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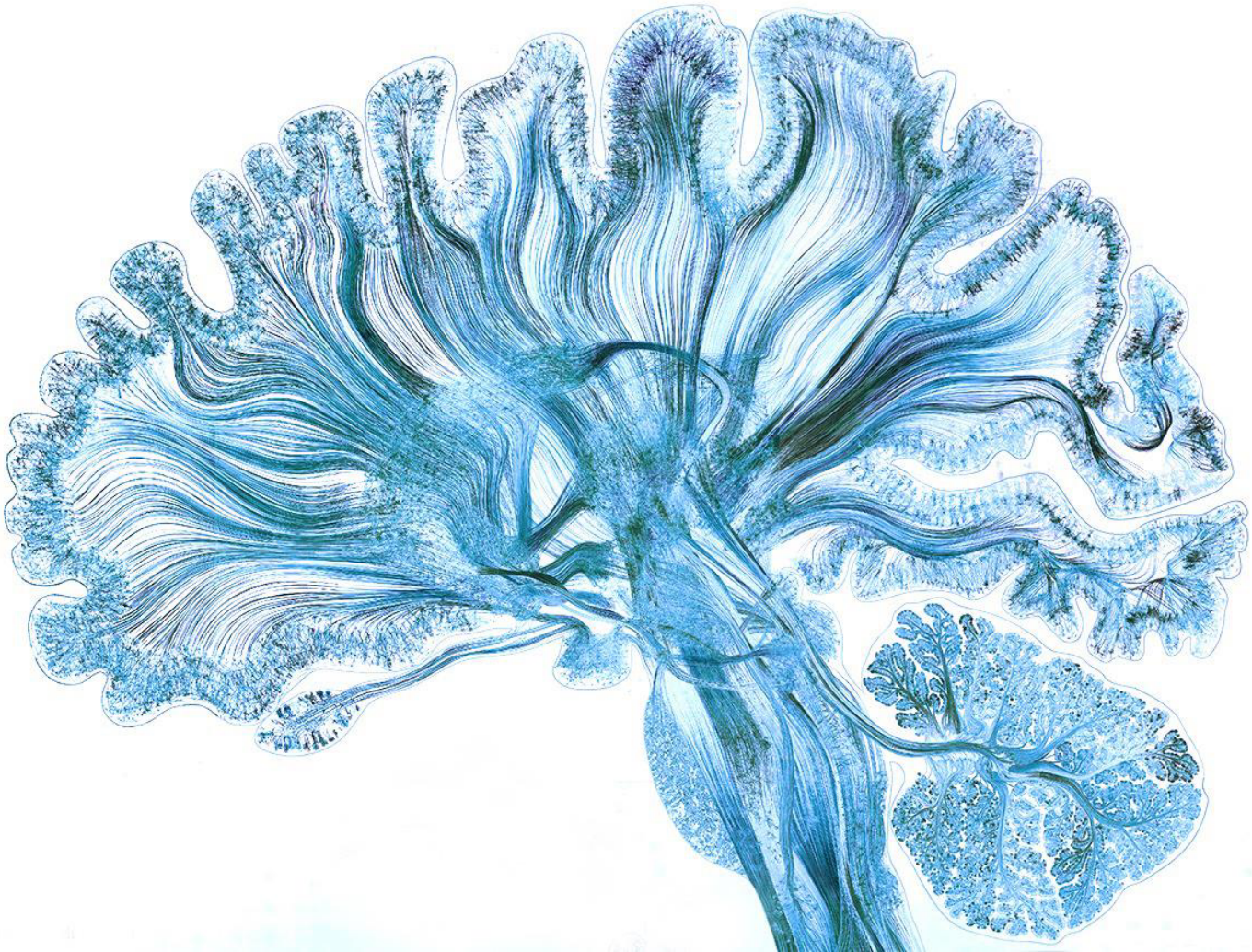
ABSTRACT BOOK

KNJIGA SAŽETAKA



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Abnormally folded alpha-synuclein protein, dysfunctional mitochondria, increased oxidative stress and reduced dopamine neurotransmitter synthesis are a
ll extremely well characterized phenomena in Parkinson's disease (PD) and are thought to be interconnected. While direct targeting of these areas has demonstrated neuroprotection *in vitro* and *in vivo*, there has been a major lack of success in clinical trials. A critical component in the failure of these clinical trials is the inability to specifically target drugs to dopamine producing neurons in the brain.
New drugs targeting the dopaminergic neurons by specific uptake through the human dopamine transporter (hDAT) could represent a viable strategy for establishing selective neuroprotection. Molecules able to increase the bioactive amount of extracellular dopamine, thereby enhancing and compensating a loss of dopaminergic neurotransmission, and to exert neuroprotective response because of their accumulation in the cytoplasm, are required.
By means of homology modeling, molecular docking and molecular dynamics simulations, we have generated 3D structure models of hDAT in complex with substrate and inhibitors. Our results clearly reveal differences in binding kinetics of these compounds to the hDAT in the open and closed conformations, critical for future drug design. The established *in silico* approach allowed the identification of three promising substrate compounds that were subsequently analyzed for their efficiency in inhibiting hDAT-dependent fluorescent substrate uptake, through *in vitro* live cell imaging experiments. Taken together, our work presents the first implementation of a combined *in silico/in vitro*-approach enabling the selection of promising dopaminergic neuron specific substrates.

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Identification of potential dual histamine H_3 receptor antagonist and serotonin reuptake inhibitors through ligand-based and structure-based approaches

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The major depressive disorder (MDD), routinely treated with selective serotonin reuptake inhibitors (SSRIs), is the second leading cause of disability worldwide. However, the treatment of MDD is complicated by high prevalence of residual symptoms connected to increased risk of relapse. Some of the most common residual symptoms are cognitive dysfunction and fatigue. Histamine H_3 receptor (H_3R) antagonists are both, pro-cognitive and wake-promoting agents. In pre-clinical study it was suggested that dual histamine H_3R antagonist and SSRI may have utility as a more efficient antidepressant therapy. The aim of this *in silico* study was identification of novel dual SSRI/H_3R antagonist using ligand-based and structure-based drug design techniques. Starting from structures and activities of known dual ligands, two GRIND-based 3D-QSAR models have been developed, SERT model (R^2 = 0.97; Q^2 = 0.79; SDEP= 0.124) and H_3R model (R^2 = 0.86; Q^2 = 0.75; SDEP= 0.184), and 3D-pharmacophores were constructed. Further, homology model of H_3R was built and refined with molecular dynamics. The hypotheses of binding modes for dual ligands were generated with molecular docking on H_3R model and X-ray structure of SERT. In the second part of this study, ligand-based and structure-based virtual screening models were generated and validated. Prospective screening of ZINC database was performed in order to extract novel chemotypes of dual ligands. Final selection of ligands was performed based on generated pharmacophore and docking models as well as predicted pharmacokinetic properties. Few novel compounds were emphasized as promising starting point for development of new classes of dual antidepressants.

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Structure and ligand based drug design strategies in the development of novel serotonin 5-HTt$_{2a}$ receptor antagonists

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The serotonin 5-HT_{2A} receptors are widely distributed throughout the central and the peripheral nervous system where they play a key role in many physiological functions. Abnormal activity of 5-HT_{2A} receptors is associated with various neurological disorders, such as depression, schizophrenia, anxiety, and Parkinson disease. In order to analyze 3D-structure of the

pharmacophore as well as binding kinetics of 5-HT_{2A}-R antagonists, we have combined ligand and structure based approaches. Three-dimensional quantitative structure-activity relationship (3D-QSAR) study in combination with molecular docking and molecular dynamic (MD) simulation was used to identify key substituents responsible for high binding affinity and selectivity of 5-HT_{2A} antagonists. The study was performed on wide range of structurally diverse antagonists that were divided into three different clusters: clozapine, ziprasidone, and CHEMBL240876 derivates. We have obtained three different inactive, antagonist-bound, conformations of this receptor by using the 50ns long MD simulations with each cluster representative. Subsequently, these conformations were used as templates for docking studies in order to find virtually bioactive conformations of ligands. Selected virtually bioactive conformations were used for calculation of specific molecular descriptors (Grid Independent Descriptors- GRIND) and 3D-QSAR model building. The 3D-QSAR approach was used to select the most influential variables which were used for clarifying the structural features required for 5-HT_{2A} antagonists. The reliability and predictive power of the model was assessed using an external test set compounds and showed reasonable external predictability. The study provides valuable information about the key structural features that are required in the rational drug design of novel 5-HT_{2A} antagonists.

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Treatment of pediatric multiple sclerosis

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Multiple sclerosis is a chronic, autoimmune, inflammatory, demyelinating disease of the central nervous system. The exact etiology of MS is still not clarified, although it is known that autoimmune, genetic and environmental factors play an important role in the development of MS, making it multifactorial disease. The disease most often begins between second and fourth decade of life, but it can also begin in the childhood. Multifocal inflammatory changes and damage to myelin are the key pathological features of MS. Clinical events that characterize MS are relapse and progression. Therapy of pediatric MS involves treatment of relapses, immunomodulatory therapy and symptomatic treatment. The disease-modifying drugs (DMDs), which are base for MS therapy, are able to modify the course of the disease i.e. immunomodulatory drugs. They are divided it to two categories: First - line and second – line immunomodulatory therapy. First line immunomodulatory therapy (Interferon beta-1a, Interferon beta-1b and Glatiramer acetate) have shown significant therapeutic effectiveness by reducing the frequency of clinical relapses and further progression of the disease. The disadvantage is that they are only allowed in children older than 12 years. Second-line immunomodulatory therapy (Natalizumab, Mitoxantrone, Fingolimod, Teriflunomide, Azathioprine, Cyclophosphamide, Rituximab, Dimethyl fumarate) are allowed in pediatric population only as a part of ongoing clinical studies. Although those medications reduce the number of relapses by 68% (First-line IMT only by 30%), they have many serious side-effects. Treatment of relapses/exacerbations also involves the use of intravenous doses of corticosteroids which are still gold standard for relapsing- remitting MS.

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Quantification of antidepressants and antipsychotics exposure increase in CYP 2C19/CYP 2D6 slow metabolizers: Systematic review and meta-analysis

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In a recent period, development of new antidepressants and antipsychotics entered a quiescent phase, so clinicians can rely only on handful of effective drugs in the treatment of psychiatric disorders. At very best, half of the psychiatric patients respond to the first administered psychotropic drug and treatment-resistant patients are also numerous. Therefore, it is of paramount importance to utilize current therapeutic options as effectively as possible. Administering appropriate dose is very important since underdosed patients will not respond to treatment, while overdose can cause serious adverse drug reactions. In order to determine the most appropriate dose, the clinicians need an accurate estimate of drug metabolism and exposure. Most of the currently used antidepressants and antipsychotics are metabolized via polymorphic CYP 2C19 and CYP 2D6 enzymes. Metabolic capacity of both enzymes is genotype-determined. Poor metabolizer status (PM) defined as complete absence of enzymatic activity and intermediate metabolizer status (IM) defined as greatly reduced enzymatic activity, can both cause a significant increase in drug exposure. When making genotype-governed dose adjustments, clinicians need to know the magnitude of exposure increase in PM and IM. However, due to small sample size of the previous studies, the exposure increase magnitude still cannot be estimated with sufficient precision. Therefore, the aim of this meta-analysis is to pool all these studies and estimate to the best possible degree the magnitude of drug exposure increase caused by the CYP 2C19 and CYP 2D6 PM and IM status compared with normal metabolizers (NM).