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Contemporary medicinal-chemistry strategies for the discovery of selective butyrylcholinesterase inhibitors

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Butyrylcholinesterase (BChE) is considered a promising drug target for the treatment of moderate to severe Alzheimer's disease (AD). Here, we review medicinal-chemistry strategies that are currently available for the discovery of selective BChE inhibitors.

Introduction

AD is a complex neurodegenerative disorder and the most common form of dementia. However, the pharmacotherapy of AD is currently limited. Of the four approved drugs for treatment of AD, memantine is an *N*-methyl-D-aspartate receptor antagonist, whereas the other three are all cholinesterase (ChE) inhibitors: the selective, reversible acetylcholinesterase (AChE) inhibitors donepezil (**1**) and galantamine (**2**), and the pseudo-irreversible dual AChE/butyrylcholinesterase (BChE) inhibitor rivastigmine (**3**) (Fig. 1a). However, inhibition of AChE in the parasympathetic autonomic nervous system and the peripheral nervous system can cause adverse effects (e.g., vomiting, diarrhea, nausea, and tremors), and limits the doses of these drugs that can be tolerated. In addition, these drugs are only effective during early stages of AD; furthermore, they only ameliorate the symptoms, rather than being curative [1,2].

The 'cholinergic hypothesis' is the oldest theory regarding AD pathophysiology [3]. Cholinergic neurotransmission in the brain is halted by hydrolysis of the neurotransmitter acetylcholine (ACh) to result in acetate and choline, which are catalyzed by both AChE and BChE. In the brain of healthy adults, AChE accounts for 80% of ChE activity, with BChE accounting for the remainder [4].

The amount of AChE in the brain decreases with the progression of AD. However, the enzymatic activity of BChE remains unchanged or even increases, and can compensate for the loss of AChE [4]. Furthermore, overexpression of BChE is involved in the transformation of nonfibrillar to fibrillary amyloid β-protein (Aβ) plaques and senile plaques. Therefore, inhibiting BChE activity could prove beneficial as a curative approach to AD [5,6], and BChE is considered a potential therapeutic target in advanced AD [7].

Nevertheless, selective targeting of BChE over AChE is challenging, because the human isoforms hAChE and hBChE share 65% amino acid sequence homology. Their overall structures are similar, and their catalytic sites, comprising a catalytic triad (the catalytic active site, CAS) and a choline-binding pocket, are both buried at the bottom of a deep gorge (~20 Å) (Fig. 1b) [8,9].

Although the CAS of both enzymes is conserved, the two enzymes show differences in the space that they provide for a substrate or inhibitor. These differences result from differences in the amino-acid residues forming the gorge and the acyl-binding pocket, which accommodates the acyl group of the substrate during catalysis. The most significant difference concerns the acyl-binding pocket, in which Phe288 and Phe290 of AChE are replaced by Leu286 and Val288 in BChE, respectively, allowing BChE to accommodate bulkier substrates (Fig. 1b).

As increasing information about the structural biology of ChE–inhibitor complexes becomes available, structure-based approaches using diverse medicinal-chemistry strategies are being adopted in the search for selective BChE inhibitors [10]. In this review, we present an overview of the strategies used for lead discovery and optimization of BChE inhibitors during the past

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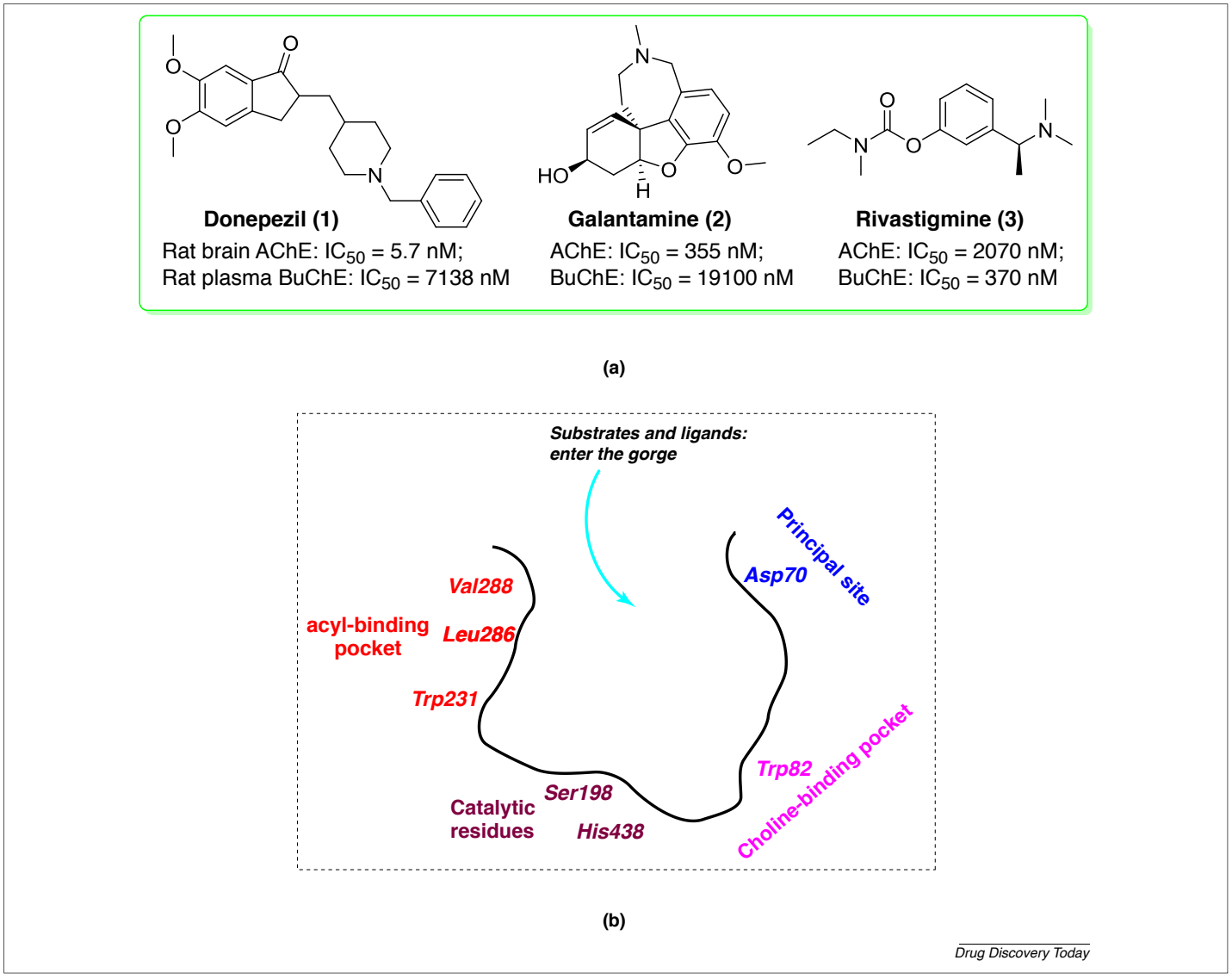

FIGURE 1

The representative ChE inhibitors and their binding mode. (a) Structures and ChE-inhibitory potencies of donepezil, galantamine, and rivastigmine. (b) Schematic representation of the active-site gorge (including key amino acid residues) of human butyrylcholinesterase (BChE), including the peripheral anionic site (Asp70), the choline-binding pocket (Trp82), and the acyl-binding pocket (Trp231, Leu286, and Val288), as well as the catalytic serine and histidine residues (Ser198 and His438).

decade. We also discuss what approaches might be most effective in the future.

Medicinal-chemistry strategies used in the discovery of BChE inhibitors

Here, with the aid of case-studies, we describe medicinal-chemistry strategies that have been used to seek BChE inhibitors, including virtual screening and structure-based optimization, mechanism-based drug design, refining of existing BChE inhibitors via the design of heterobivalent ligands and homobivalent dimers, molecular hybridization, systematic structure–activity relationship (SAR) studies via a substituent-decorating approach, and multi-target-directed ligands.

Virtual screening and structure-based optimization

Using a hierarchical virtual-screening method, the hit compound **4** was identified as a potent, selective, slowly reversible, tight-binding inhibitor of hBChE (Fig. 2) [11]. Subsequent modification (bioisosteric replacement) of **4** generated the sulfonamide-based *in vivo*-active noncovalent BChE inhibitor **5**. The crystal structures of the hBChE complexes with **4** [Protein Data Bank (PDB) code: 4TPK] and **5** (PDB code: 5DYW) revealed that the naphthalene moiety and the 2,3-dihydro-1H-indene (or benzyl) moiety of these inhibitors kept close to the acyl-binding and choline-binding pockets, respectively [12]. Even so, the crystal structures suggested several opportunities for structural optimization of these compounds to enhance the hBChE-inhibitory activity. In particular, the catalytic residues (in purple in Fig. 2) and Trp82 (in orange in Fig. 2) might form hydrogen bonds and/or cation–π interactions.

With these considerations in mind, a focused library of amide derivatives of **4** was designed, synthesized, and bioassayed. Among them, compound **6** showed selective, reversible hBChE inhibition in the picomolar concentration range. The crystal structure of its complex with hBChE (PDB code: 5NN0) revealed that two strong

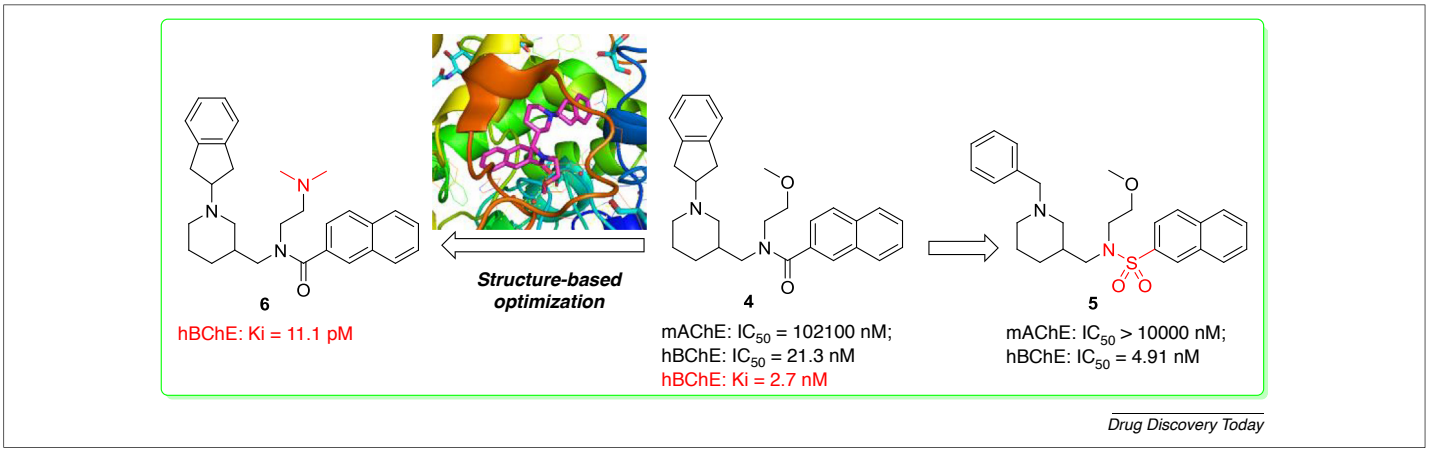

FIGURE 2

Structure-based optimization of hit compound **4**. The modifications introduced during each design step are indicated in red. The figure showing the binding mode was generated using PyMol (www.pymol.org).

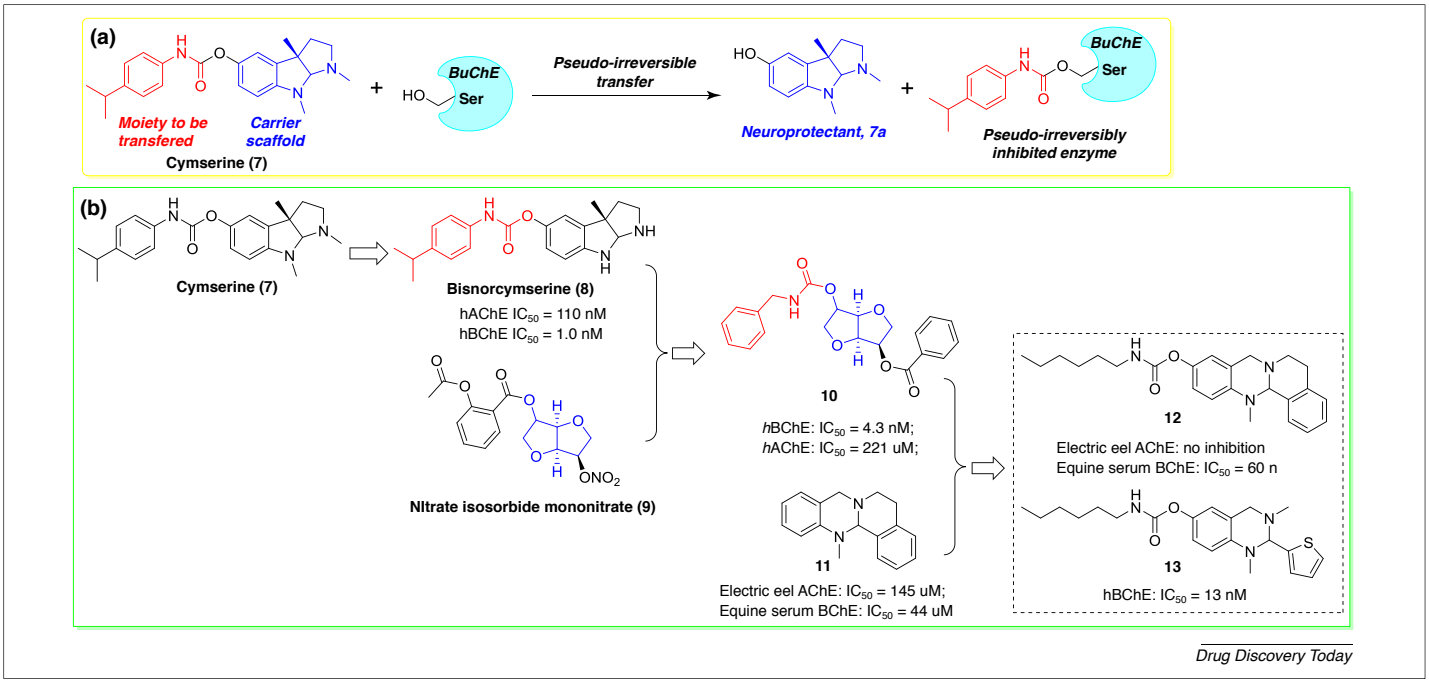

FIGURE 3

Discovery of carbamate-based ChE inhibitors via mechanism-based drug design. (a) Mode of inhibition of butyrylcholinesterase (BChE) by the hexahydropyrrolo [2,3-b]indol-5-ol-based carbamate **7**. The inhibitor transfers the carbamate group to the active center with release of the phenolic hexahydropyrrolo[2,3-b]indol-5-ol scaffold, which itself acts as a neuroprotectant. (b) Development of tetrahydroquinazoline-based carbamate derivatives **10**, **12**, and **13**.

cation–π interactions are the basis of its significantly improved inhibitory potency. Administration of compound **6** in a cholinergic-deficit AD mouse model resulted in improvements of memory, learning ability, and cognitive functions without any acute cholinergic adverse effects [13].

Mechanism-based drug design: carbamate inhibitors of cholinesterases

Selective inhibition of BChE can be achieved by targeting the catalytic active site with carbamate-based pseudo-irreversible inhibitors, as exemplified by cymserine (**7**) and its analog bisnorcymserine (**8**, an *in vivo*-active BChE inhibitor that has entered clinical trials). In general, these inhibitors feature a carrier scaffold guiding a carbamate group into the correct site in ChE, followed by transfer of the carbamate group to the catalytic serine in the active center of ChE with release of the carrier scaffold (Fig. 3a). However, concerns remain over potential off-target reactivity-based or non-specific toxicities [14].

In most cases, the development of pseudo-irreversible ChE inhibitors has been limited to modification of the transferable carbamate group, because, up to now, little information about the binding mode of the carrier scaffold itself has been available. However, in 2008, based on the clinically used nitrate isosorbide mononitrate (**9**), isosorbide-2-carbamate esters were reported as

potent and selective BChE inhibitors with a time-dependent, competitive, and reversible mode of action. The most potent and selective molecule was isosorbide-2-benzyl carbamate-5-benzoate **10** with an IC_{50} of 4.3 nM for BChE and >50 000 fold selectivity over human erythrocyte AChE (Fig. 3b) [15].

In 2016, using tetrahydroquinazoline-derived AChE and BChE inhibitors as templates, a series of bicyclic derivatives were reported as pseudo-irreversible BChE inhibitors with high selectivity over hAChE (exemplified by **12**); these were designed to explore the binding mode of the carrier scaffold at the active site of BChE by chemical optimization of the scaffold [16]. 2-Thiophenyl compound **13** proved to be an inhibitor of eqBChE (KC = 14.3 nM) and hBChE (KC = 19.7 nM) (Fig. 3b) [16].

Greater knowledge of the interactions between BChE and the carrier scaffold will facilitate the rational design of carbamate-based BChE inhibitors, likely enabling the discovery of other types of pseudo-irreversible inhibitor.

Further refining of existing BChE inhibitors: heterobivalent ligands and homobivalent dimers

On the basis of the investigations of the active-site gorge topology of BChE and AChE, tacrine (in red in Fig. 4) heterobivalent ligands (**14**, **15**), homobivalent dimers of tricyclic quinazolinimines (**16**–**18**) and homobivalent (-)-nor-meptazinols (**19a**–**c**) were identified as inhibitors with well-balanced potency and selectivity (Fig. 4a) [17–21].

Further refining of existing BChE inhibitors: molecular hybridization

Molecular hybridization is a useful strategy for drug discovery by combining pharmacophoric fragments of different bioactive compounds to provide new hybrids with improved affinity and potency, compared with the parent molecules [22]. Based on the structures of NP61 (**20**) and donepezil (**1**), a set of indolylpiperidines hybrids **21**–**25** were discovered to be potent and selective hBChE inhibitors (Fig. 4b) [23].

Further refining of existing BChE inhibitors: analog-based drug discovery

Within a particular class of BChE inhibitors, small structural changes can improve the potency and selectivity [12,13]. Consequently, the use of analog-based drug discovery, involving systematic SAR studies via decorating existing BChE inhibitors with peripheral substituents while maintaining the basic scaffold, continues to be one of the most fruitful strategies affording more potent and selective BChE inhibitors, as exemplified by tetrahydrocarbazole benzyl pyridine **26** and tricyclic pyrazolo[1,5-d][1,4] benzoxazepin-5(6H)-one **27** (Fig. 4c) [24,25].

Multitarget-directed ligands

Although increasing evidence supports the idea that BChE might be a better target than the well-known AChE for treating advanced AD, hitting a single pathological target is unlikely to be curative. Thus, based on the concept of multitarget-directed ligands [26], multifunctional BChE inhibitors might offer an attractive therapeutic strategy. Although the identification of multifunctional anti-AD agents has long been the result of serendipity, medicinal chemists are now attempting to design polypharmacology.

In recent years, the grafting onto BChE inhibitors of pharmacophores that chelate metal ions and/or inhibit monoamine oxidase (MAO), inhibit fatty acid amide hydrolase (FAAH), or act on several receptor systems, has been explored for the design of multifunctional BChE inhibitors [27]. These multifunctional ligands comprise two drug entities either as a hybrid linked by a spacer or merged into a single chemical entity. Recently, various new multifunctional compounds have been obtained by merging other well-known molecular scaffolds with anti-AD properties (metal chelators **28**–**30**, MAO inhibitor **31**), as exemplified by hBChE inhibitors **32** and **33**, which display antioxidant and neuroprotective activities [28,29], and dual hBChE and MAO-B inhibitors **34**–**36** (Fig. 4d) [30]. The crystal structure of two of these inhibitors (compounds **32** and **34**) complexed with hBChE revealed their binding mode (PDB codes: 4XII, 5LKR).

This pharmacophore-merging approach yielded the benzimidazole derivative **37** as an *in vivo*-effective, balanced dual-acting inhibitor targeting BChE and the human cannabinoid receptor 2 [31]. Furthermore, the carbamate-bearing compound **38** was identified as a potent dual fatty acid amide hydrolase (FAAH)/BChE inhibitor, with well-balanced nanomolar-level activities by means of a networked targets approach (Fig. 4d) [32].

For most of the academic multifunctional BChE inhibitors, detailed absorption, distribution, metabolism, and excretion and toxic (ADMET) data are not yet available, which makes it difficult to predict whether these agents will be effective clinical candidates for improving memory and cognitive deficits in patients with progressive AD.

Concluding remarks and prospects

BChE is considered to be potentially a better target than the well-known AChE for the treatment of later-stage cognitive decline in AD. Thus, the discovery of *in vivo*-active, selective, reversible, and pseudo-irreversible BChE inhibitors is desirable to provide not only drug candidates, but also chemical probes for studying the potential of BChE as a therapeutic target. Here, we reviewed the medicinal-chemistry strategies currently available for the discovery of selective BChE inhibitors. As increasing structural biology information becomes available, we envision that the development of novel and selective BChE inhibitors will move on from trial-and-error approaches to increasingly sophisticated strategies. Based on the solved crystal structures of AChE (or BChE)–ligand complexes, (un)favorable interactions can now be analyzed by means of molecular simulation. It will be possible to use BChE-biased interactions or critical residues to design molecular scaffolds with suitable 3D shapes to afford improved binding affinity and selectivity for BChE [33].

In target-based drug discovery, the insightful application of critical molecular design elements is an effective strategy to improve success rates. In particular, the cation–π interaction has emerged as a high-impact design element in the optimization of specific noncovalent BChE–inhibitor interactions. Undoubtedly, further exploitation of nonbonding cation–π interactions will provide significant opportunities to take full advantage of the chemical space of the binding pocket in the rational design of next-generation BChE inhibitors [34,35].

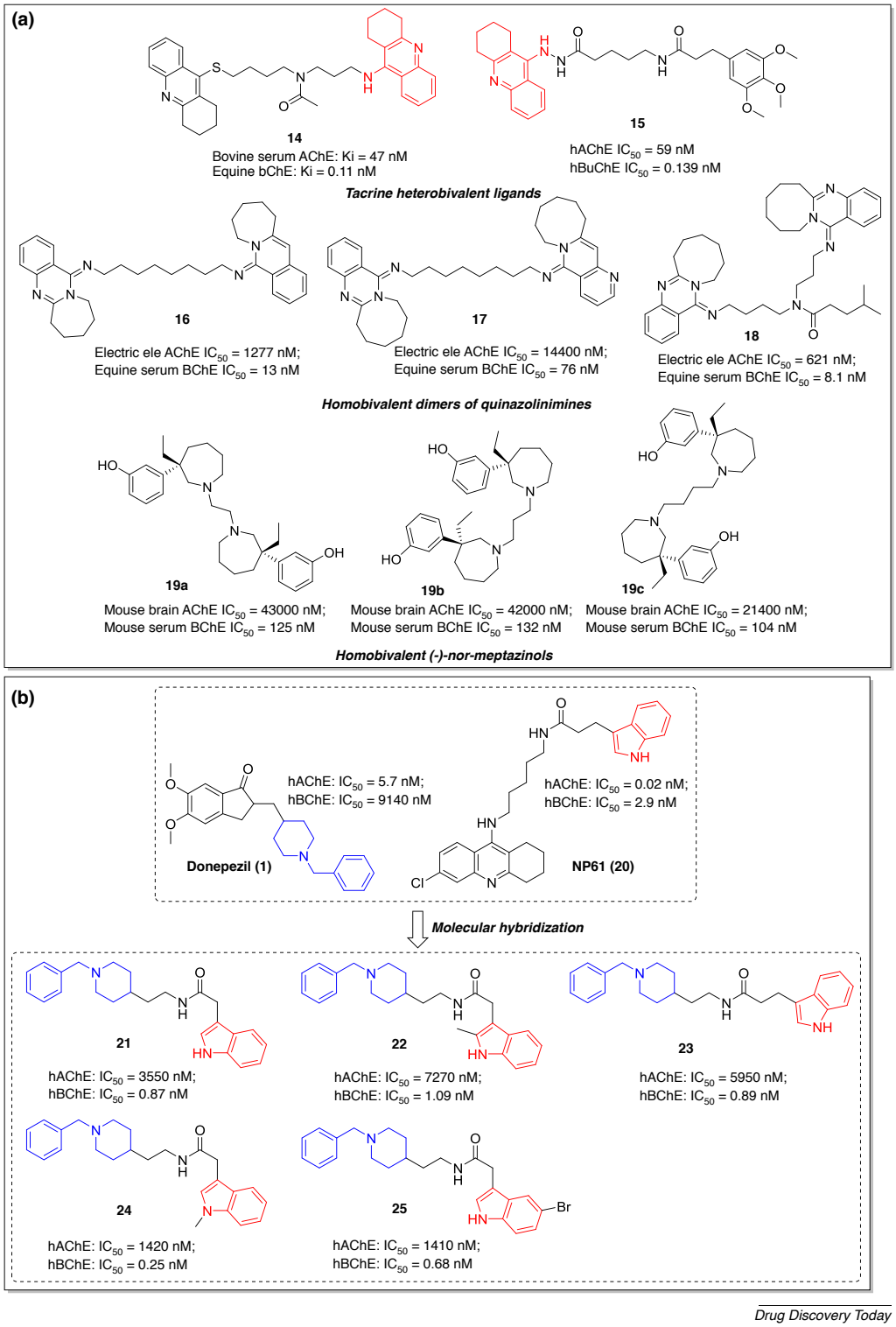

FIGURE 4

Other medicinal-chemistry strategies for the discovery of selective butyrylcholinesterase (BChe) inhibitors: (a) Design of heterobivalent ligands and homobivalent dimers as selective BChE inhibitors; (b) hybrid indolylpiperidine molecules **21**–**25**, obtained by hybridization of NP61 (**20**) and donepezil; (c) novel BChE inhibitors obtained by refining existing compounds; (d) multitarget-directed ligands as BChE inhibitors.

In addition, new medicinal chemistry concepts and techniques, such as crystallographic overlay-based pharmacophore hybridization, dynamic combinatorial chemistry [36], privileged substructure-based diversity-oriented synthesis (pDOS) [37], click chemistry-based combinatorial library assembly [38], and DNA-encoded libraries [39], can also be utilized for the discovery of novel and selective BChE inhibitors.

Finally, drug discovery is a multifactorial optimization campaign, but avoidance of toxicity in particular is a key issue at the early stage of drug discovery [40,41]. Thus, it might be desirable to

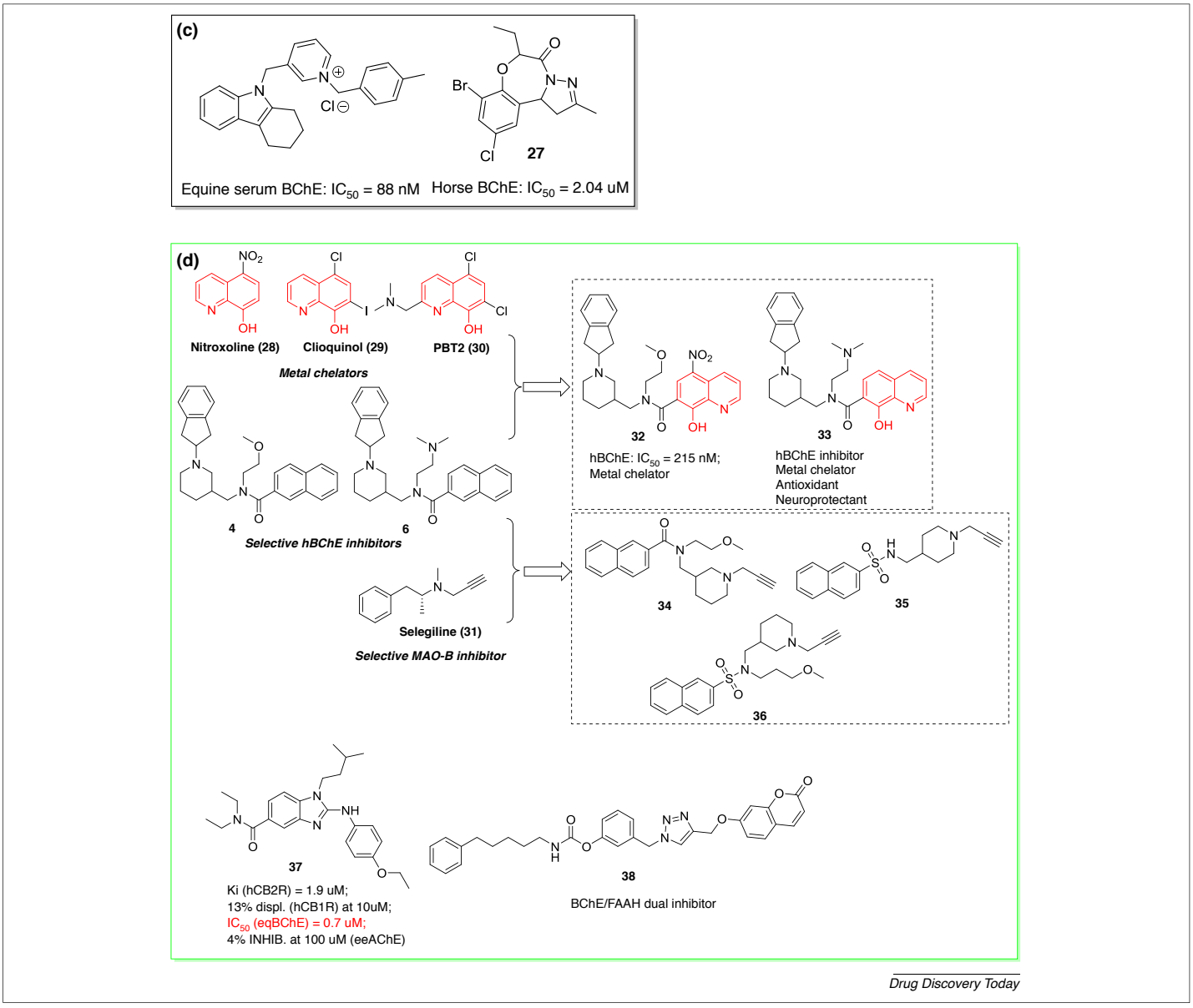

Fig. 0020. (*Continued*).

pay more attention to early ADMET assessment (especially, the drug-likeness central nervous system multiparameter optimization) in the structure optimization of BChE inhibitors [42].

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