Study Concerning the Inhibitory Activity Upon Myeloperoxidase of Some Oxicams Class Derivatives Using the Docking Molecular Technique

DENISA-CONSTANTINA AMZOIU[1], ANCA-MIHAELA (BULEARCA) STOIAN[2], EMILIA AMZOIU[3]*, GABRIELA RAU[2]

[1] University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 2–4 Petru Rareº Str., 200349, Craiova, Romania

[2] University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, Department of Organic Chemistry, 2–4 Petru Rareº Str., 200349, Craiova, Romania

[3] University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy, Department of Physics Chemistry, 2–4 Petru Rareº Str., 200349, Craiova, Romania

"*Molecular docking" was an useful tool for selecting potential therapeutic agents. This technique allowed assessment of the proper way for bonding a ligand molecule inside the active site of the biological receptor and moreover enabled the evaluation of minimum bonding energy. The method was used for testing some nonsteroidal anti-inflammatory drugs from the oxicam class (meloxicam, piroxicam and tenoxicam), as potential inhibitors of myeloperoxidase (MPO). Net charges distributed on the atoms from the studied molecules, which have appeared as result of the chemical bonds formation, gave information about the nature and the reactivity of the atoms from each molecule. They also indicated the atoms that contributed in the formation of the biological response of the substances studied class. In the present study, the results have shown that meloxicam represented the best inhibitory agent of myeloperoxidase because Meloxicam–MPO had the lowest energy value.*

Keywords: molecular docking, oxicams, myeloperoxidase, inhibitors, NSAIDs

Osteoarthritis (OA) was defined as a heterogeneous group of disorders, characterized by joints events, associated with alterations of the cartilage integrity, subchondral bone and periarticular structures [1].

Reactive oxygen (ROS) and nitrogen species (RNS) (radicals – superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical ($HO^\bullet$), peroxyl radical ($ROO^\bullet$), nitric oxide ($NO^\bullet$), and also non-radicals – hypochlorous acid (HOCl), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$), ozone (O_3)) were involved in the OA pathogenesis. Markers level of oxidative stress was observed to be higher in patients with arthrosis, as compared to that for healthy persons [2, 3].

Hypochlorous acid is a reactive non-radical oxygen species, contributing to bactericidal effect of oxygen reactive species in phagocytes. During the respiratory blast, the production of $O_2^{\bullet -}$, H_2O_2, $HO^\bullet$, $^1O_2$ and NO with antimicrobial and antitumoral effects increases in macrophages and neutrophils, and the presence of myeloperoxidase (MPO) determines the formation of hypochlorite, by Cl^- oxidation, using H_2O_2. Hypochlorite contributes to pathogens destruction [4-6]:

$$H_2O_2 + Cl^- \xrightarrow{MPO} H_2O + OCl^- \text{ or}$$

$$H_2O_2 + Cl^- \xrightarrow{MPO} OOCl^- + {}^1O_2 + 2H^+ \text{ or}$$

$$H_2O_2 + Cl^- \xrightarrow{MPO} OCl^- + {}^1O_2 + H_2O$$

Or by protonation of OCl^-:

$$OCl^- + H^+ \rightarrow HOCl$$

The most part from the generated ion OCl^- is converted into toxic chloramines [7]:

$OCl^- + H^+ + R–NH_2 \rightarrow R–NHCl + H_2O$

or it could react with other compounds [4]:

$OCl^- + {}^1O_2 +$ amino acids + fatty acids $\rightarrow$ chloramines, peroxides, aldehydes

HOCl is a strong oxidizing agent, responsible for antimicrobial effects and for those that involve injuries of chronic inflammation mediated by polymorphonuclear cells (PMN) [7]. MPO is the only peroxidase that catalyses the Cl^- ion oxidation to hypochlorite, thereby converting 20-70% of H_2O_2 released by activated neutrophils [8].

$$H_3O^+ + H_2O_2 + Cl^- \xrightarrow{MPO} HClO + 2\,H_2O$$

Besides this halogenation activity, MPO acts as a classical peroxidase. Ferric MPO reacts with H_2O_2 produced by stimulated neutrophils in order to form the redox–compound I intermediate, which is a strong oxidising agent, able to react with different substrates. The compound I could be reduced by organic substrates (RH), resulting free radicals and the compound II, which reacts with second RH molecule, remaking the enzyme [9]:

Compound I + RH $\rightarrow$ Compound II + $R^\bullet$

Compound II + RH $\rightarrow$ MPO + $R^\bullet$

It was discovered that MPO is implicated in tyrosine nitration reaction, as catalyst, presence of 3-nitrotyrosine at the inflammation site involving $ONOO^-$ in the tissue injuries from inflammation [6, 9].

* *email: emanro2002@yahoo.com; Tel.: 0745226647*

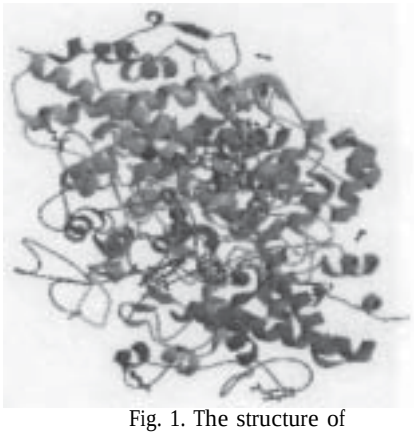

Fig. 1. The structure of myeloperoxidase (code ICXP - Protein Data Bamk)

Table 1
BONDING ENERGIES VALUES FOR OXICAM – MPO COMPLEXES

Oxicam	Meloxicam	Piroxicam	Tenoxicam
Value of the bonding energy (kJ/mol)	-409.6	-392.2	-286.1

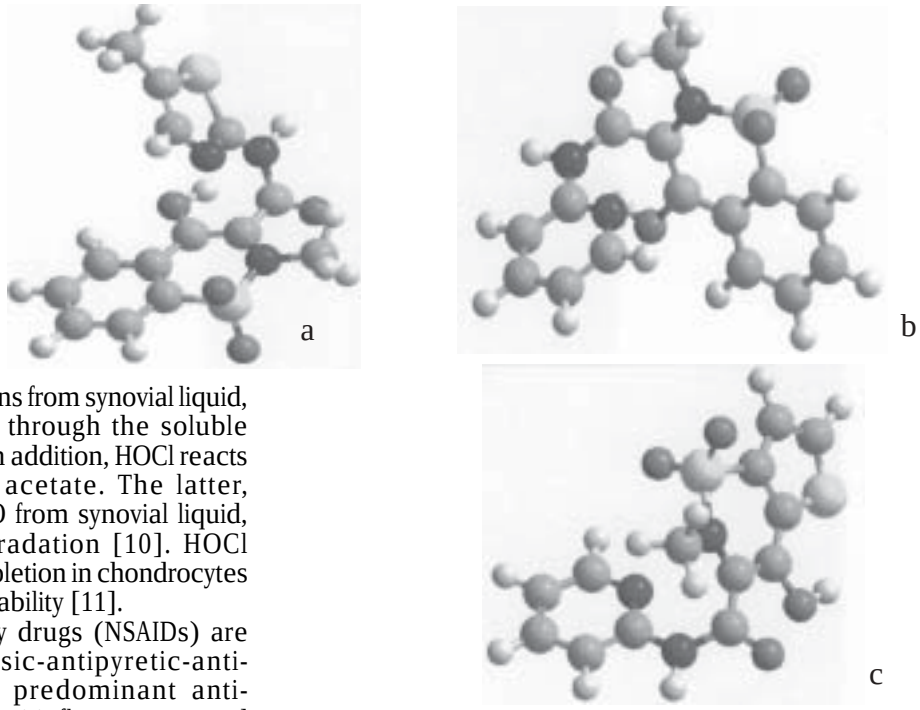

Fig. 2. The optimized structures for meloxicam (a), piroxicam (b) and tenoxicam (c)

HOCl attacks preferentially proteins from synovial liquid, reducing its viscosity, especially through the soluble hyaluronan precipitation process. In addition, HOCl reacts with polysaccharides, forming acetate. The latter, correlated with the activity of MPO from synovial liquid, involves HOCl in cartilage degradation [10]. HOCl determines ATP and glutathione depletion in chondrocytes and decreases the chondrocytes viability [11].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are substances from the analgesic-antipyretic-anti-inflammatory group, having a predominant anti-inflammatory effect, used with anti-inflammatory and analgesic purpose. They have a rapid antirheumatic effect, but its duration is short when administration ends. NSAIDs are substances with symptomatic-pathogenic action, diminishing or eliminating some inflammation symptoms in chronic rheumatic diseases, but giving no modification in the pathologic process evolution [12, 13]. NSAIDs most important effect is cyclooxigenase (COX) inhibition, thus decreasing the PG biosynthesis. NSAIDs mechanism anti-inflammatory action is not yet completely known. Besides the inhibitory effect upon COX, different NSAIDs have possible other additional action mechanisms [14].

Experimental part

Materials and methods

"Molecular docking" was used for testing some oxicams (meloxicam, piroxicam and tenoxicam), as potential inhibitors of myeloperoxidase (MPO, code 1CXP-Protein Data Bank [15, 16]), an enzyme produced by leukocytes activation [17]. Both enzyme and oxicams structures were prepared for the docking process, using Hex 5.0 program [18]. They were modelled using 3D parametric functions, which encodes in the same time surface shape, electrostatic charge and potential distribution. These parametric functions were based on orthogonal spherical or polar basic functions.

In order to obtain an efficient "docking", the Fourier algorithm was used together with the spherical polar approximation. Fourier algorithm allows the acceleration for searching the most favourable orientations for "docking" inside the receptor molecule with the ligand translation. Spherical polar approximation permits both ligand translation and rotation for generating and evaluating the optimum orientations.

Results and discussions

Knowing oxicam anti-inflammatory, analgesic and antipyretic activity [17], meloxicam, piroxicam and tenoxicam were tested as potential myeloperoxidase inhibitors (fig. 1).

Structures of meloxicam, piroxicam and tenoxicam were presented in figure 2. Structures were optimized with the ArgusLab program [19].

"Docking" process was more complete through ligand translation and rotation. Table 1 presented bonding energies (affinities) values, measured in kJ/mol, for the oxicam – MPO complexes.

Regarding net atomic charges inside molecule, for each compound the following electronic distribution was determined (fig. 3).

Main action mechanism of NSAIDs consisted in COX inhibition. It was suggested that their anti-inflammatory activity might also be due to the capacity for scavenging ROS and inhibiting neutrophils respiratory blast [7]. It was demonstrated that NSAIDs inhibited HOCl formation and scavenged it. Paino et al. suggested that NSAIDs did not allow HOCl formation through competition with chloride ion for MPO active site, without inhibiting NADPH-oxidase enzymatic complex, excepting indomethacin [20]. Kato et al. observed that phenols were typical MPO substrates, thus piroxicam and tenoxicam were more efficient than compounds without phenol structure as diclofenac and naproxen [21], while indomethacin, another MPO substrate, was as efficient as piroxicam and tenoxicam [7, 22].

Van Antwerpen and Nève studied *in vitro* ROS scavenging effect of piroxicam, tenoxicam, lornoxicam, meloxicam, nimesulid and ibuprofen. They discovered that piroxicam had an antioxidant activity higher than tenoxicam, ibuprofen and nimesulid. It was expected that molecules containing thiol groups had a more pronounced antioxidant

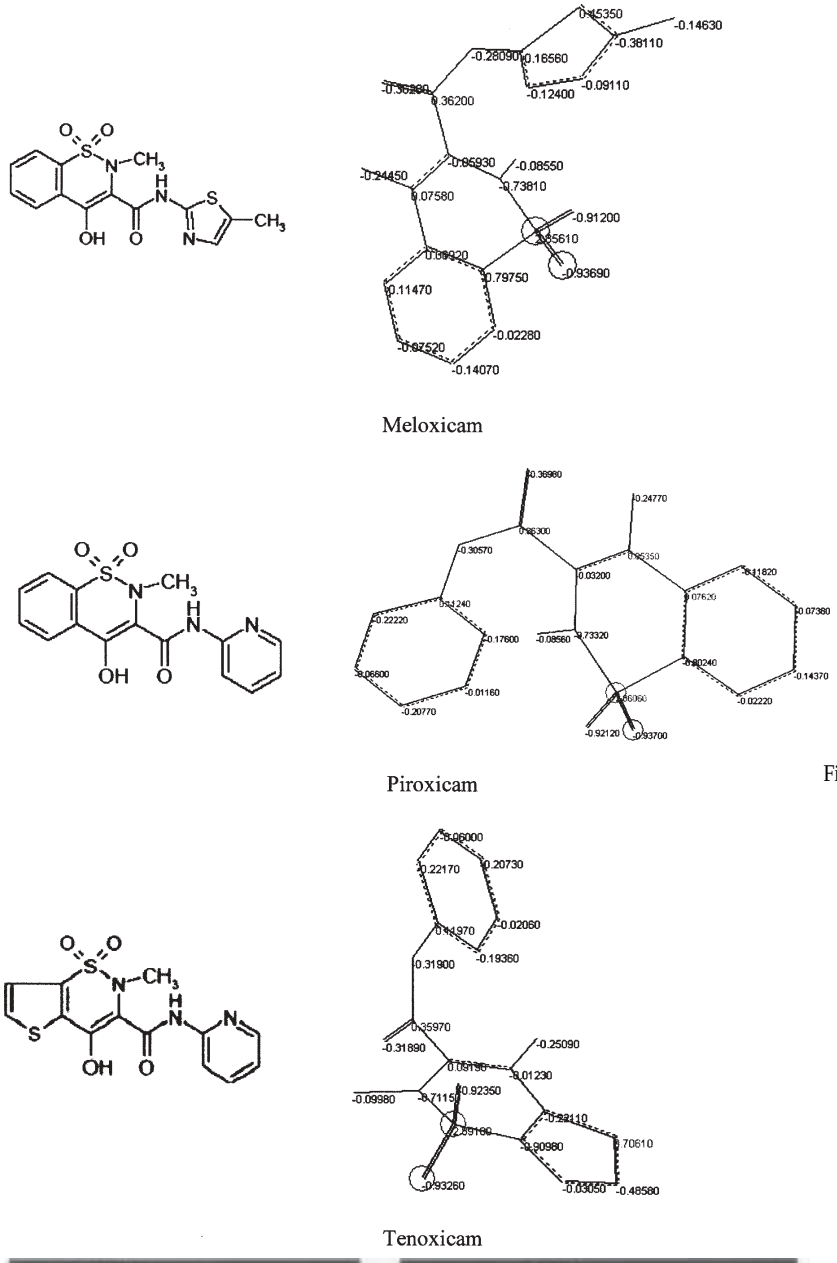

Fig. 3. Values of the net charges on atoms from the three oxicams

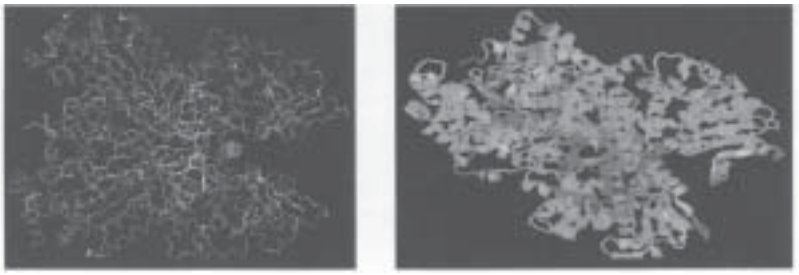

Fig. 4. The structure of the Mel - MPO complex

capacity due to HO˙ radical transformation into sulphoxide or sulphone. A diminished activity might be connected to HO˙ radical attack upon NSAIDs aromatic ring (for example aryl acetic, aryl propionic or indolic acid) or upon sulphoanilide group. In oxicams case, it was proposed a radical attack on the benzothiazine (piroxicam) or thienothiazine (tenoxicam) rings, cumulated with impairment of the enol group, with intermediary reactivity. Therefore, taking into consideration the reactivity towards HO˙ radical, NSAIDs might be classified in the following order: molecules containing thiol > oxicams > other NSAIDs [23, 24].

It was demonstrated that HOCl scavenging effect of oxicams was linked with C3 oxidation. Ibuprofen did not interact at a detectable level with HOCl, and nimesulid had a weaker antioxidant capacity, linked to C6 oxidation, resulting a chlorinated product. Thus, the examined oxicams and nimesulid were weak HOCl scavenging agents, while NSAIDs from the aryl acetic, aryl propionic or indolic acids group did not react with HOCl [23, 24].

Data presented in table 1 suggested that Meloxicam – MPO molecular edifice (fig. 4), which had the lowest energy value, represented potential myeloperoxidase inhibitory agent.

In electrophilic type interaction between drug substance (oxicam) and biologic receptor, the most reactive positions were those with maximum charge density, which means with maximum negative net charge in absolute value. Thus, in this type of ligand – receptor interaction, oxygen atom might be responsible for the inhibitory activity, with net charges -0.93690 (meloxicam), -0.93700 (piroxicam) and -0.93260 (tenoxicam).

In nucleophilic type interaction between ligand (oxicam) and biologic receptor, the most reactive positions were those with minimum charge density, which means those with maximum positive net charge. Therefore, in this type of interaction the sulphur atom might be responsible for the biological activity, with net charges 2.85610 (meloxicam), 2.86060 (piroxicam) and 2.89100 (tenoxicam).

Conclusions

"Molecular docking" is an efficient method for selecting potential therapeutic agents. This technique permits choosing the proper way for bonding a ligand molecule in biological receptor active site. In addition, it allows minimum bonding energy evaluation. When the synthesis of potential therapeutic agents consists in high financial costs, this technique could be used in order to give a helping hand to researchers for deciding whether to continue the synthesis or not. When applying the docking method, it is necessary to know different data about the biological target.

Net charges distribution on atoms of studied molecules, which appeared after chemical bonds formation, described both nature and reactivity of atoms from molecules and indicated the atoms that contribute to biological response formation for the studied substances class. Therefore, oxygen (sp^2) and sulphur (sp^2) atoms had the greatest contribution to inhibitory activity shown by the oxicam class studied compounds.

Results obtained in this study suggested that meloxicam is the best myeloperoxidase inhibitory agent, because Meloxicam – MPO complex had the lowest energy value. This conclusion sustained the hypothesis of an additional action mechanism for NSAIDs of oxicam class to diminish the oxidative stress in osteoarthritis.

References

1. RO^{a}U, A., VREJU, F., Reumatologie, Ed. Medicalã Universitarã, CIUREA, P., Craiova, 2007, p. 387.
2. MANEESH, M., JAYALEKSHMI, H., SUMA, T., CHATTERJEE, S., CHAKRABARTI, A., SINGH, T.A., Indian J. Clin. Biochem., **20**, no. 1, 2005, p. 129.
3. ARUOMA, O.I., JAOCS, **75**, no. 2, 1998, p. 199.
4. DEJICA, D., COMES, L., DUMA, L., MURE^{a}AN, A., POP, S., PORR, P.J., SZANTO, P., TACHE, S., Stresul oxidative în bolile interne, Ed. Casa Cãrþii de ^{a}tiinþã, Cluj-Napoca, 2000.
5. PASE, L., LAYTON, J.E., WITTMANN, C., ELLETT, F., NOWELL, C.J., REYES-ALDASORO, C.C., VARMA, S., ROGERS, K.L., HALL, C.J., KEIGHTLEY, M.C., CROSIER, P.S., GRABHER, C., HEATH, J.K., RENSHAW, S.A., LIESCHKE, G.J., Curr. Biol., **22**, no. 19, 2012, p. 1818, doi: 10.1016/j.cub.2012.07.060.
6. SILVESCU, C.I., SACKSTEIN, R., PNAS, **111**, no. 29, 2014, p. 10696, www.pnas.org/cgi/doi/10.1073/pnas.1320833111.
7. SAEED, S.A., MESAIK, M.A.; QUADRI, J., TASNEEM, S., MOTIWALA, A., KHALID, U., ALI, S.U., HUSSAIN, F., CHOUDHARY, M., J. Pharmacol. Toxicol., **1**, no. 2, 2006, p. 115.
8. TAFAZOLI, S., O2 BRIEN, P.J., Drug Discovery Today, **10**, no. 9, 2005, p. 617.
9. VAN DALEN, J.C., WINTERBORN, C., SENTHILMOHAN, R., KETTLE, A., J. Biol. Chem., **275**, 2000, p. 11638.
10. REES, M.D., KENNETT, E.C., WHITELOCK, J.M., DAVIES, M.J., Free Radical. Biol. Med., **44**, 2008, p. 1973.
11. HENROTIN, Y., KURZ, B., AIGNER, T., Osteoarthritis Cartilage, **13**, 2005, p. 643.
12. PAVELESCU, M., Tratat de farmacologie, Ed. Medicalã, CRISTEA A.N., Bucure^{o}ti, 2005, p. 617.
13. NITA, S., ANDRIES, A.A., PATRON, L., ALBULESCU, R., RADULESCU, F., TANASE, C., VINTILA, M., Rev. Chim. (Bucharest), **62**, no. 5, 2011, p. 549.
14. HAKAN, T., TOKLU, H.Z., BIBER, N., OZEVREN, H., SOLAKOGLU, S., DEMIRTURK, P., AKER, F.V., Neurol. Res., **32**, no. 6, 2010, p. 629, doi: 10.1179/016164109X12464612122731.
15. *** Protein Data Bank, http://www.pdb.org/pdb/home/home.do.
16. FIEDLER, T.J., FENNA, R.E., J. Biol. Chem., **275**, 2000, p. 11964.
17. MATHY-HARTERT, M., BOURGEOIS, E., GRÜLKE, S., DEBY-DUPONT, G., CAUDRON, I., DEBY, C., LAMY, M., SERTEYN, D., Can. J. Vet. Res., **62**, no. 2, 1998, p. 127.
18. *** RITCHIE, D., Hex Protein Docking, http://www.csd.abdn.ac.uk/hex/.
19. *** www.arguslab.com
20. PAINO, I.M., XIMENES, V.F., FONSECA, L.M., KANEGAE, M.P.P., KHALIL, N.M., BRUNETTI, I.L., Braz. J. Med. Biol. Res., **38**, 2005, p. 543.
21. KATO, Y., NAGAO, A., TERAO, J., OSAWA, T., Biosci. Biotechnol. Biochem., **67**, 2003, p. 1136.
22. SUTA, L.M., VLAIA, L., FULIAS, A., LEDETI, I., HADARUGA, D., MIRCIOIU, C., Rev. Chim. (Bucharest), **64**, no. 11, 2013, p. 1279.
23. VAN ANTWERPEN, P., NÈVE. J., Eur. J. Pharmacol., **496**, 2004, p. 55.
24. VAN ANTWERPEN, P., GELBEKE, M., DUBOIS, J., NÈVE, J., Free Radic. Res., **38**, 2004, p. 251

Manuscript received: 18.12.2014