530 is absent in compounds **GA-23** and **PA-82**; this could rationalize the low activity of **GA-23** and **PA-82** towards COX-1 inhibition observed in *ex vivo* experiments. On the other hand, the scoring function of **GA-23** and **PA-82** complexes with COX-2 confirms that both compounds are preferred ligands for COX-2 rather than for COX-1 and provides a good rational for the selectivity found in the *ex vivo* experiments. Taken together, our findings evidence the existence of a correlation between the presence of the isopentenyl chain and of a 3'-OH group on the B ring of the molecule skeleton of the dihydrostilbenes under investigation.

Of course we cannot exclude that, besides COX-2 inhibition, other mechanisms can undergo the observed decrease in LPS-stimulated PGE2 release (an index of the COX-2 activity of blood monocytes) in human whole blood samples. In fact PGE2 production is related to COX-2 expression elicited by LPS, and several natural drugs are able to modulate cell signaling pathways and gene expression; thus the effect of **GA-23** and **PA-82** could be due, partially at least, to a down regulation of COX-2 expression.

Stilbenoids act as natural protective agents to defend the plant against viral and microbial attack, excessive ultraviolet exposure, and disease [9]. More than 1,000 compounds belonging to this group have been discovered and, depending on their chemical structure, demonstrate different biological activities. The most known stilbene, resveratrol (*trans*-3,5,4′-trihydroxystilbene), demonstrates anti-inflammatory activity, but it has also been shown to be a non-selective inhibitor of COX-1 and COX-2. [20,21] Murias *et al*. [22] described a series of hydroxylated resveratrol analogues selective COX-2 inhibitors with potency comparable to or better than the clinically established celecoxib; furthermore, more recently, Bouaziz-Terrachet *et al* [23] showed that a series of stilbene analogs is able to selectively inhibit COX-2. Interestingly this is the first report which describes naturally occurring dihydrostilbenes able to selectively inhibit COX-2. Finally, these molecules are isolated from liquorice leaves that are considered to be an agrochemical waste because no intended use for such material has been envisaged; thus this by-product of the agro-food industry may be useful as sources of bioactive molecules, giving the opportunity to obtain added-value products.

Experimental

General: Optical rotations were measured on a Jasco DIP-370 digital polarimeter, and UV and FTIR spectra on a Perkin-Elmer model Lambda 25 and model Spectrum BX spectrophotometers, respectively. ^{1}H NMR spectra were measured on a Varian INOVA operating at 499.883 MHz and a Bruker AC-250 at 250 MHz, whereas ^{13}C NMR spectra were run at 63 MHz on a Bruker AC-250 instrument. High resolution electron impact mass spectra (HREIMS) were obtained at 70 eV on a Kratos M50S mass spectrometer. TLC was carried out on pre-coated silica gel F254 plates (Merck); flash chromatography on Diol and LiChroprep (Merck). All solvents used in this study were high purity American Chemical Society (ACS) solvents from Carlo Erba (Milano, Italy). Lipopolysaccharide derived from *Escherichia coli* 026:B6, sodium heparin, aspirin, nimesulide, indomethacin and diphenylpicrylhydrazyl (DPPH) were purchased from Sigma (Sigma-Aldrich Milan, Italy).

Plant material: *Glycyrrhiza glabra* L. was collected on the bank of the Simeto river in April 2011. A voucher specimen was deposited in the Herbarium of the Department of Botany, Catania, Italy.

Extraction and isolation: Fresh plant was ground and freeze-dried to obtain 100 g of dried material, which was defatted 3 times with *n*-hexane and the residual material extracted with ethyl acetate 3 times at room temperature with continuous stirring. After removing the extract by filtration, the material was further extracted with *n*-butanol. All the extractions were made in triplicate, at room temperature with continuous stirring, and, after evaporation of each solvent under reduced pressure, 16 and 2.2 g of the ethyl acetate and *n*-butanol extract, respectively, were obtained: 10 g of ethyl acetate extract was subjected to chromatography over MN Polyammide SC6<0.07 mm. Elution with a stepwise gradient from 60% MeOH/H_2O to 100% MeOH gave 15 fractions (A-Q). Fraction H (1 g) was submitted to repeated chromatographic purifications, according to the previous procedure [6] to obtain compound **PA-82** (47 mg) and **GA-23** (207 mg). The *n*-butanol extract (2.2 g) was chromatographed over polyamide, using as eluents acetone-*n*-hexane (10:90→100:0), MeOH-acetone (10:90), MeOH (100%), and then H_2O-MeOH (1:1), obtaining 13 fractions (A-O BuOH). Fraction M was submitted to several chromatographic separation steps, according to the previous reported procedure [6] to obtain pure compound **DO-07** (10 mg).

DPPH test: The free radical-scavenging capacity of the dihydrostilbenes was tested as bleaching of the stable radical DPPH [8,24]. The reaction mixture (3.5 mL of methanol) contained 100 μM DPPH and different concentrations of the compounds; an equal volume of the solvent employed to dissolve the drugs tested (37.5 μL) was added to control tubes. After 20 min at room temperature, the absorbance was recorded at 517 nm. All experiments were carried out in duplicate and repeated at least 3 times. Results were expressed as per cent decrease with respect to control values; mean scavenging concentrations (SC_{50}) and 95 % confidence limits (95% C.L.) were calculated by using the Litchfield and Wilcoxon test.

Release of PGE2 and TxB2 in human whole blood: In order to characterize the anti-inflammatory properties of the liquorice dihydrostilbenes under study, we used a human whole blood assay as adapted in our laboratories [8] to assess their capability to act as *in vitro* selective inhibitors of COX-1 or COX-2 pathways,[14,25] by decreasing release of TxB2 and PGE2, respectively. Peripheral blood samples were drawn from healthy volunteers who had taken no anti-inflammatory drug during the last two weeks before the

study. Informed consent was obtained from each subject. To evaluate changes in PGE2 release, 1 mL peripheral blood aliquots containing 10 IU of sodium heparin were incubated both in the absence and in the presence of lipopolysaccharide (LPS; 10 µg/mL) for 24 h at 37°C. The contribution of platelet prostaglandin endoperoxide synthase-1 was suppressed by adding aspirin (10 µg/mL) at time 0. Then plasma was separated by centrifugation (10 min at 1600 x *g*) and kept at -30°C until assayed for the content of PGE2; this is an index of the COX activity of blood monocyte prostaglandin endoperoxide synthase-2. To evaluate changes in TxB2 release, 1 mL whole blood aliquots were immediately transferred into glass tubes and allowed to clot at 37°C for 1 h. Serum was separated by centrifugation (10 min at 1600 x *g*) and kept at -30°C until assayed for TxB2; this is an index of endogenously formed thrombin-stimulated COX activity of platelet prostaglandin endoperoxide synthase-1. Plasma PGE2 and serum TxB2 were measured by enzyme immunoassay (EIA). To evaluate the effects of the liquorice dihydrostilbenes on PGE2 and TxB2 in whole blood, the compounds were dissolved in DMSO and 2 µL of these solutions was pipetted directly into test tubes to give a final concentration in whole blood of 50-800 µM. The same volume of the vehicle alone (DMSO) was used in control tubes. The experiments were then carried out as described above. Each determination was performed in triplicate; results were expressed as per cent inhibition with respect to control values, and mean inhibitory concentrations (IC_{50}) and 95 % confidence limits (95 % C.L.) were calculated by using the Litchfield and Wilcoxon test.

As reference drugs, the classical non-steroidal anti-inflammatory drugs indomethacin (30 µM), as a non-selective COX inhibitor, and nimesulide (30 µM), as a selective COX-2 inhibitor, have been used. Each determination was performed in triplicate; results were expressed as percent inhibition with respect to control values and are reported as mean ±S.D. of 3 experiments.

Supplementary Information: Characterization of compounds PA-82, GA-23 and DO-07 and Docking study.

Acknowledgments - This work was supported by the Consiglio Nazionale delle Ricerche (CNR Rome, Italy). We wish to thank Mr Felice Rao (Rao Erbe, Valverde CT, Italy) for collecting the plant material. We are also grateful to Ms Tonia Strano (ICB-CNR, CT, Italy) for her skillful technical assistance.

References

[1] Fiore C, Eisenhut M, Ragazzi E, Zanchin G, Armanini D. (**2005**) A history of the therapeutic use of liquorice in Europe. *Journal of Ethnopharmacology*, ***99***, 317-324.

[2] Fugh-Berman, A. (**2000**) Herb-drug interactions. *Lancet*, ***355***, 134-138.

[3] Tang W, Eisenbrand G. (**1992**) *Chinese Drugs of Plant Origin*. Springer-Verlag, Berlin, 567-588.

[4] Fukui H, Goto K, Tabata M. (**1998)** Two antimicrobial flavanones from the leaves of *Glycyrrhiza glabra*. *Chemical and Pharmaceutical Bulletin*, ***36***, 4174-4176.

[5] Hayashi H, Yasuma M, Hiraoka N, Ikeshiro Y, Yamamoto H, Yesilada E, Sezik E, Honda G, Tabata M. (**1996**) Flavonoid variation in the leaves of *Glycyrrhiza glabra*. *Phytochemistry*, ***42***, 701-704.

[6] Biondi DM, Rocco C, Ruberto G. (**2003**) New dihydrostilbene derivatives from the leaves of *Glycyrrhiza glabra* and evaluation of their antioxidant activity. *Journal of Natural Products*, ***66***, 477-480.

[7] Biondi DM, Rocco C, Ruberto G. (**2005**) Dihydrostilbene derivatives from *Glycyrrhiza glabra* leaves. *Journal of Natural Products*, ***68***, 1099-1102.

[8] Siracusa L, Saija A, Cristani M, Cimino F, D'Arrigo M, Trombetta D, Rao F, Ruberto, G. (**2011**) Phytocomplexes from liquorice (*Glycyrrhiza glabra* L.) leaves--chemical characterization and evaluation of their antioxidant, antigenotoxic and anti-inflammatory activity. *Fitoterapia*, ***82***, 546-556.

[9] Greger H. (**2012**) The diversity of Stemona stilbenoids as a result of storage and fungal infection. *Journal of Natural Products*, ***75***, 2261-2268.

[10] Recio MC, Andujar I, Rios JL. (**2012**) Anti-inflammatory agents from plants: progress and potential. *Current Medicinal Chemistry*, ***19***, 2088-2103.

[11] Rouzer CA, Marnett LJ. (**2009**) Cyclooxygenases: structural and functional insights. *Journal of Lipid Research*, ***50***, Suppl:S29-34.

[12] Rainsford, K.D. (**2007**) Anti-inflammatory drugs in the 21st century. *Subcellular Biochemistry*, ***42***, 3-27.

[13] Takeuchi K, Tanaka A, Kato S, Amagase K, Satoh H. (**2010**) Roles of COX inhibition in pathogenesis of NSAID-induced small intestinal damage. *Clinica Chimica Acta*, ***411***, 459-466.

[14] Zha S, Yegnasubramanian V, Nelson WG, Isaacs WB, De Marzo AM. (**2004**) Cyclooxygenases in cancer: progress and perspective. *Cancer Letters*, ***215***, 1-20.

[15] von Aulock S, Hermann C, Hartung T. (**2003**) Determination of the eicosanoid response to inflammatory stimuli in whole blood and its pharmacological modulation ex vivo. *Journal of Immunological Methods*, ***277***, 53-63

[16] Romeo R, Giofrè SV, Macchi B, Balestrieri E, Mastino A, Merino P, Carnovale C, Romeo G, Chiacchio U. (**2012**) Truncated reverse isoxazolidinyl nucleosides. A new class of allosteric HIV-RT inhibitors, *ChemMedChem*, ***7***, 565–569.

[17] Michaux C, Charlier C. (**2004**) Structural approach for COX-2 inhibition. *Mini-Reviews in Medicinal Chemistry*, ***4***, 603-615.

[18] Navya A, Jayasimha RD, Uma Maheswari Devi P. (**2011**) Docking studies on xanthones of mangosteen as COX-2 inhibitors. *International Journal of Applied Biology and Pharmaceutical Technology*, ***2***, 263-268.

[19] Kaur J, Bhardwaj A, Huang Z, Knaus EE. (**2012**) Aspirin analogues as dual cyclooxygenase-2/5-lipoxygenase inhibitors: Synthesis, nitric oxide release, molecular modeling, and biological evaluation as anti-inflammatory agents. *ChemMedChem*, ***7***, 144-150.

[20] Calamini B, Ratia K, Malkowski MG, Cuendet M, Pezzuto JM, Santarsiero BD, Mesecar AD. (**2010**) Pleiotropic mechanisms facilitated by resveratrol and its metabolites. *Biochemical Journal*, ***429***, 273-282.

[21] Cao H, Yu R, Tao Y, Nikolic D, van Breemen RB. (**2011**) Measurement of cyclooxygenase inhibition using liquid chromatography-tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, ***54***, 230-235.

[22] Murias M, Handler N, Erker T, Pleban K, Ecker G, Saiko P, Szekeres T, Jäger W. (**2004**) Resveratrol analogues as selective cyclooxygenase-2 inhibitors: synthesis and structure-activity relationship**.** *Bioorganic & Medicinal Chemistry*, ***12***, 5571-5578.

[23] Bouaziz-Terrachet S, Toumi-Maouche A, Maouche B, Taïri-Kellou, S. (**2010**) Modeling the binding modes of stilbene analogs to cyclooxygenase-2: a molecular docking study. *Journal of Molecular Modeling*, ***16***, 1919-1929.

[24] Trombetta D, Cimino F, Cristani M, Mandalari G, Saija A, Ginestra G, Speciale A, Chirafisi J, Bisignano G, Waldron K, Narbad A, Faulds CB. (**2010**) *In vitro* protective effects of two extracts from bergamot peels on human endothelial cells exposed to tumor necrosis factor-alpha (TNF-alpha). *Journal of Agricultural and Food Chemistry*, ***58***, 8430-8436.

[25] Patrignani P, Panara MR, Sciulli MG, Santini G, Renda G, Patrono C. (**1997**) Differential inhibition of human prostaglandin endoperoxide synthase-1 and -2 by nonsteroidal anti-inflammatory drugs. *Journal of Physiology and Pharmacology*, ***48***, 623-631.