STRUCTURE-ACTIVITY RELATIONSHIPS OF ANTIOXIDANT/ANTIHYPERLIPIDEMIC AROMATIC (BENZO)THIAZINES/BENZOXAZINES LEADING TO VARIABLE MULTIPOTENT ACTIVITY

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The multifactorial nature of many complex diseases, such as metabolic syndrome, atherosclerosis, neurodegeneration and cancer, justifies the development of multitarget agents, i.e. compounds bearing a combination of two or more pharmacophores, each with a distinct pharmacological action.

A series of new potentially multipotent molecules was designed that combine moieties with antioxidant and anti-inflammatory properties, as well as a structure with potent squalene synthase inhibitory activity.[1-2] The new compounds were synthesized in good yields, characterized via ^{1}H and ^{13}C NMR spectroscopy and pharmacologically evaluated - both in vitro and in vivo - and compared with parent compounds.

Oxidative stress is key to a plethora of pathological conditions, rendering antioxidant activity an asset for a multipotent agent. The compounds' antioxidant activity was assessed via the inhibition of microsomal lipid peroxidation and via their scavenging potency on the free radical DPPH. Substitution on the benzoxazine ring led to a decreased activity in both assays, whereas increasing the lipophilicity of the benzothiazine derivatives by adding an extended aromatic substituent, had a positive effect. Moreover, the introduction of a known antioxidant moiety on the otherwise inactive thiazine structure led to a very potent antioxidant analogue.

Inflammation has been correlated with many multifactorial diseases. Anti-inflammatory activity was studied via in vitro inhibition of the enzyme lipoxygenase (LOX) and via in vivo reduction of carrageenan-induced paw edema. All new derivatives maintained or even exceeded the parents' activity. Again, the substitution on the benzoxazine ring slightly decreased the anti-inflammatory activity, whereas the incorporated (methoxy)naphthyl substructure, a naproxen-like moiety, on the benzothiazine ring increased both LOX inhibition and in vivo potency. The thiazine parent compounds did not have significant activity in vitro, possibly due to decreased lipophilicity, compared to the benzo(oxa/thia)zine analogues. However, both the parent thiazine and its derivatives demonstrated a good in vivo anti-inflammatory profile.

Imbalance in cholesterol levels leads to hyperlipidemia, resulting primarily in CVD/atherosclerosis and secondarily, via also alterations in cell membrane structure and function, to neurodegeneration and other disorders.[2]The new derivatives were assessed in an in vivo hyperlipidemia protocol, in which they showed to maintain the activity of the parent molecules, reducing significant lipid parameters. In this case, the benzoxazine core seemed to exhibit a greater potency, than the (benzo)thiazine.

Finally, the lipid imbalance-related neuronal cell damage[3] may imply a potential beneficial effect of the new molecules on neurodegeneration. Thus, compounds were further evaluated for their inhibition of acetylcholinesterase (AChE), as well as iron-chelation activity (ferrozine assay). Preliminary data showed no significant activity on AChE, whereas the presence of an N-substituted aromatic hydroxyl moiety (on the thiazine ring) enhanced chelating properties.

In conclusion, the improved antioxidant and anti-inflammatory activity of the new derivatives may be promising against multifactorial disorders involving

inflammation (e.g. rheumatoid arthritis and neuroinflammation), while hypolipidemic and chelating activity may contribute towards a multipotent profile. Further structural optimization to increase AChE inhibitory activity is underway.

[1]Matralis, AN; Kourounakis, AP. ACS Med. Chem. Lett. 2019, 10(1), 98-104. [2]Kourounakis, A.P., Matralis, A.N. et al. Bioorg. Med. Chem. 2010, 18, 7402-7412. [3]Kourounakis AP; Bavavea E. Arch. Pharm. (Weinheim) 2020, 353(9):e2000085.