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An evaluation of neonicotinoids' potential to inhibit human cholinesterases: Protein–ligand docking and interaction profiling studies

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ABSTRACT

Many so-called neuroactive insecticides target invertebrate neurotransmitter systems, including the cholinergic system. With their relatively low toxicity to vertebrates, neonicotinoids represent a new class of neuroactive insecticides that bind to nicotinic receptors for acetylcholine in the insect central nervous system and result in paralysis and eventual death due to receptor overstimulation. On the understanding that, today, cholinesterase inhibitors are used to obtain the symptomatic relief of Alzheimer disease (AD), the aforementioned direct cholinomimetic action of neonicotinoids could, perhaps, confer anti-AD drug-like attributes to these compounds. It is shown here, using protein–ligand docking and interaction profiling, that neonicotinoids penetrate deep into the active-site gorge of both acetylcholinesterase and butyrylcholinesterase and that they form relatively strong noncovalent bonds with multiple critical residues that normally bind/hydrolyze choline esters. With their gorge-spanning shape and dual-binding specificity, neonicotinoids (first-generation compounds in particular) represent promising leads for the development of reversible, mixed-type cholinesterase inhibitors in the fight against AD.

1. Introduction

A large number of so-called neuroactive insecticides target neurotransmission, particularly GABAergic and cholinergic processes [1]. Cholinergic neurotransmission is a key player in a myriad of pysiological phenomena such as voluntary/involuntary muscle contraction and glandular secretion (in the periphery), as well as cognition and behavior (in the central nervous system) [2]. It is mediated by the binding of the neurotransmitter acetylcholine (ACh) to nicotinic/muscarinic ACh receptors (AChRs). While the synthesis of ACh is catalyzed by the enzyme choline acetyltransferase (ChAT), the hydrolysis of ACh after its dissociation from AChRs is brought about by the enzyme acetylcholinesterase (AChE). Another enzyme which is loosely related to the mammalian cholinergic system is butyrylcholinesterase (BChE). It protects the neurotransmitter function of its sister enzyme AChE by neutralizing the chemical reactivity of neurotoxins and serves as a backup to AChE by hydrolyzing ACh that has diffused out of the synaptic cleft [3].

Organophosphates and *N*-methylcarbamates target cholinesterases through respectively phosphorylating or carbamylating the serine residue from the catalytic (esteratic) subsite [4]. This causes ACh to accummulate, thereby leading to immoderate excitation of AChRs. On the other hand, neonicotinoids target nicotinic AChRs through exerting ACh agonistic effects [5]. To date, seven different neonicotinoid insecticides have been made available to the global market [6]. These are acetamiprid (ACE; developed by Nippon Soda), clothianidin (CLO; Bayer CropScience and Sumitomo Chemical), dinotefuran (DIN; Mitsui Chemicals), imidacloprid (IMI; Bayer CropScience), nitenpyram (NIT; Sumitomo Chemical), thiacloprid (THI; Bayer CropScience), and thiamethoxam (TMX; Syngenta) (Fig. 1). The common structure of neonicotinoids consists of three parts, namely a pharmacophore (which bears an electron-withdrawing functional group designated =X–Y), a bridging chain, and a heterocyclic moiety. Regarding their pharmacophores, neonicotinoids fall into three categories: *N*-cyanoamidines (=N–CN; ACE and THI), *N*-nitroguanidines (=N–NO_2; CLO, DIN, IMI, and TMX), and nitromethylenes (=CH–NO_2; NIT) [7]. The coplanarity between the amidine/guanidine moiety and the cyano/nitro substituent yields an electronegative tip on the pharmacophore, which allows for hydrogen bonding and π–π stacking with

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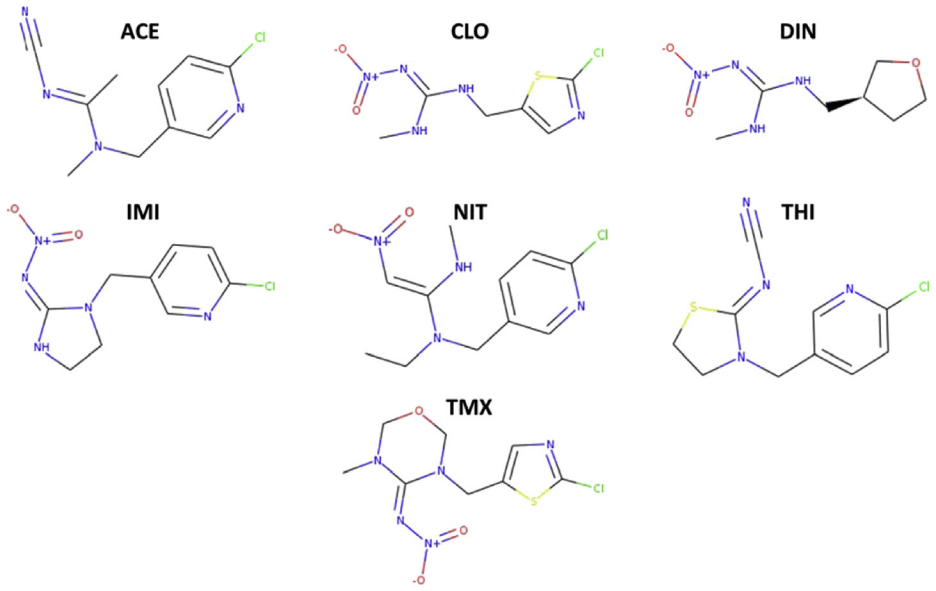

Fig. 1. Molecular structures of the seven commercially available neonicotinoids. Each neonicotinoid is made up of three essential parts: a pharmacophore, a bridging chain, and a heterocyclic moiety.

the receptor subsites [8]. Although 90% of the parent compound nicotine ($pK_a = 7.9$) is protonated at physiological pH in mammals, neonicotinotids remain unprotonated at this pH [9]. In ACE, IMI, NIT and THI (all first-generation neonicotinoids), the essential heterocyclic structural element is a 6-chloro-3-pyridylmethyl group. In CLO and TMX (both second-generation neonicotinoids), this group is replaced by a 2-chloro-5-thiazolyl group. DIN (a third-generation neonicotinoid) lacks the aromatic chloropyridylmethyl group altogether and possesses an aliphatic oxolane (tetrahydrofuran) ring instead. The heterocyclic moiety is believed to enhance the binding of a neonicotinoid to its target receptor [10]. The relatively low toxicity of neonicotinoids in mammals is attributed to different binding specificity and affinity for mammalian nicotinic AChRs, rapid biotransformation in the mammalian system, and poor penetration of the mammalian blood–brain barrier [11-13]. Although there exist several reports investigating the effects of acute or chronic neonicotinoid exposure on human health, more studies are warranted to better understand the impact of neonicotinoids on human health, especially due to chronic exposure (reviewed by Ref. [14]).

Alzheimer disease (AD) is a neurodegenerative disorder pathologically characterized by the loss of neurons, formation of neurofibrillary tangles inside neurons and deposition of amyloid-β (Aβ) proteins (aka senile plaques) between neurons — all leading to the progressive loss of memory and other cognitive functions [15]. Today, symptoms of mild-to-moderate AD are mostly treated by cholinesterase inhibitors (ChEIs) that delay the breakdown of ACh at the synapse; however, more recent research suggests that nicotine metabolites/analogs too may offer symptomatic relief (reviewed by Ref. [16]). The relative persistence of ACh in the synaptic cleft eventually improves cholinergic neurotransmission and thus motor function and cognition. Given that neonicotinoids mimic the action of ACh which normally binds to nicotinic AChRs, it is tempting to speculate that these insecticides may also guide the structure-based design of more potent and selective ChEIs that hold promise as anti-AD drugs.

Here, evidence is provided from protein–ligand docking and interaction profiling studies suggesting that neonicotinoids are well anchored in the active-site gorge of both human acetylcholinesterase and human butyrylcholinesterase mainly through hydrogen-bonding and π–π stacking interactions between their pharmacophores/heterocycles and several key residues from the substrate-binding and/or substrate-transforming subsites of cholinesterases. This implies a mixed type of cholinesterase inhibition by commercial neonicotinoid products. To my knowledge, the present computational study is the first, and most comprehensive, one in the relevant scientific literature to focus on the inhibitory activity of neonicotinoids against human cholinesterases.

2. Materials and methods

2.1. Target/ligand selection and preparation

The crystal structure of recombinant human AChE in complex with huprine W and the black mamba toxin fasciculin-2 (PDB ID: 4BDT; resolution: 3.1 Å; R-value, free: 0.219; R-value, work: 0.159) [17] was downloaded from the RCSB Protein Data Bank [18] and provided as input to the Dock Prep tool of UCSF Chimera, Version 1.11.2 [19] for preparation purposes. AChE was prepared by: (i) the removal of fasciculin-2, non-protein parts, and solvent (water) molecules; (ii) the addition of hydrogen atoms in a process where the hydrogen-bonding network was optimized and the determination of the protonation states of residues at physiological pH; and (iii) the assignment of correct formal charges to individual atoms in standard residues based on the AMBER ff14SB force field. The crystal structure of recombinant human BChE in complex with tacrine (PDB ID: 4BDS; resolution: 2.1 Å; R-value, free: 0.209; R-value, work: 0.175) [17] was also downloaded from the RCSB Protein Data Bank [18] and provided as input to the Dock Prep tool of UCSF Chimera, Version 1.11.2 [19] for preparation purposes. During BChE preparation, non-protein parts and water molecules were removed, and hydrogens, protonation-state information and correct formal charges were added in the same manner as AChE preparation. The ready-to-dock, three-dimensional structures of ACE (ZINC ID: 13827890), CLO (ZINC ID: 13827936), DIN (ZINC ID: 13827799), IMI (ZINC ID: 4474604), NIT (ZINC ID: 2381598), THI (ZINC ID: 13828082) and TMX (ZINC ID: 31361189) were downloaded from the ZINC Database [20].