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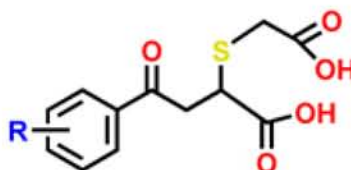
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2-[(CARBOXYMETHYL)SULFANYL]-4-OXO-4-ARYLBUTANOIC ACIDS. PART 3. PHARMACOLOGY AND CONFORMATIONAL PREFERENCES IN DIFFERENT SOLVENTS. THE NMR/MD STUDY

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2-[(Carboxymethyl)sulfanyl]-4-oxo-4-arylbutanoic acids (Figure) exerts antiproliferative potency toward human tumor cells *in vitro* in low micromolar to submicromolar concentrations [1].



Compounds show selective toxicity *in vitro* toward healthy human cells non-stimulated, or stimulated for growth, with selectivity indexes 2-30. So far we showed that selectivity of compounds correlate with their flexibility. Linear correlation was found between selectivity and the time needed for conformational change, as obtained by molecular dynamics simulations in explicit *n*-octanol-water as a solvent, and by applying biasing force to speed-up sampling and to map free-energy surfaces [2, 3]. Congeners having similar substitution pattern on phenyl ring, which exert high or low selectivity, have significantly different free-energy surfaces, even conformations of global minima were comparable [3]. Acute toxicity of selected compounds, *in vivo*, was obtained on mice. Remarkably, compounds that exert high selectivity *in vitro*, also show low toxicity ($LD_{50} > 2\text{g/kg}$), while the first signs of the toxicity of compounds having low selectivity *in vitro*, appear on 100 mg/kg. When applied intravenously as disodium salt, the major amount of the one of the most selective compound (2,4,6-tri-Et- derivative) was excreted unchanged. Conformational properties of selected compounds, in different isotropic or anisotropic solvents, were examined by the NMR and the NAMFIS analysis [4]. In non-polar solvents extended conformations predominate; while in polar solvent predominate folded conformations. DOSY-NMR spectra were used to differentiate shape of major conformers in solvents of different polarity and HBA/HBD abilities. Those results were compared with the conformational behavior of compounds obtained by molecular dynamics simulations in the model of the explicit solvents, without or with applied biasing forces. Distribution of the conformation-dependent molecular properties is discussed.

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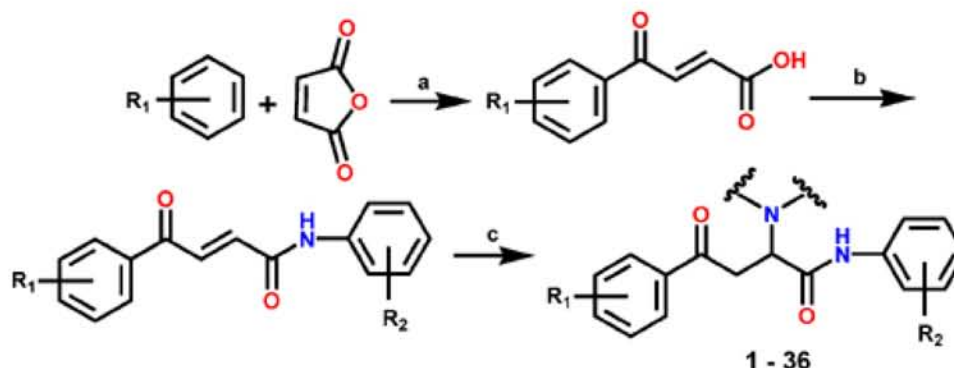
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4-ARYL-4-OXO-N-PHENYL-2-AMINYLBUTYRAMIDES WITH INTERESTING AChE/BChE SELECTIVITY PROFILE

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Recent findings that acetylcholinesterase (AChE) is involved in A β peptide deposition and aggregation through its peripheral anionic site (PAS) [1] revoked the interest in synthesizing novel AChE reversible inhibitors with better pharmacokinetic profile than those currently available, FDA approved drugs, for the treatment of Alzheimer's disease (AD). Butyrylcholinesterase (BChE) was also proved as important target for AD, since it plays the supportive role in cholinergic neurotransmission. Recently, we reported congeneric series of the twenty 4-aryl-4-oxo-N-phenyl-2-aminylbutyramides with varying substitution on the aroyl phenyl ring [2]. Alkyl-substituted congeners inhibited both AChE and BChE in micromolar concentrations, while chloro- or methoxy-substituted compounds were proved to be inactive. In this communication, we have investigated the influence of the amide portion of the molecule on the activity toward AChE and BChE. We systematically changed the amine used to obtain aroylacrylic acid amides (3,5-dimethoxyaniline, 4-isopropylaniline and cyclohexylamine), keeping the favourable alkyl substituents on the aroyl part of the molecule. For the Michael addition to the activated double bond, four amines were used: piperidine, imidazole, morpholine and N-methylpiperazine (Scheme).



Scheme. Synthesis of **1-36**. Reagents: (a) AlCl₃; (b) POCl₃, THF and 3,5-dimethoxyaniline, 4-*i*Pr-aniline or cyclohexylamine; (c) piperidine, imidazole, morpholine or *N*-methylpiperazine in CH₂Cl₂/toluene. R₁= 4-*i*Pr, 2,4-di-*i*Pr or 1,2,3,4-tetrahydronaphthalenyl.

The inhibitory activity of 36 novel compounds was tested on both AChE and BChE. Compounds have shown interesting selectivity profile with piperidino and imidazolo derivatives being active toward AChE, and some morpholino and *N*-methylpiperazino derivatives being active toward BChE, both in low micromolar concentrations. Possible interactions of the compounds with AChE were investigated by docking calculations and MD simulations.

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