

Accepted Manuscript

Discovery of small molecule inhibitors for the snake venom metalloprotease BaP1 using *in silico* and *in vitro* tests

Fabian Villalta-Romero, Luiz Borro, Boris Mandic, Teresa Escalante, Alexandra Rucavado, Jose María Gutiérrez, Goran Neshich, Ljubica Tasic

PII: S0960-894X(17)30242-1
DOI: <http://dx.doi.org/10.1016/j.bmcl.2017.03.007>
Reference: BMCL 24759

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 18 January 2017
Revised Date: 2 March 2017
Accepted Date: 3 March 2017

Please cite this article as: Villalta-Romero, F., Borro, L., Mandic, B., Escalante, T., Rucavado, A., Gutiérrez, J.M., Neshich, G., Tasic, L., Discovery of small molecule inhibitors for the snake venom metalloprotease BaP1 using *in silico* and *in vitro* tests, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: <http://dx.doi.org/10.1016/j.bmcl.2017.03.007>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

**Discovery of small molecule inhibitors for
the snake venom metalloprotease BaP1 using
in silico and *in vitro* tests**

Fabian Villalta-Romero, Luiz Borro, Boris Mandic, Teresa Escalante, Alexandra Rucavado, Jose María Gutiérrez, Goran Neshich and Ljubica Tasic

Leave this area blank for abstract info.

ACCEPTED MANUSCRIPT



Discovery of small molecule inhibitors for the snake venom metalloprotease BaP1 using *in silico* and *in vitro* tests

Fabian Villalta-Romero^a, Luiz Borro^b, Boris Mandic^c, Teresa Escalante^d, Alexandra Rucavado^d, Jose María Gutiérrez^d, Goran Neshich^e and Ljubica Tasic^{a*}

^aChemical Biology Laboratory, Organic Chemistry Department, Institute of Chemistry, UNICAMP, Campinas, SP, Brazil

^bInstitute of Biology, UNICAMP, Campinas, SP, Brazil

^cFaculty of Chemistry, University of Belgrade, 11158, Belgrade, Serbia

^dInstituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica

^eBrazilian Agricultural Research Corporation (EMBRAPA), National Center for Agricultural Informatics, Computational Biology Research Group, Campinas, SP, Brazil

ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Snake venom metalloproteases

BaP1

Inhibitors

High Through Virtual Screening

In vitro interaction studies

ABSTRACT

Snakebites represent an important public health problem, with a great number of victims with permanent sequelae or fatal outcomes, particularly in rural, agriculturally active areas. The snake venom metalloproteases (SVMPs) are the principal proteins responsible for some clinically-relevant effects, such as local and systemic hemorrhage, dermonecrosis, and myonecrosis. Because of the difficulties in neutralizing them rapidly and locally by antivenoms, the search and design of small molecules as inhibitors of SVMPs are proposed. The *Bothrops asper* metalloprotease P1 (BaP1) is hereby used as a target protein and by High Throughput Virtual Screening (HTVS) approach, the free access virtual libraries: ZINC, PubChem and ChEMBL, were searched for potent small molecule inhibitors. Results from the aforementioned approaches provided strong evidences on the structural requirements for the efficient BaP1 inhibition such as the presence of the pyrimidine-2,4,6-trione moiety. The two proposed compounds have also shown excellent results in performed *in vitro* interaction studies against BaP1.

2017 Elsevier Ltd. All rights reserved.

Snake venom is a complex mixture of numerous bioactive components, mainly proteins and peptides that contribute to subdue, kill and/or digest the prey¹⁻⁵. Composition of the most snake venoms counts with about 30% or more of metalloproteases (SVMPs), thus pointing to their significant role in the envenomation-related pathogenesis, such as bleeding, coagulopathies, edema, inflammation and necrosis^{1,3,5,6}.

SVMPs are responsible for local and systemic hemorrhage. This process is an extremely rapid event *in vivo*, with capillary endothelial cells showing drastic structural alterations just within few minutes^{6,7}. Because of the difficulty in neutralizing or locally inhibiting the action of SVMPs by antivenoms, the search for specific inhibitors may represent a new alternative or an add in treatment of envenomations^{7,8}.

Some synthetic substances like *O*-phenathroline, EDTA^{8,9} and Batimastat, because of their ability to chelate metals (Zn²⁺ ion in the case of SVMP) have been shown to inhibit local hemorrhage induced by the venom of *Bothrops asper*, were tested. It was

demonstrated that these compounds were effective at inhibiting both the isolated SVMPs and the hemorrhagic activity of crude venoms in animal models^{9,10}. Also, some hydroxamate-containing tripeptides with sequence Phe-Leu-Phe showed good results *in vitro*^{10,11}. Although hydroxamates are often pointed out as very potent SVMPs inhibitors, several challenges need to be addressed in the context of producing new drugs for these enzymes. First, their relatively low selectivity is a definite problem, which may cause several adverse effects. As broad-spectrum inhibitors, hydroxamates lead to musculoskeletal syndrome side effects, mainly due to the presence of hydroxamic acid. By proposing inhibitors with more specific activity against the SVMPs some of the low-selectivity associated problems can be solved. But still, research has been so far focused mainly on highly efficient Zn²⁺ ion binding groups^{11,12}. Pharmacokinetics and toxicological issues of the potential inhibitors represent other problems that should be considered in the design of specific SVMPs inhibitors. Hydroxamic acids may hydrolyze to the corresponding carboxylic acids under physiological conditions. Some hydroxamates are prone to hydrolysis in plasma. This

influences their distribution and efficiency because the corresponding carboxylic acids are generally less active. Hydrolysis may also contribute to the toxicity of the compounds because of the fact that the byproduct, hydroxylamine¹²⁻¹⁴, have been shown to have mutagenic characteristics¹³⁻¹⁵. Thus, the inability of hydroxamates to be used as clinically viable compounds against SVMPs has been attributed to above noted effects. In addition, the public access to metalloprotease inhibitors, designed by the pharmaceutical industry, is limited.

In order to find potential inhibitors to the BaP1 enzyme, a structure-based virtual screening (SBVS) campaign was conducted on a library constructed from the public databases ZINC12 (Drug-Like subset), ChEMBL_17 and PubChem (known inhibitors for metalloproteases). The final compound library was designed considering molecules containing only organic atom types (H, C, N, O, P, S, F, Cl, Br and I). Since starting ligand structures can decisively impact on the virtual screening results¹⁵⁻¹⁷, each library entry was prepared using the protocol "Prepared Ligand" as implemented in Discovery Studio (DS) version 4.0 (BIOVIA/Accelrys Inc., San Diego, CA, USA) with default parameters. This protocol performs tasks such as removing duplicates, enumerating isomers and tautomers, and generating viable 3D conformations.

The crystal structure of the BaP1 enzyme was retrieved from the Protein Data Bank (PDB: 2W14). More precisely, this PDB file describes a structure of the BaP1 enzyme in complex with a peptidomimetic inhibitor with very high resolution (1.08 Å). This structure was later prepared in order to delete alternate conformations (disorder), remove water molecules and ligands (except the Zn²⁺ ion), and finally protonate titratable residues using predicted pK_as at pH 7.4. All these tasks were performed using "Prepare Protein" protocol available in DS 4.0 with default parameters. Employing STING's¹⁶⁻¹⁹ Java Protein Dossier¹⁸⁻²⁰, structural analysis of the prepared enzyme allowed the characterization of BaP1's active site, including the zinc(II) coordination site and the deep hydrophobic pocket located at the S1' subsite. This pocket was later refined using the module "Define Site - From Receptor Cavities" from DS 4.0. Considering the amino acid residues that compose the peptidomimetic inhibitor binding site on the BaP1 enzyme, a structure-based pharmacophore model was built using Ludi algorithm as implemented in DS 4.0. Ludi creates a pharmacophore model by identifying potential interactions sites (features), which can be classified as hydrogen bond donors (HBD), hydrogen bond acceptors (HBA) and hydrophobic (HY). Because of the importance of the zinc(II) coordination site, the obtained pharmacophore model was customized to also characterize zinc(II) interaction features regarding the following functional groups: carboxyl, phosphate, imidazole, 1,2,3-triazole, 1,2,4-triazole,^{20,21} tetrazole, thiaziazole, 1-hydroxy-2-oxo derivatives.

Once the compound library, pharmacophore model and target enzyme were validated, a SBVS campaign consisting of five steps was carried out. Firstly, a fast pharmacophore-based screening was performed in order to prioritize compounds containing functional groups that can bind to zinc(II). In the second step, using LibDock^{21,22}, filtered compounds were docked into a region corresponding to the binding site of the co-crystallized ligand described in the 2W14.pdb. Then, aiming to find ligands with high specificity and affinity to the BaP1 enzyme, successfully docked compounds in step two were reevaluated considering also the spatial region delimited by the highly hydrophobic S1' subsite tunnel. For that, a second docking round was performed using LigandFit a shape-based

molecular docking program suitable for well-characterized pocket regions²²⁻²⁴. In step 4, a consensus scoring procedure was employed to rank the compounds in terms of binding affinity. Affinity metrics calculated at the three previous steps were combined into a single scoring function, thus allowing identifying compounds that were well evaluated through the entire screening process. More precisely, for each of the considered metrics, compounds were listed in descending order of predicted binding affinity. The consensus score of a given molecule was then defined by the number of times it had been ranked at the top binding affinity percentile (top 20%). Consensus score approaches balance errors in single affinity predictions, thus improving the probability of identifying 'true' ligands²³⁻²⁵.

The fifth step consisted of a re-ranking procedure. The top 500 compounds were re-docked using CDOCKER, a CHARMM-based molecular dynamics (MD) docking program. Considering the re-docking results, compounds were then analyzed in terms of protein ligand-interactions, being discarded the non-satisfactory ones. Finally, the compounds were re-ranked in ascending order of CDOCKER energy, which is calculated based on the internal ligand strain energy and the protein-ligand interaction energy (data organized in Table S1 in Supplementary material section). After the SBVS campaign was completed, the five best compounds in terms of CDOCKER energy were assayed for inhibition of BaP1's proteolytic activity in experiments performed *in vitro*. From those, only two showed inhibition *in vitro* evaluation: ZINC06812429 and ZINC08767570 (from now on referred as compounds A1 and A2, respectively).

In order to shed some light on the binding modes of compounds A1 and A2, a quantum and molecular mechanical (QM/MM) docking approach (see Supplementary Material) was performed. Figure 1 depicts a comparison between the co-crystallized peptidomimetic inhibitor (PDB: 2W14) and QM/MM docking predicted binding modes for the compounds A1 and A2.

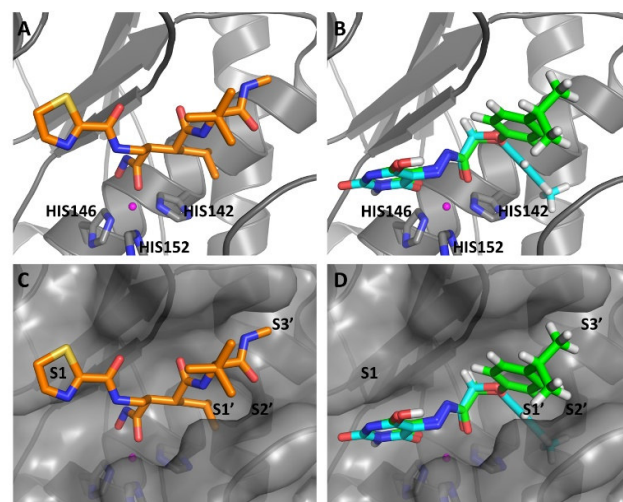


Figure 1 Enlarged view of the molecular interactions of three compounds with the BaP1 metalloprotease (PDB: 2W14). (A, C): The co-crystallized peptidomimetic inhibitor (carbon atoms colored in orange). (B, D): *In silico* binding modes for compounds A1 (carbon atoms colored in cyan) and A2 (carbon atoms colored in green), both with the **pyrimidine-2,4,6- trione** ring and placed within the catalytic enzyme site (parts of the molecular structures of A1 and A2 overlap). Catalytic zinc ion is shown as a magenta sphere. Zinc-coordinating histidines side chains are shown as light gray sticks. BaP1 subsites are denoted with black bold letters on the molecular surface (only at C and D). Figure generated using PyMOL.

Based on the *in silico* results, one may suggest that the binding of the **pyrimidine-2,4,6-trione ring** into the catalytic BaP1 site S1 occurs through the **keto groups** of the ligands differently of what was shown for some barbiturate-metal complexes^{25,26} that presented tautomeric (enol) forms before bonding with the metal. Crystal structure of MMp-9 and mmP-13 had shown that the N-3 atom of the **pyrimidine-2,4,6-trione ring** is in close contact with the catalytic zinc ion and the oxygen atoms O-2 and O-4 belong to the second coordination shell^{27,28}. The docking solutions also showed these coordination modes. Also, favorable interactions of ligands (A1 and A2) with the BaP1 are established with the **His142**, **His146** and **Arg110** residues that are present in the subsite S1. Other hydrophobic interactions with **Leu170**, **Ser168**, **Gly109**, **Thr107** and **Ile108** occur principally in subsite S1 (see Supplementary Material, Figure S3).

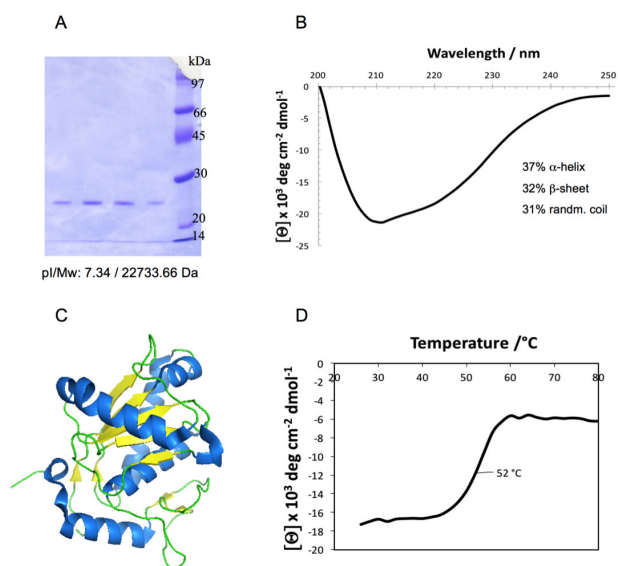


Figure 2 (A) SDS-PAGE (12%) results: Samples as fractions collected from the two-step purification of BaP1 were run on 12% gel, and stained with Coomassie Brilliant Blue. The protein marker is given at right in kDa (LMW-SDS Marker kit, Amersham Bioscience). (B) Circular dichroism spectrum of BaP1 protein ($4 \mu\text{mol}\cdot\text{L}^{-1}$, Tris-HCl $25 \text{ mmol}\cdot\text{L}^{-1}$, pH 8). (C) BaP1 (PDB: 2W14) cartoon representation with illustration of secondary structure elements: α -helix (in blue), β -sheet (in yellow) and random coil (in green). (D) Circular dichroism data at 222 nm during the heating of the BaP1 protein (25–80 °C and 10 °C/h) showed that BaP1 has a melting point at 52 °C.

The target SVMP enzyme BaP1 was isolated from the venom of *Bothrops asper* as described by Gutiérrez et al.^{26,29} and Watanabe et al.^{29,30}. A combination of ion-exchange chromatography on CM-Sepharose, followed by affinity chromatography on Affi-gel Blue for the enzyme BaP1 purification was employed. Gel electrophoresis (SDS-PAGE, 12%) was used for protein detection and Circular Dichroism for accessing the protein secondary structure features as illustrated in Figure 2. The BaP1 showed to be folded protein, with one third of its structure being folded as α -helix, as β -sheet or presented itself in random coil, thus corroborating with the 3D PDB structure features (Figure 2B). Also, BaP1 exhibited the relatively high melting point with the T_m at 52 °C, but was not stable thermally as it was not possible to refold it after heating protein sample up to 80 °C.

The inhibition of proteolytic activity of the BaP1 with the docked ligands A1 and A2, was assayed by following the cleavage of azocaseine ($1 \text{ mmol}\cdot\text{dm}^{-3}$), a nonspecific protease substrate³⁰. Almost complete inhibition of the proteolytic activity of the BaP1 was obtained by both compounds A1 (98% of inhibition) and A2 (95% inhibition) in concentrations of $100 \mu\text{mol}\cdot\text{dm}^{-3}$ as presented in Table 1. Also using the same substrate, IC_{50} were determined as $20 \mu\text{mol}\cdot\text{dm}^{-3}$ for A1 and $17 \mu\text{mol}\cdot\text{dm}^{-3}$ for A2.

Table 1 Results for *in vitro* inhibition³⁰⁻³⁵ of BaP1's proteolytic activity over azocasein at $1 \text{ mmol}\cdot\text{dm}^{-3}$:

Symbol	Compound	IC_{50} $\mu\text{mol}\cdot\text{dm}^{-3}$	Inhibition <i>in vitro</i> %
A1	ZINC06812429	20 ± 2	98
A2	ZINC08767570	17 ± 2	95

The **BaP1-A1** and **BaP1-A2** protein-ligand interactions were monitored *in vitro* and analyzed by applying the fluorescence quenching experiments (Figure 3). These enabled us to evaluate changes in the environment of the BaP1 tryptophan residues when A1 or A2 compounds were present. Both compounds, A1 and A2 caused quenching in fluorescence when compared with the intrinsic fluorescence of the BaP1 protein. In addition, it was observed that the maximums of fluorescence were shifted to the red region. In the case of the compound A1 this shift was around $\Delta = +11 \text{ nm}$, and in the case of A2, the shift in Λ_{max} was $\Delta = +7 \text{ nm}$.

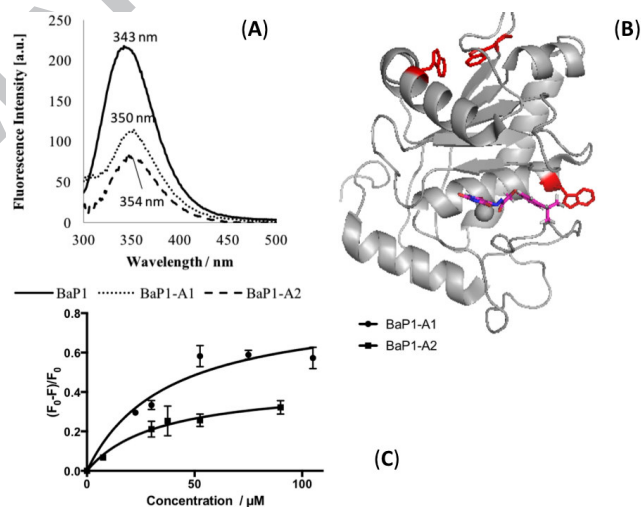


Figure 3 Fluorescence spectroscopy interaction studies: (A) spectra of BaP1 ($2 \mu\text{mol}\cdot\text{L}^{-1}$, Tris-HCl $25 \text{ mmol}\cdot\text{L}^{-1}$, pH 8.0) free and in the presence of inhibitors: BaP1-A1 (···) or BaP1-A2 (- - -), (B) BaP1 protein cartoon representation (PDB: 2W14) with illustration of its tryptophan (Trp, W) residues in red, and (C) Stock-Volmer graphs and K_d constants obtained from fluorescence quenching studies for the two pairs BaP1-A1 and BaP1-A2 were 37.1 and $33.6 \mu\text{mol}\cdot\text{dm}^{-3}$, respectively. The experiments were repeated three times, and plot is showing mean values with error bars.

The obtained results of the *in vitro* assays confirmed that compounds A1 and A2 are viable BaP1 inhibitors (Table 1 and Figure 3). The Stock-Volmer K_d constants obtained from the fluorescence quenching studies were 37.1 (BaP1-A1) and $33.6 \mu\text{mol}\cdot\text{dm}^{-3}$ (BaP1-A2), together with the inhibition of BaP1 activity of almost 100% during *in vitro* experiments make the pyrimidine-2,4,6-trione structure moiety very interesting for further investigation aimed to improve its binding and affinity properties against BaP1. Also, when compared with the known peptidomimetic inhibitor³¹ (Fig. 1. B and D), we can say that the

compounds A1 and A2 exhibited similar IC_{50} values of around $20 \mu\text{mol}\cdot\text{dm}^{-3}$ and when compared to other type of inhibitors³², even had lower IC_{50} and performed an excellent inhibition of BaP1 proteolytic activity against azocasein.

Results obtained are not very surprising and are in agreement with some previous studies³²⁻³⁷, as one pyrimidine-2,4,6-trione has been reported as a promising effective and selective inhibitor of cell matrix metalloproteases^{38,39}. Other studies have also shown the efficiency of pyrimidine-2,4,6-trione derivatives, particularly one named RO 28-2653 developed by Hoffman-La Roche research group, in anticancer therapy³³⁻³⁷. Nevertheless, none of the two presented compounds (A1 and A2) have ever been pointed as an SVMP BaP1 inhibitors or been used in the neutralization of snake venom enzymes.

In summary, this study proposes two commercially available compounds, A1 and A2 with pyrimidine-2,4,6-trione ring, as new inhibitors of the snake venom metalloprotease BaP1. In addition, BaP1 protein subsites important for the interaction with the A1 and A2 were identified. The *in vitro* tests showed that A1 and A2 can inhibit the activity of the BaP1 by 95-98%, thus indicating that have potential for the development of novel snake venom metalloprotease inhibitors. We expect that the results presented herein can motivate future efforts in finding potent pyrimidine-2,4,6-trione derivatives that can be used for SVMPs inhibition *in vivo*.

Acknowledgments

This research received financial supports from *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP). We also thank Organization for the Prohibition of Chemical Weapons (OPCW) for Dr. Mandic grant. At last, but not at least, we thank Jose Augusto Salim, Inacio Yano and Ivan Mazoni from GPBC, Embrapa CNPTIA for their help with *in silico* experiments.

References and notes

- Zeller, E. A. *Experientia* **1977**, *33*, 143–150.
- Kang, T. S.; Georgieva, D.; Genov, N.; Murakami, M. T.; Sinha, M.; Kumar, R. P.; Kaur, P.; Kumar, S.; Dey, S.; Sharma, S.; Vrieling, A.; Betzel, C.; Takeda, S.; Arni, R. K.; Singh, T. P.; Kini, R. M. *FEBS J.* **2011**, *278*, 4544–4576.
- Bjarnason, J. B.; Fox, J. W. *Pharmacol. Ther.* **1994**, *62*, 325–372.
- Calvete, J. J.; Sanz, L.; Cid, P.; la Torre, de, P.; Flores-Diaz, M.; Santos, Dos, M. C.; Borges, A.; Bremono, A.; Angulo, Y.; Lomonte, B.; Alape-Girón, A.; Gutiérrez, J. M. *J. Proteome Res.* **2010**, *9*, 528–544.
- Alape-Girón, A.; Sanz, L.; Escolano, J.; Flores-Diaz, M.; Madrigal, M.; Sasa, M.; Calvete, J. J. *J. Proteome Res.* **2008**, *7*, 3556–3571.
- Gutiérrez, J. M.; Rucavado, A.; Escalante, T.; Díaz, C. *Toxicol. Chem.* **2005**, *45*, 997–1011.
- Gutiérrez, J.; Rucavado, A.; Ovadia, M. *Drug Discovery Today* **1999**, *4*, 532–533.
- Borkow, G.; Gutiérrez, J. M.; Ovadia, M. *Toxicol. Appl. Pharmacol.* **1997**, *147*, 442–447.
- Escalante, T.; Franceschi, A.; Rucavado, A.; Gutiérrez, J. M. *Biochem. Pharmacol.* **2000**, *60*, 269–274.
- Villalta-Romero, F.; Gortat, A.; Herrera, A. E.; Arguedas, R.; Quesada, J.; de Melo, R. L.; Calvete, J. J.; Montero, M.; Murillo, R.; Rucavado, A.; Gutiérrez, J. M.; Pérez-Payá, E. *ACS Med. Chem. Lett.* **2012**, *3*, 540–543.
- Bourguet, E.; Alix, A.; Moroy, G.; Sapi, J.; Hornebeck, W. *Pharmacomodulation of Broad Spectrum Matrix Metalloproteinase Inhibitors Towards Regulation of Gelatinases*. INTECH Open Access Publisher, 2012.
- Flipo, M.; Charton, J.; Hocine, A.; Dassonneville, S.; Deprez, B.; Deprez-Poulain, R. *J. Med. Chem.* **2009**, *52*, 6790–6802.
- Weisburger, J. H.; Weisburger, E. K. *Pharmacological reviews* **1973**, *25*, 1–66.
- Putrament, A.; Baranowska, H.; Pachecka, J. *Mol. Gen. Genet.* **1973**, *122*, 61–72.
- Madhavi Sastry, G.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. *J. Comput. Aided Mol. Des.* **2013**, *27*, 221–234.
- Neshich, G.; Borro, L. C.; Higa, R. H.; Kuser, P. R.; Yamagishi, M. E. B.; Franco, E. H.; Krauchenco, J. N.; Fileto, R.; Ribeiro, A. A.; Bezerra, G. B. P.; Velludo, T. M.; Jimenez, T. S.; Furukawa, N.; Teshima, H.; Kitajima, K.; Bava, A.; Sarai, A.; Togawa, R. C.; Mancini, A. L. *Nucleic Acids Res.* **2005**, *33*, W29–W35.
- Neshich, G.; Mazoni, I.; Oliveira, S. R. M.; Yamagishi, M. E. B.; Kuser-Falcão, P. R.; Borro, L. C.; Morita, D. U.; Souza, K. R. R.; Almeida, G. V.; Rodrigues, D. N.; Jardine, J. G.; Togawa, R. C.; Mancini, A. L.; Higa, R. H.; Cruz, S. A. B.; Vieira, F. D.; Santos, E. H.; Melo, R. C.; Santoro, M. M. *Genet. Mol. Res.* **2006**, *5*, 717–722.
- Neshich, G.; Rocchia, W.; Mancini, A. L.; Yamagishi, M. E. B.; Kuser, P. R.; Fileto, R.; Baudet, C.; Pinto, I. P.; Montagner, A. J.; Palandrani, J. F.; Krauchenco, J. N.; Torres, R. C.; Souza, R. C.; Togawa, R. C.; Higa, R. H. *Nucleic Acids Res.* **2004**, *32*, W595–W601.
- Neshich, G. *Nucleic Acids Res.* **2004**, *33*, D269–D274.
- Al-Balas, Q.; Hassan, M.; Al-Oudat, B.; Alzoubi, H.; Mhaidat, N.; Almaaytah, A. *Molecules* **2012**, *17*, 13740–13758.
- Diller, D. J.; Merz, K. M. *Proteins* **2001**, *43*, 113–124.
- Venkatachalam, C. M.; Jiang, X.; Oldfield, T.; Waldman, M. J. *Mol. Graph. Model.* **2003**, *21*, 289–307.
- Charifson, P. S.; Corkery, J. J.; Murcko, M. A.; Walters, W. P. *J. Med. Chem.* **1999**, *42*, 5100–5109.
- Kitehen, D. B.; Decomez, H.; Furr, J. R.; Bajorath, J. *Nat. Rev. Drug Discov.* **2004**, *3*, 935–949.
- Shaban, N. Z.; Masoud, M. S.; Awad, D.; Mawlawi, M. A.; Sadek, O. M. *Chem. Biol. Interact.* **2014**, *208*, 37–46.
- Gutiérrez, J. M.; Romero, M.; Díaz, C.; Borkow, G.; Ovadia, M. *Toxicol.* **1995**, *33*, 19–29.
- Blagg, J. A.; Noe, M. C.; Wolf-Gouveia, L. A.; Reiter, L. A.; Laird, E. R.; Chang, S.-P.; Danley, D. E.; Downs, J. T.; Elliott, N. C.; Eskra, J. D.; Griffiths, R. J.; Hardink, J. R.; Haugeto, A. I.; Jones, C. S.; Liras, J. L.; Lopresti-Morrow, L. L.; Mitchell, P. G.; Pandit, J.; Robinson, R. P.; Subramanyam, C.; Vaughn-Bowser, M. L.; Yocum, S. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1807–1810.
- Tochowicz, A.; Maskos, K.; Huber, R.; Oltenfreiter, R.; Dive, V.; Yiotakis, A.; Zanda, M.; Bode, W.; Goettig, P. *J. Mol. Biol.* **2007**, *371*, 989–1006.
- Watanabe, L.; Shannon, J. D.; Valente, R. H.; Rucavado, A.; Alape-Girón, A.; Kamiguti, A. S.; Theakston, R. D. G.; Fox, J. W.; Gutiérrez, J. M.; Arni, R. K. *Protein Sci.* **2003**, *12*, 2273–2281.
- Wang, W.-J.; Shih, C.-H.; Huang, T.-F. *Biochem. Biophys. Res. Commun.* **2004**, *324*, 224–230.
- Villalta-Romero, F.; Gorat, A.; Herrera, A. E.; Arguedas, R.; Quesada, J.; de Melo, R. L.; Calvete, J. J.; Montero, M.; Murillo, R.; Rucavado, A.; Gutiérrez, J. M.; Pérez-Payá, E. *ACS Medicinal Chemistry Letters* **2012**, *3*, 540–543.
- Lingott, T.; Schleberger, C.; Gutiérrez, J. M.; Merfort, I. *Biochemistry* **2009**, *48*, 6166–6174.
- Lein, M.; Jung, K.; Ortel, B.; Stephan, C.; Rothaug, W. *Oncogene* **2002**, *21*, 2089–2096.
- Abramjuk, C.; Jung, K.; Krell, H.-W.; Juchem, R.; Peters, R.; Taymoorian, K.; Staack, A.; Stephan, C.; Schnorr, J.; Loening, S. A.; Lein, M. *Anticancer Drugs* **2005**, *16*, 855–861.
- Kilian, M.; Gregor, J. I.; Heukamp, I.; Hanel, M.; Ahlgrimm, M.; Schimke, I.; Kristiansen, G.; Ommer, A.; Walz, M. K.; Jacobi, C. A.; Wenger, F. A. *Prostaglandins Leukot. Essent. Fatty Acids* **2006**, *75*, 429–434.
- Nagel, S.; Heinemann, von, P.; Heiland, S.; Koziol, J.; Gardner, H.; Wagner, S. *Brain Res.* **2011**, *1368*, 264–270.
- Maquoi, E.; Sounni, N. E.; Devy, L.; Olivier, F.; Frankenne, F.; Krell, H.-W.; Grams, F.; Foidart, J.-M.; Noël, A. *Clin. Cancer Res.* **2004**, *10*, 4038–4047.
- Grams, F.; Brandstetter, H.; D'Alò, S.; Geppert, D.; Krell, H. W.; Leinert, H.; Livi, V.; Menta, E.; Oliva, A.; Zimmermann, G.; Gram, F.; Brandstetter, H.; D'Alò, S.; Geppert, D.; Krell, H. W.; Leinert, H.; Livi, V.; Menta, E.; Oliva, A.; Zimmermann, G. *Biol. Chem.* **2001**, *382*, 1277–1285.
- Vandenbroucke, R. E.; Libert, C. *Nat. Rev. Drug Discov.* **2014**, *13*, 904–927.

Supplementary Material

Supplementary material is provided as a separate electronic file in PDF format.

ACCEPTED MANUSCRIPT

