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Review Article

Dual inhibition: a novel promising pharmacological approach for different disease conditions

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Abstract

To overcome the problems associated with polypharmacy, which include medication non compliance, adverse drug reactions, drug–drug interactions and increased pill-burden, various strategies, such as sustained-release drugs and fixed-dose combination regimens (polypills), have been developed. Out of these, a novel and very much promising approach is the use of dual-action drugs. Amongst the dual-action drugs, there is a class of compounds known as dual inhibitors, which possess the dual inhibitory activity. The most common examples of dual inhibitors are rivastigmine, ladostigil, asenapine, phenserine, amitriptyline, clomipramine, doxepin and desipramine. This review article focuses on the conventional drugs used in different diseases which possess dual inhibition activity as well as those which are still in the preclinical/clinical phase.

Keywords dual cholinesterase inhibitors; dual inhibitors; dual noradrenergic and specific serotonergic antidepressants; dual serotonin–norepinephrine reuptake inhibitors

Abbreviations AD-Alzheimer's disease, ACh-Acetylcholine, AChE-Acetylcholinesterase, AChE-I-Acetylcholinesterase-inhibitor, APP-Amyloid precursor protein, Abeta-β amyloid, ACP-Acyl carrier protein, ACE-Angiotensin-converting enzyme, ADP-Adenosine diphosphate, AML-Acute myeloid leukemia, BuChE-Butyrylcholinesterase, BuChE-I-Butyrylcholinesterase-inhibitor, β-Secretase-BACE, ChE-I-Cholinesterase-inhibitor, CD-Circular dichroism, cAMP-Cyclic adenosine monophosphate, cGMP-Cyclic guanosine monophosphate, COX-Cyclooxygenase, CCB-Calcium channel blocker, CDK-Cyclin-dependent kinase, CypA- Human cyclophilin A, DNA-Deoxyribonucleic acid, ETs-Endothelins, ECE-Endothelin-converting enzyme, EGFR-Epidermal growth factor receptor, FDA-Food and Drug Administration, FLT3-Fms-like tyrosine kinase 3, GP-Glycoprotein, GABA-Gamma-Aminobutyric acid, HuAChE-Human recombinant acetylcholinesterase, HIV-1-Human immunodeficiency virus type 1, HRV-Human rhinovirus, CA-HIV-1 capsid, HERG-Human ether-a-go-go-related gene, HupB-Huperzine B, IC50-Inhibition concentration 50, IKK2-IkappaB kinase-2, LOX-Lipoxygenase, MAOI-Monoamine oxidase inhibitor, mTOR-Mammalian target of rapamycin, NMDA-N-Methyl-D-aspartate inhibitor, NaSSA-Dual noradrenergic and specific serotonergic antidepressant, NSAIDs-Non steroidal anti-inflammatory drugs, NA-Noradrenaline, NEP-Neutral endopeptidase, PD-Parkinson's disease, PDE10A-Phosphodiesterase 10A, PAH-Pulmonary artery hypertension, PI3K/AKT-Phosphatidylinositol-3-kinase/Akt, SERT-Serotonin transporter, SSRI-Selective serotonin reuptake inhibitor, SNRI-Serotonin-norepinephrine reuptake inhibitor, 5-HT-Serotonin, SRI-Serotonin reuptake inhibitor, THC-Delta9-tetrahydrocannabinol, TCA-Tricyclic antidepressant, TXA_2-Thromboxane A_2, VPI-Vasopeptidase inhibitor, V-Vasopressin receptor, VLDL-Very low density lipoprotein.

Introduction

Polypharmacy, a widespread treatment regimen used especially for geriatric and psychiatric patients, is a major risk factor for medication non-compliance. Besides this, it can cause adverse drug reactions and drug–drug interactions, and suffers from pill burden and high cost.[1,2] Other appealing advances made in the field of drug delivery to ensure consistent drug dosing are sustained-release drugs and fixed-dose combination regimens (polypills). However, along with many apparent advantages, these possess certain

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disadvantages as compared to single-drug therapy. To ameliorate these problems a promising approach is the use of dual-action drugs.

A dual-action drug is a compound that combines two different desired pharmacological actions at a similar efficacious dose. In other words, a single compound possesses dual mechanistic action due to targeting of different effector mechanisms. Hence the use of such drugs leads to efficacious outcomes due to reduced pill burden, improved medication compliance and decreased adverse effects or drug–drug interactions. A single molecule with dual activity is considered better than combination therapy from developmental and clinical perspectives, as this approach can be assessed by simple toxicology studies and avoids the pharmacokinetic disadvantages arising from the combination of two separate agents with differing absorption and distribution properties.

Amongst dual-action drugs, a class of compounds known as dual inhibitors (a single molecule possessing dual inhibitory activity) is experiencing a surge in interest in both scientific and clinical fields. Dual inhibitors possess two different biological activities, which may be due to inhibition of two different enzymes, neurotransmitters or effector mechanisms. Some examples of dual inhibitors are rivastigmine, ladostigil, asenapine, pheneserine, amitriptyline, clomipramine, doxepin and desipramine.

Due to their diverse nature, dual inhibitors are finding roles in the treatment of many diseases. The aim of this review is to highlight the applications of this novel pharmacological approach in different diseases. Since there is as yet no recognized classification of dual inhibitors in the literature, an attempt has been made to rectify this situation in this article.

Role of Dual Inhibitors in Different Diseases

Neural diseases: Alzheimer's disease

Dual cholinesterase inhibitors

Many of the dual cholinesterase inhibitors (ChE-Is) used for treating patients with Alzheimer's disease (AD) selectively inhibit acetylcholinesterase (AChE). Selective butyrylcholinesterase-inhibitors (BuChE-Is) are also considered attractive options since they raise acetylcholine (ACh) in the brain, augment long-term potentiation, improve cognitive performance in rodents and are devoid of the classic adverse actions of acetylcholinesterase-inhibitors (AChE-Is) and ChE-Is.[3] However, the emergence of a new hypothesis regarding the more sustained efficacy provided by dual inhibitors of AChE and BuChE has shifted the focus towards the latter class of compounds.[4] Quantification of density changes in brain grey matter has provided empirical evidence for a neuroprotective potential of dual cholinesterase inhibition in AD patients. Patients on dual ChE-Is showed none of the cortical atrophic changes in parietotemporal regions that were invariably reported in untreated AD patients and those treated with selective AChE-Is.[5]

Rivastigmine, a Food and Drug Administration (FDA) approved AChE-I, is reported to exhibit dual inhibitor activity (Figure 1a). It inhibits AChE as well as BuChE enzyme taking care of other neurotransmitters. It has demonstrated beneficial effects on AD severity as well as its cognitive, functional and behavioral domains.[6] An observational study has concluded that AD patients deteriorating on selective AChE-I treatment can benefit from switching to a dual AChE–BuChE-I regime, such as rivastigmine – effects include stabilization of disease, improvement in cognitive function and reduction in the burden of concomitant psychoactive treatment.[7]

Tacrine is a dual AChE–BuChE-I, and various huprine–tacrine heterodimers have been developed by linking huprine Y with tacrine through a linker containing hetero atoms, which provide simultaneous interaction with both binding sites (Figure 1b and 1c). Huprine Y possesses one of the highest affinities for the active site of AChE, while tacrine has affinity for the peripheral site of the enzyme. These compounds have an IC50 in the sub-nanomolar range for human AChE and in the low nanomolar range for human BuChE.[8]

Other such emerging dual inhibitors are listed in Table 1. Studies have found that 10-methylacridinium iodide (methylacridinium; MA) inhibits AChE as well as BuChE. In addition, its ability to cross the blood–brain barrier makes it an attractive option for use in the treatment of neural disorders.[9] A novel class of isaindigotone derivatives has also shown AChE and BuChE inhibitory activity.[10] Investigations have shown that xyloketal A-D – metabolites of mangrove fungus *Xylaria sp*. from the coast of the South China Sea – can inhibit AChE as well as BuChE *in vitro*. Hence these compounds are also considered potential AD drug candidates.[11]

Dual AChE and monoamine oxidase inhibitors

Recent research has revealed the complex nature of AD and suggested the involvement of multiple neurotransmitter systems, including serotonin, glutamate and neuropeptides. Oxidative stress has also been implicated and monoamine oxidase inhibitors (MAOIs) are therefore considered to be potential candidates as anti-Alzheimer drugs because of their capacity to inhibit oxidative damage.[53] Moreover, observations have suggested that MAO and AChE inhibition might decrease β-amyloid deposition, therefore compounds with dual AChE and MAO inhibitory activity are likely to be more effective against AD.

Ladostigil (TV3326) – (N-propargyl-(3R) aminoindan-5yl)-ethyl methyl carbamate – is a novel drug that combines AChE/MAO inhibition and a neuroprotective ability (Figure 1d). This compound is a combination of active components from rasagiline (MAOI and neuroprotective) and rivastigmine (AChE-I). It possesses multiple therapeutic activities as it reduces apoptosis and stimulates the processing of amyloid precursor protein (APP) alpha, thereby reducing the possibility of generation of toxic β-amyloid. The S-enantiomer of ladostigil, TV3279, which is a ChE-I but is devoid of MAO inhibitory activity, exerts neuroprotective properties and regulates APP processing, indicating that these effects are independent of MAO inhibition (Figure 1e). Thus, ladostigil is more effective than TV3279 for Alzheimer's patients.[54]

It has been suggested that accumulation of iron in brain regions associated with neurodegenerative diseases, such as Parkinson's disease (PD), AD, amyotrophic lateral sclerosis and Huntington's disease, is involved in Fenton chemistry

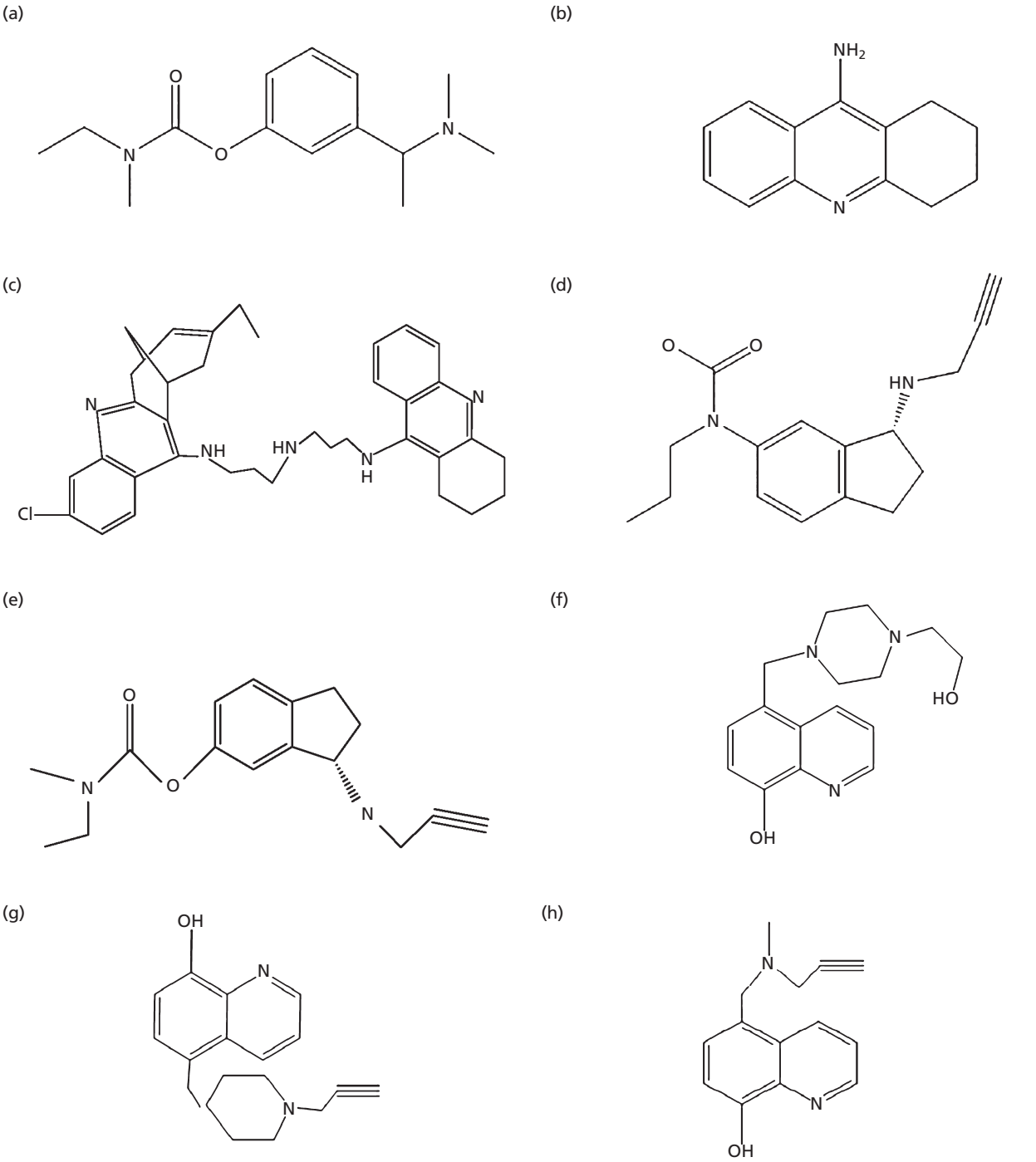
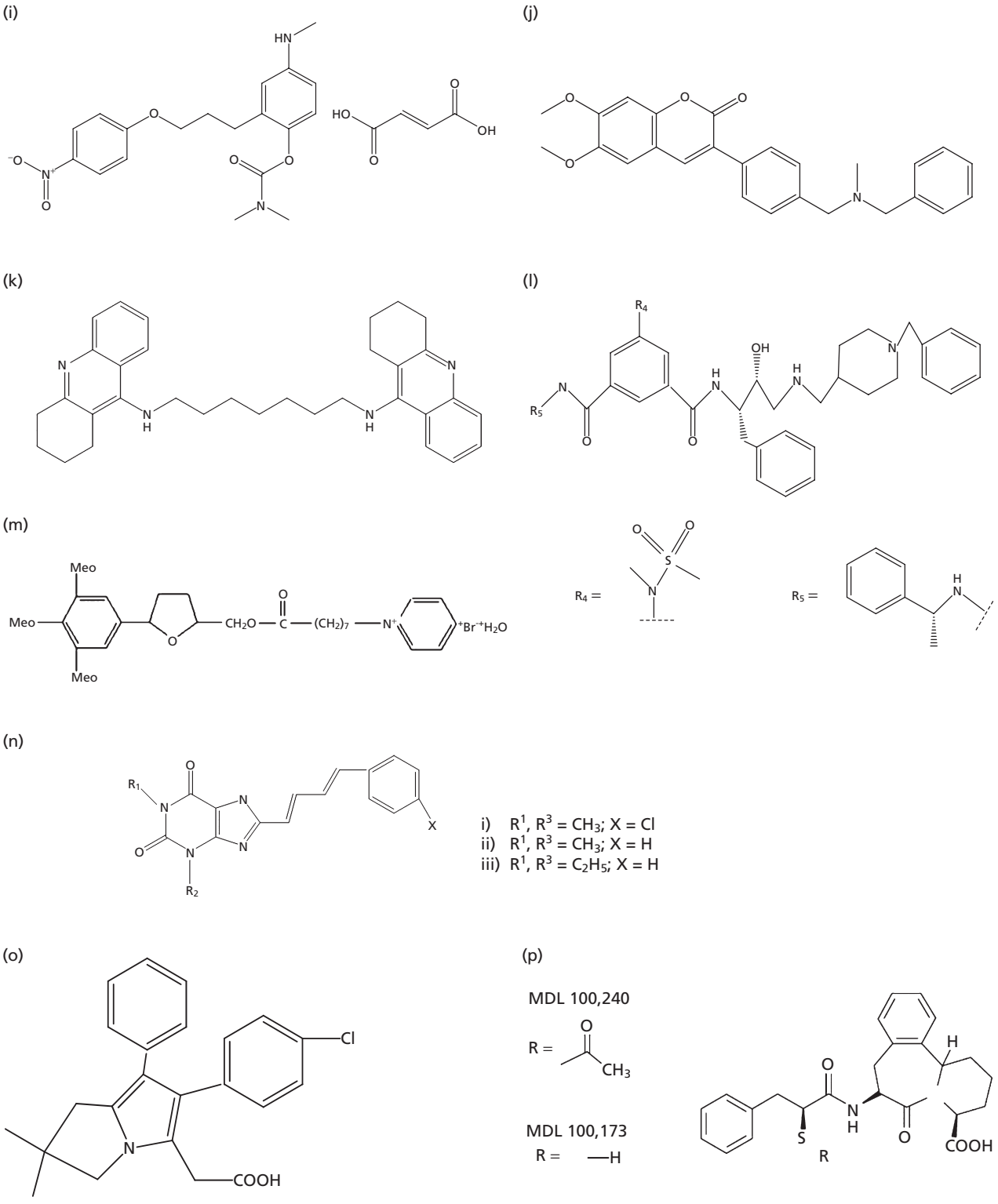


Figure 1 Structural formulae for various dual inhibitors. (a) Rivastigmine: 3-(1-(dimethylamino)ethyl)phenyl ethyl(methyl)carbamate. (b) Tacrine: 1,2,3,4-tetrahydroacridin-9-amine. (c) Huprine-tacrine heterodimer. (d) TV3326 (Ladostigil): [(3R)-3-(prop-2-ynylamino)indan-5-yl]-N-propylcarbamate. (e) TV-3279. (f) VK 28. (g) HLA 20. (h) M30. (i) RS-1259: 4-[(1S)-methylamino-3-(4-nitrophenoxy)] propylphenyl N,N-dimethylcarbamate (fumaric acid). (j) AP2238: 3-(4-{[Benzyl(methyl)amino]methy[}phenyl)-6,7-dimethoxy-2H-2-chromenone. (k) bis (7)-Tacrine: N,N′-bis(1,2,3,4-tetrahydro-9-acridinyl)-1,7-heptanediamine, dihydrochloride. (l) An inhibitor coded as 28 i.e. (1S,2R)-N-{1-Benzyl-2-hydroxy-3-(S)-[2-(1-benzylpiperidin-4-yl)ethylamino]-propyl}-5-[methyl(methylsulfonyl)amino]-N′-[(R)-1-phenylethyl]isophthalamide. (m) PMS777. (n) (E,E)-8-(4-phenylbutadien-1-yl)caffeine analogues (i–iii). (o) ML 3000. (p) MDL 100,240, a prodrug, and its active metabolite MDL 100, 173. (q) SLV 306: 2-[3(S)-[1-[2(R)-(Ethoxycarbonyl-4-phenylbutyl]cuclopentan-1-ylcarboxamido]-2-oxo-2,3,4,5-tetrahydro-1H-1benzazepin-1-yl]acetic acid. (r) NVP BEZ 235: 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile. (s) BAG956: α,α,-Dimethyl-4-[2-methyl-8-[2-(3-pyridinyl)ethynyl]-1Himidazo[4,5-c]quinolin-1-yl]-benzeneacetonitrile. (t) TAS-103: 6-[2-(dimethylamino) cthylamino]-3-hydroxy-7H-indeno[2,1-e]quinolin-7-one dihydrochloride.



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oxidative stress observed in these diseases. The neuroprotective activity of propargylamines has led to the development of several novel bifunctional iron chelators from the prototype brain permeable iron chelator, VK-28, possessing propargylamine moiety (HLA-20 and M30) (Figure 1f–1h). These compounds have shown iron-chelating and MAO-A and MAO-B selective brain inhibitory and neuroprotective-antiapoptotic actions.[55] In M30, the pharmacophore of brain-permeable iron chelator, VK-28, plus the MAO-inhibitory neuroprotective propargylamine moiety of rasagiline are

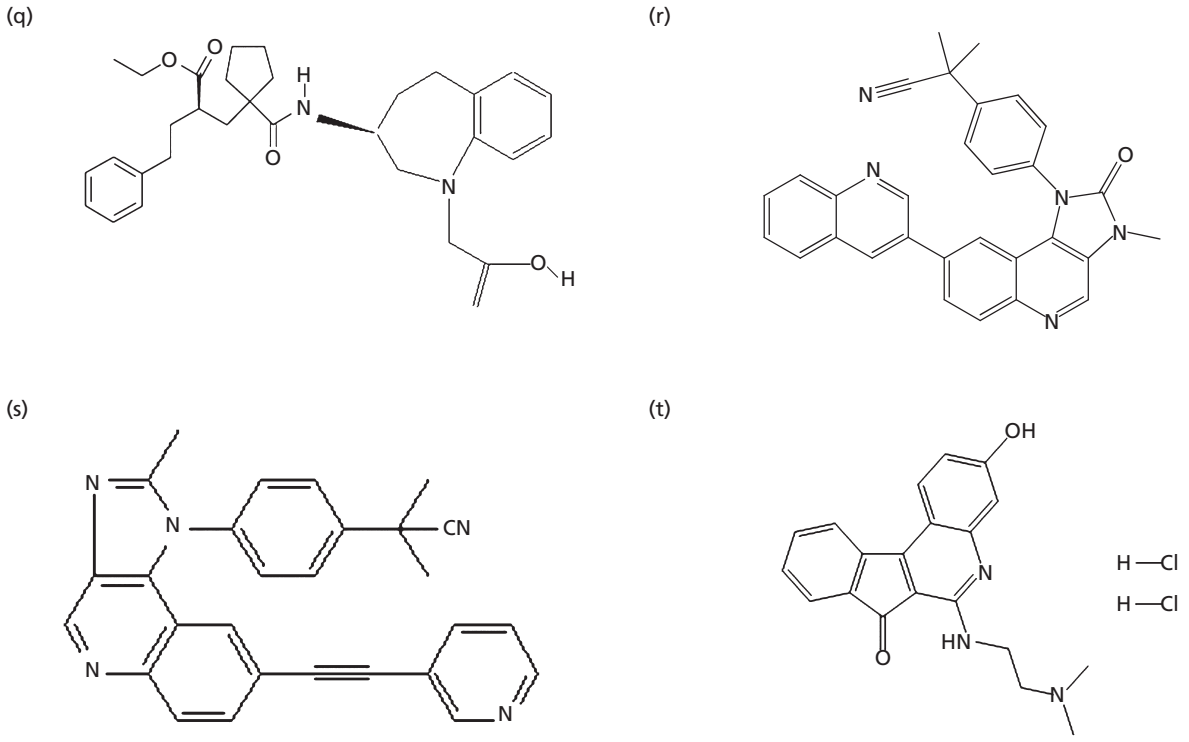

Figure 1 Continued.

combined into a single molecule as a potential treatment for AD, Lewy body disease and PD with dementia.[56]

A set of coumarin derivatives with known inhibitory activity towards MAO-A and MAO-B have been screened for AChE inhibitory activity.[12] More recently studies have reported 1-N-substituted thiocarbamoyl-3-phenyl-5-thienyl-2-pyrazoline derivatives as attractive options for AD treatment, since they have shown MAO-B and ChE inhibitory activity.[13] Thorough screening of a library of non-alkaloidal natural compounds has resulted in the emergence of four xanthones making them dual AChE/MAO-Is of great interest.[14]

Dual AChE and serotonin transporter inhibitors

The use of AChE-Is is a very successful strategy but it lags behind in treating the depression commonly seen in AD patients. Because of this, dual inhibitors of AChE and serotonin transporter (SERT) have been synthesized as a novel class of drugs for AD treatment. They are thought to bring about greater therapeutic effects than AChE inhibition alone, and to avoid their adverse peripheral effects. Combining rivastigmine and fluoxetine, various dual inhibitors have been designed and evaluated for their inhibitory activities. A compound (S)-2j (RS-1259) has been reported to possess balanced inhibitory activities of AChE (IC50 = 101 nM) and SERT (IC50 = 42 nM) (Figure 1i). Ex-vivo experiments conducted in mice have also demonstrated the dual inhibitory action of AChE and SERT in the brain following oral administration.[15]

Dual binding site AChE-Is

Biochemical and crystallographic studies of AChE have revealed two potential binding regions at both anionic and peripheral binding sites. The 'peripheral anionic site' has been implicated in promoting aggregation of β-amyloid peptide, which is responsible for the neurodegenerative process in AD, while the active catalytic anionic site is responsible for the termination of nervous signals through the hydrolysis of Ach, thereby leading to reduced cholinergic transmission and cognitive deficits.[21] Thus dual binding site AChE-Is targeting both locations could simultaneously alleviate cognitive deficits and function as disease-modifying agents by diminishing the β-amyloid aggregation. 3-[4-{(benzylmethylamino) methyl}phenyl]-6,7-dimethoxy-2H-2-chromenone (AP2238) is the first compound published to bind both anionic sites of human AChE, and allows the simultaneous inhibition of the catalytic and β-amyloid pro-aggregating activities of AChE (Figure 1j).[16] Many new dual binding site AChE-Is have been designed and synthesized. These include hybrids of tacrine or 6-chlorotacrine with an indole moiety, capable of binding simultaneously to both catalytic and peripheral sites of AChE.[57]

Recently designed tacrine–thiadiazolidinone hybrids are new leads in the optimization of AD modifying agents as they have exhibited significant AChE inhibitory activity. Competition assays of selective ligands for the peripheral anionic site on AChE, using propidium as reference, have indicated the influence of these compounds over the peripheral site.[17] Recently, two isomeric series of dual binding site AChE-Is have been designed and synthesized by hybridizing a unit of 6-chlorotacrine and pyrano[3,2-c]quinoline scaffold as the active site and peripheral site interacting moieties, respectively. These moieties are connected through an oligomethylene linker containing an amido group at a variable position.

Table 1 List of emerging dual inhibitors in different disease conditions

Emerging dual inhibitors	
Alzheimer's disease	
Dual cholinesterase inhibitors	Huprine-tacrine heterodimers,[8] 10-methylacridinium iodide,[9] a novel class of isaindigotone derivatives[10] and xyloketal A-D, i.e. metabolites of mangrove fungus *Xylaria* sp.[11]
Dual AChE and MAO inhibitors	Coumarin derivatives,[12] 1-N-substituted thiocarbamoyl-3-phenyl-5-thienyl-2-pyrazoline derivatives,[13]xanthones[14]
Dual AChE and serotonin transporter inhibitors	(S)-2j (RS-1259)[15]
Dual binding site AChE inhibitors	AP2238,[16] tacrine-thiadiazolidinone hybrids[17]
Dual AChE and Abeta aggregation inhibitors	Propidium–tacrine heterodimer,[18] tacripyrines (tacrine-dihydropyridine hybrids obtained by combining tacrine with nimodipine),[19] Congo red dye,[20] huperzine A (HupA)-based bivalent ligands[21]
Dual AChE and NMDA inhibitors	Bis (7)-tacrine, i.e.(1,7-*N*-heptylene-bis-9,9′-amino-1,2,3,4-tetrahydroacridine)[22]
Dual AChE and β-secretase (BACE) inhibitors	(1S,2R)-N-{1-benzyl-2-hydroxy-3-(S)-[2-(1-benzylpiperidin-4-yl)ethylamino]-propyl} -5-[methyl(methylsulfonyl)amino]-N'-[(R)-1-phenylethyl]isophthalamide[23]
Dual acetylcholinesterase inhibitors and antioxidants	PMS777[24]
Psychosis	
Dual cAMP and cGMP phosphodiesterase 10A (PDE10A) inhibitors	Papaverine and MP-10 [2-{[4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy]methyl}quinoline][25]
Parkinsonism	
Dual monoamine oxidase B inhibitors and adenosine A_{2A} receptor antagonists	Methylxanthines[26] and (E,E)-8-(4-phenylbutadien-1-yl)caffeine[27]
Inflammation	
Dual cyclooxygenases (COX-1 and COX-2) and 5-lipoxygenase (5-LOX) inhibitors	Flavocoxid[28] and ML3000 (2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-H-pyrrolizine-5-yl) acetic acid[29]
Hypertension	
Dual inhibitors of neprilysin and angiotensin-converting enzyme	BMS-182657,[30] MDL-100173[31] and S21402 (RB105) {N-[2S,3R-(2-mercaptomethyl-1-oxo-3-phenylbutyl)-L-alanine]}[32]
Dual inhibitors of the angiotensin II receptor and neprilysin	LCZ696[33]
Dual inhibitors of angiotensin-converting and endothelin-converting enzymes	CGS 26303[34] and SLV 306 (Daglutril)[35]
Dual vasopressin receptor (V_1/V_2) antagonists	(RWJ-676070)[36]
Thrombosis	
Dual-acting anticoagulant/antiplatelet inhibitors	MC45301, MC45308, MC45350, and MC45403 derived from vitamin B6 (pyridoxine)[37]
Microbial infections	
Dual inhibitors of type I and type II DNA topoisomerases	Some novel indolyl quinoline analogs [38]
Dual inhibitors of β-ketoacyl-[acyl carrier protein (ACP)] synthase II (FabF) and III (FabH)	Platencin[39]
Viral infections	
Dual inhibitors of human immunodeficiency virus type 1 (HIV-1) integrase and RNase H	Madurahydroxylactone derivatives[40]
Dual inhibitors of 2A and 3C proteases encoded by human rhinoviruses (HRVs)	LY343814[41]
Cancer	
Dual PI3K and mTOR inhibitors	PI-103,[42] NVP-BEZ235,[43] WJD008[44] and BAG956 [45]
Dual topoisomerases I and II inhibitors	Benzophenanthridine alkaloids, indolocarbazoles and lipophilic bis(naphthalimides), anthraquinones, pyridoindoles, indenoquinolones, acridines, TAS-103, leptosins (Leps) F and C, tafluposide (F 11782) and XR11576[46–50]
Dual inhibitors of epidermal growth factor receptor, ErbB-2, and vascular endothelial growth factor receptor-2	NVP-AEE788[51]
Dual inhibitors of IkappaB kinase-2 (IKK2) and Fms-like tyrosine kinase 3 (FLT3)	AS602868[52]

The new hybrids retain the potent and selective human AChE-inhibitory activity of the parent 6-chlorotacrine, while exhibiting a significant in-vitro inhibitory activity towards AChE/self-induced β-amyloid aggregation, and toward β-secretase (BACE)-1, as well as an ability to enter the central nervous system. This makes them promising anti-AD lead compounds.[8]

Dual AChE and β-amyloid aggregation inhibitors

AChE colocalizes with β-amyloid deposits in the brains of AD patients. It is suggested that AChE has secondary non-cholinergic functions, including the processing and assembly of β-amyloid peptides. The role of AChE in an early step during the development of the senile plaque has also been demonstrated.[58] The incorporation of AChE into amyloid aggregates results in the modification of the biochemical properties of the enzyme, which include sensitivity to low pH, inhibition at high substrate concentrations and increased β-amyloid neurotoxicity.[20] Thus dual AChE and β-amyloid aggregation inhibitors may prove very useful for AD treatment. Such inhibitors prevent the aggregation of β-amyloid into Alzheimer's fibrils. Furthermore, inhibition studies conducted by means of circular dichroism and thioflavin T fluorescence spectroscopy have confirmed the inhibition of human recombinant acetylcholinesterase-induced β-amyloid aggregation by AChE-Is (propidium, decamethonium, donepezil and physostigmine).[59]

Phenserine, a derivative of physostigmine, is a new, potent and highly selective AChE-I. It has been reported to reduce β-amyloid formation *in vitro* and *in vivo*. Thus it may be a promising drug to attenuate the progression of AD.[60] An active component of marijuana, delta9-tetrahydrocannabinol (THC), competitively inhibits AChE as well as preventing AChE-induced β-amyloid aggregation, the key pathological marker of AD. Computational modeling of the THC–AChE interaction revealed that THC binds in the peripheral anionic site of AChE, the critical region involved in amyloidgenesis. Compared to currently approved drugs prescribed for the treatment of AD, THC is a considerably superior inhibitor of β-amyloid aggregation.[61]

Some emerging dual inhihitors are discussed as follows. Propidium-tacrine heterodimer is an effective AChE and β-amyloid aggregation inhibitor. IC50 for AChE and Abeta aggregation as revealed by in-vitro biological studies is in the low nanomolar range.[18] Tacripyrines, the first tacrine-dihydropyridine hybrids obtained by combining an AChE-I (tacrine) with a calcium antagonist such as nimodipine, are selective and potent AChE-Is. The mixed-type inhibition of AChE activity has been associated with inhibition of the pro-aggregating action of AChE on β-amyloid, and a moderate inhibition of β-amyloid self-aggregation. In addition, their neuroprotective action, Ca^{2+} channel-blocking effect and ability to cross the blood–brain barrier makes them potential candidates for treating AD.[19] Congo red dye reportedly stabilizes β-amyloid monomer, and inhibits oligomerization and the binding of AChE to β-amyloid.[20] Huperzine A-based bivalent ligands have been developed with the aim of concomitantly increasing AChE inhibition potency by utilizing the chelate effect and protecting neurons from β-amyloid toxicity.[21]

Dual AChE and N-methyl-D-aspartate inhibitors

Several studies have implicated abnormal release of glutamate as a contributing factor for neuronal apoptosis, which is further associated with deterioration of cognition and memory in AD. This released glutamate causes an overactivation of glutamate receptors of the N-methyl-D-aspartate (NMDA) subtype, leading to an abnormal influx of Ca^{2+} in the viable neurons and subsequent neuronal death.[62,63] Thus AChE-Is possessing anti-apoptotic activity may prove very useful in the treatment as well as the prevention of the pathogenesis of AD. Recently it has been reported that bis (7)-tacrine, i.e. (1,7-*N*-heptylene-bis-9,9′-amino-1,2,3,4-tetrahydroacridine), a novel dimeric AChE-I derived from tacrine, inhibits glutamate-mediated apoptosis in neurons through the blockade of NMDA receptors at the MK-801-binding site, and by a mechanism independent of AChE inhibition (Figure 1k). Thus the evidence of its dual anti-NMDA and anti-AChE activities makes it a potential drug candidate for the treatment of AD.[22]

Dual AChE and β-secretase inhibitors

β-secretase (BACE-1), an aspartyl protease, generates β-amyloid residues after cleaving APP. Although BACE-1 inhibitors have shown good results in AD, the multifactorial nature of its pathogenesis demands the drugs possess a dual mode of action. Since AChE is the most successful target for symptomatic treatment of AD, and BACE-1 is a crucial factor of β-amyloid formation for the pathogenesis, dual inhibitors simultaneously targeting both AChE and BACE-1 have been synthesized. The first dual inhibitors of AChE and BACE-1 were reported by Lorna Piazzi and co-workers.[64] Recently, a series of novel dual inhibitors have been reported to possess in-vitro enzyme-inhibitory potency and cellular activity, and in-vivo functional efficacy. Among them, an inhibitor coded as 28, i.e. (1S,2R)-N-{1-benzyl-2-hydroxy-3-(S)-[2-(1-benzylpiperidin-4-yl)ethylamino]-propyl}-5-[methyl(methylsulfonyl)amino]-N′-[(R)-1-phenylethyl]isophthalamide, has exhibited good dual potency in an enzyme-inhibitory potency assay (BACE-1: IC50 = 0.567 μM; AChE: IC50 = 1.83 μM), and has showed excellent inhibitory effects on β-amyloid production of APP-transfected HEK293 cells (IC50 = 98.7 nM) (Figure 1l). Furthermore, intracerebroventricular injection of this compound in APP-transgenic mice caused a 29% reduction of β-amyloid production, thereby indicating that it is a good lead compound for further evaluation.[23]

Dual AChE-Is and antioxidants

The dual AChE-inhibitory and free radical scavenging activity of tacrine and melatonin is well documented. Recently, some new hybrids of both drugs have displayed in-vitro properties that are greater than the sum of their parts, thereby indicating their potential as future drugs for AD.[65] PMS777, a tetrahydrofuran derivative, designed as a novel dual PAF and AChE-I, has demonstrated a potential to fight oxidative injury (Figure 1m), therefore PMS777 could be a dual inhibitor of interest for patients with cognitive impairment and inflammatory damage, as in AD.[24]

Psychosis

Generally, antipsychotic drugs are not specific for the type of psychosis to be treated. Moreover, a number of side effects

have been observed with many of these medications. Thus dual inhibitors for the treatment of psychosis are being investigated. Amisulpride is one such compound with a dual mode of action. It has a high selectivity for D_2 and D_3 receptors, and acts preferentially on the mesocortical and mesolimbic systems. At low doses, it blocks presynaptic dopamine autoreceptors, which induce an increased dopaminergic neurotransmission. At high doses it blocks postsynaptic dopaminergic activity.[66]

Dual cAMP and cGMP phosphodiesterase 10A (PDE10A) inhibitors

It has been reported that dual cAMP and cGMP-PDE10A inhibitors may present a novel mechanism to treat positive symptoms of schizophrenia. Two such compounds, papaverine [1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline] and MP-10 [2-{[4-(1-methyl-4-pyridin-4-yl-1H-pyrazol-3-yl)phenoxy]methyl}quinoline] have demonstrated the alleviation of both dopaminergic and glutamatergic dysfunction, which are thought to underlie schizophrenia, in a variety of in-vivo and in-vitro assays.[25]

Dual dopamine and serotonin antagonists

Atypical antipsychotic drugs have a blocking effect on D_2 receptors. Some also block or partially block serotonin receptors (particularly $5HT_{2A,C}$ and $5HT_{1A}$ receptors), e.g. risperidone. Asenapine is a 5-HT_{2A}- and D_2-receptor antagonist under development for the treatment of schizophrenia and acute mania associated with bipolar disorder. Flupenthixol is a type of thioxanthene drug that acts by antagonism of D_1 and D_2 receptors as well as serotonin. Paliperidone, an FDA-approved drug for the treatment of schizophrenia, is believed to act via similar pathways, i.e. its therapeutic effect is suggested to be due to a combination of D_2 and 5-HT_{2A} receptor antagonism. Iloperidone also acts by antagonizing specific neurotransmitters, particularly multiple dopamine and serotonin receptor subtypes. The antipsychotic effect of zotepine is thought to be mediated through antagonistic activity at dopamine and serotonin receptors. Zotepine has a high affinity for the D_1 and D_2 receptors. It also affects the $5HT_{2A}$, $5HT_{2C}$, $5HT_6$, and $5HT_7$ receptors. In addition, it inhibits the reuptake of noradrenaline.[67]

Miscellaneous compounds

Bifeprunox is a partial D_2 and serotonin 5-HT_{1A} receptor agonist, which shows average therapeutic efficacy, but may offer safety advantages in terms of reduced risk of metabolic complications. Norclozapine (N-desmethylclozapine), a major metabolite of clozapine, possesses partial agonist activity at D_2 receptors. In addition, it appears to have muscarinic agonist activity, which is believed to be responsible for its observed positive effects on cognition.[67]

Depression

Various neurotransmitters are involved in the pathophysiology of depression, e.g. 5HT, NA, dopamine and β-adrenoceptors. Uptake and reuptake of these neurotransmitters may lead to depression by interacting with presynaptic and postsynaptic receptors. Blocking the neurotransmitters that are acting on the receptors can be beneficial against depression. There are various drugs approved for this indication. These either block the neurotransmitters or block the transporters of the neurotransmitters to the receptor. The single-action serotonin-reuptake inhibitors (SSRIs) are still the most commonly prescribed antidepressants. They are generally considered safer and more tolerable than tricyclic antidepressants (TCAs) and MAOIs because of their selectivity and better side effect profiles. However, the selectivity of SSRIs, which is responsible for their safety, may limit the therapeutic efficacy in some cases. In addition, SSRIs may have a slower onset of action, resulting in lower remission rates, and be less effective for the physical symptoms associated with depression than TCAs and MAOIs. On the other hand, TCAs and MAOIs are effective in treating depression; they have an inferior side-effect profile that makes them less tolerable. Thus dual-action antidepressants are experiencing a surge in scientific and clinical interest.[68,69]

Dual serotonin–norepinephrine reuptake inhibitors

Since serotonin is not the only neurotransmitter implicated in the pathophysiology of depression, newer dual-action medications have been developed to inhibit the reuptake of both serotonin and norepinephrine. These dual serotonin–norepinephrine reuptake inhibitors (SNRIs) have shown improved side-effect profiles and tolerability. Moreover, it is suggested that they may have prophylactic properties, preventing major depressive episodes. Examples of SNRIs include venlafaxine, desvenlafaxine, duloxetine and milnacipram.[70]

Some TCAs also block the reuptake of certain neurotransmitters, such as norepinephrine (noradrenaline) and serotonin. They are used less commonly now, due to the development of more selective and safer drugs. These include amitriptyline, clomipramine, doxepin, desipramine and nortriptyline. More recently, novel arylthiomethyl morpholines have been investigated as potent dual SNRIs.[70]

Dual noradrenergic and specific serotonergic antidepressants

Dual noradrenergic and specific serotonergic antidepressants (NaSSAs) belong to a newer class of antidepressants in which norepinephrine (noradrenaline) and serotonin neurotransmission is increased by blocking presynaptic alpha-2 adrenergic receptors while at the same time blocking certain serotonin receptors. Examples include mianserin and mirtazapine.[71]

Dual dopamine–norepinephrine reuptake inhibitors

Dual dopamine–norepinephrine reuptake inhibitors (DNRIs) inhibit the neuronal reuptake of dopamine and norepinephrine (noradrenaline). Bupropion is such a dual-acting DNRI, possessing overall efficacy similar to SSRIs and TCAs. It has been suggested that bupropion targets specific major depressive disorder symptoms, which, in some instances, may offer therapeutic advantage over other antidepressants (e.g. loss of pleasure).[72]

Dual serotonin (5-HT) reuptake inhibitors and 5-HT$_{1A}$ receptor antagonists

Newly synthesized 2-piperazin-1-yl quinoline derivatives have been reported to exhibit potent functional activities at both the 5-HT transporter and 5-HT$_{1A}$ receptors. Furthermore, preclinical characterization of a novel compound, WAY-211612, has demonstrated dual 5-HT uptake inhibitor and 5-HT$_{(1A)}$ receptor antagonist activities.[73]

Dual serotonin/norepinephrine reuptake/ phosphodiesterase inhibitors

The new dual serotonin/norepinephrine reuptake/ phosphodiesterase inhibitors (SNRI/PDE4-Is), obtained after chemically linking balanced serotonin/norepinephrine reuptake inhibitors (i.e. (R)- and (S)-norduloxetine) to a PDE4 inhibitor via a five-carbon bridge, have shown synergistic functional activity and may be effective in treating diseases such as depression.[74]

Miscellaneous compounds

Agomelatine (a melatonin agonist/5-HT$_{2C}$ antagonist) has clinically proven activity in major depression. Dual neurokinin$_1$ antagonists/5-HT reuptake inhibitors (SRIs), melanocortin$_4$ antagonists/SRIs, histamine H$_3$ antagonists/SRIs, GABA$_B$ antagonists/SRIs, glutamatergic/SRIs and cholinergic agents/ SRIs are being investigated as novel drugs.[69]

Parkinsonism

Dual MAO-B inhibitors and adenosine A$_{2A}$ receptor antagonists

Both adenosine A$_{(2A)}$ receptor antagonists and MAO-B inhibitors are considered useful for the treatment of PD as they potentiate the motor-restorative effects of levodopa by acting at different targets. In addition, they have exhibited neuroprotective properties. Because of this, dual-target-directed drugs obtained by the combination of these two activities in a single drug are being investigated. A number of methylxanthines exhibiting such dual action are being optimized.[26] Moreover (E,E)-8-(4-phenylbutadien-1-yl)caffeine analogues are also considered promising candidates in the class of dual-acting compounds (Figure 1n).[27]

Inflammatory diseases

Dual-acting anti-inflammatory drugs, which inhibit both cyclooxygenases (COX-1 and COX-2) as well as 5-lipoxygenase (5-LOX), are currently undergoing clinical development. Such compounds retain the activity of classical NSAIDs, while avoiding their main drawbacks.[75] These drugs can alleviate symptoms of rheumatic diseases and may satisfy some criteria of curative drugs. Since both COX-2 and 5-LOX are also involved in the development and progression of several types of cancer; these dual-acting drugs may produce a better anticancer response. Furthermore, the dual inhibition of both COX and 5-LOX is neuroprotective, and thus these drugs can be beneficial, particularly in ADs and PDe.[76] Other emerging dual anti-inflammatory drugs are flavocoxid, ML3000 (2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-H-pyrrolizine-5-yl) acetic acid and some virtually designed thiazolidinones (Figure 1o).[28,29]

Cardiovascular diseases: congestive heart failure

Dual β and α$_1$ adrenergic receptor antagonists

Some third-generation β-receptor antagonists also block β_1 adrenergic receptors. One representative of this class of compounds is labetalol. It acts as competitive antagonist at both β_1 and β receptors. The action of labetalol on both β_1 and β receptors contributes to a fall in blood pressure in patients with hypertension. β_1 receptor blockade leads to relaxation of arterial smooth muscle and vasodilation, while the β_1 blockade also contributes to a fall in blood pressure, in part by blocking reflex sympathetic stimulation of the heart. Furthermore, the intrinsic sympathomimetic activity of labetalol at β_2 receptors may contribute to vasodilation.

Like labetalol, carvedilol blocks β_1, β_2, and β_1 receptors. In addition, it has antioxidant and antiproliferative activities. Hence it also produces vasodilation. Bucindolol, a third-generation non-selective β-receptor antagonist, possesses some β_1 receptor blocking, as well as β_2 and β_3 agonistic, properties. Bucindolol increases left ventricular systolic ejection fraction and decreases peripheral resistance, thereby reducing afterload. Other drugs belonging to this class are bevantolol and nipradilol.[77]

Hypertension

Dual calcium channel blockers

Cilnidipine is a novel dihydropyridine calcium channel blocker (CCB) with a dual mechanism of action. It acts on both L- and N-type calcium channels. It possesses all the advantages of long-acting vasculo-selective dihydropyridine calcium channel blockers. Moreover, it is as effective as amlodipine and long-acting nifedipine in controlling blood pressure. Since it inhibits cardiac sympathetic over-activity and prevents reflex tachycardia, it is reported to be useful in patients with effort angina.[33] Efonidipine hydrochloride is a dual L- and T-type CCB. As T-type calcium channels in the sinoatrial node attenuate reflex tachycardia, this may favourably affect cardiac pacing, thereby reducing reflex tachycardia. It was found to be effective in patients with mild to severe essential hypertension and angina pectoris.[78]

Dual inhibitors of neprilysin and angiotensin-converting enzyme

Vasopeptidase inhibitors (VPIs) are a new class of antihypertensive agent that simultaneously inhibit angiotensin-converting enzyme (ACE) and neutral endopeptidase (NEP). ACE inhibition is an effective therapeutic target for hypertension. Inhibition of neprilysin (an enzyme involved in the degradation of natriuretic peptides, also known as NEP) is also emerging as an antihypertensive strategy as it potentiates the diuretic, natriuretic and vasorelaxant effects of natriuretic peptides.[79]

Fasidotril, the first dual NEP/ACE inhibitor, has been found to be effective in various experimental models of rat hypertension, and in patients with essential hypertension.[80,81] Omapatrilat inhibits both NEP and ACE and was found to be more effective than ACE inhibitors alone. However, it has not received FDA approval due to angioedema safety concerns.[82] Other dual NEP/ACE inhibitors are BMS-182657, MDL-100173 and S21402

(RB105) {N-[2S,3R-(2-mercaptomethyl-1-oxo-3-phenylbutyl)-L-alanine]} (Figure 1p).[30–32] Mixanpril, a lipophilic prodrug of RB 105, is the first dual NEP/ACE inhibitor that is potentially useful for clinical investigations.[83]

Dual inhibitors of the angiotensin II receptor and neutral endopeptidase

LCZ696 is a first-in-class dual inhibitor of the angiotensin II receptor and NEP. A randomized, double-blind, placebo-controlled, active comparator study has reported complementary and fully additive reduction of blood pressure, thereby indicating its potential for the treatment of hypertension and cardiovascular diseases.[84]

Dual inhibitors of angiotensin-converting enzyme and endothelin-converting enzyme

Since endothelins (ETs) are potent vasoconstrictors, promitogens, and inflammatory mediators, the inhibitors of endothelin-converting enzyme (ECE) may act as therapeutic agents for various cardiovascular, renal, pulmonary, and central nervous system diseases.[85] CGS 26303, a vasopeptidase inhibitor that simultaneously inhibits ECE and NEP, was found to be beneficial in the transition from hypertrophy to heart failure.[34] Another dual NEP/ECE inhibitor is SLV 306 (Daglutril) (Figure 1q).[35]

Dual vasopressin receptor (V_1/V_2) antagonists

As vasopressin has significant effects on the regional regulation of vascular tone, blood pressure and the progression of renal disease, dual V_1/V_2 receptor antagonists are being tested, alone or in combination with ACE inhibition/angiotensin II type 1 receptor blockade. Conivaptan (YMO87) is the first orally active non-peptidic dual V_1/V_2 receptor antagonist. In early clinical trials, its use resulted in promising haemodynamic effects, with a reduction in pulmonary capillary wedge pressure and an increase in urine output, without causing significant hypotension or tachycardia.[86] Another dual $V_{(1a)}$ and $V_{(2)}$ vasopressin receptor antagonist, RWJ-676070, has been suggested as an additional therapeutic agent in the treatment of chronic proteinuric nephropathy (Table 1).[36]

Pulmonary hypertension

Dual endothelin receptor antagonists

Bosentan is a dual ET receptor antagonist used in the treatment of pulmonary artery hypertension. It is a competitive antagonist of ET-1 at the ET-A and ET-B receptors. Under normal conditions, ET-1 binding of ET-A or ET-B receptors causes pulmonary vasoconstriction. By blocking this interaction, bosentan decreases pulmonary vascular resistance. Bosentan has shown a slightly higher affinity for ET-A than ET-B.[87]

Thrombosis

Dual glycoprotein II_b/III_a antagonists

Glycoprotein (GP) II_b/III_a receptor antagonists are a new class of platelet aggregation inhibitors that act by blocking the main receptor involved in platelet aggregation. GP II_b/III_a is an adhesive receptor (integrin) for fibrinogen and von Willebrand factor through which agonists like collagen, thrombin, TXA_2, ADP, etc. induce platelet aggregation. Because of this, GP II_b/III_a receptor antagonists inhibit platelet aggregation induced by various agonists.

Abciximab (previously known as c7E3 Fab), a platelet aggregation inhibitor, is a GP II_b/III_a receptor antagonist. It is the Fab fragment of a chimeric monoclonal antibody against GP II_b/III_a. This is mainly used during and after coronary artery procedures, such as angioplasty, to prevent platelets from sticking together and causing thrombus (blood clot) formation within the coronary artery.[88] Other peptide and nonpeptide GP II_b/III_a receptor antagonists are epitifibatide and tirofiban, respectively. However, oral GP II_b/III_a receptor inhibitors (orbofiban, sibrafiban and xemilofiban) were not found to be effective in reducing ischemic events when used on a long-term basis after acute coronary syndrome.[89]

Dual-acting anticoagulant/antiplatelet inhibitors

Four lead compounds (MC45301, MC45308, MC45350, and MC45403) derived from vitamin B6 (pyridoxine) have been reported to selectively inhibit thrombin and platelet aggregation, thereby indicating their potential in treating venous and arterial thrombosis.[37]

Hyperlipidemia

Dual inhibitors of lipolysis and triglyceride synthesis

Niacin (nicotinic acid), a group B vitamin, reduces production of very low density lipoprotein in liver by inhibiting triglyceride (TG) synthesis. In addition, it inhibits intracellular lipolysis in adipose tissue and increases the activity of lipoprotein lipase, which clears TGs.[90]

Infectious conditions: microbial infections

Some novel indolyl quinoline analogs developed as dual inhibitors of type I and type II DNA topoisomerases enzymes have been indicated in human leishmaniasis.[38] Platencin, a novel natural product, is a dual inhibitor and targets two essential proteins: β-ketoacyl-[acyl carrier protein] synthase II and III. It has exhibited a broad-spectrum Gram-positive antibacterial activity through inhibition of fatty acid biosynthesis.[39]

Viral infections

A series of 29 madurahydroxylactone derivatives have demonstrated dual inhibition of human immunodeficiency virus type 1 (HIV-1) integrase and RNase H, which are novel antiviral targets.[40] LY343814, a homophthalimide belonging to a group of novel non-peptidic antirhinovirus agents, has been reported as the most potent dual inhibitor of 2A and 3C proteases encoded by human rhinoviruses. These proteases have important roles in viral replication.[41] A newly synthesized class of thiourea derivatives has been reported to bind to HIV-1 capsid and human cyclophilin A, which are involved in HIV-1 assembly and disassembly processes.[91]

Cancer

Dual phosphatidylinositol-3-kinase and mammalian target of rapamycin inhibitors

The phosphatidylinositol-3-kinase (PI3K/AKT) and mammalian target of rapamycin (mTOR) signaling pathways are

activated in acute myeloid leukemia (AML) and contribute to the proliferation of blast cells as well as leukemic progenitors. Since the inhibition of each pathway alone does not induce significant apoptosis, dual PI3K and mTOR inhibitors are being developed. PI-103, the first dual PI3K and mTOR inhibitor has shown potent anti-leukemic activity. It also induces significant apoptosis in blast cells and in immature leukemic cells, due to synergistic effects between PI-103 and etoposide.[42] But its bioavailability is not optimal.

A new dual inhibitor of PI3K and mTOR, NVP-BEZ235 is currently in phase I trials for breast cancer treatment, and has shown better bioavailability than alternatives. It has been suggested for testing against AML (Figure 1r).[43] Another example is WJD008.[44] Lastly, BAG956, a dual PI3K/PDK-1 inhibitor, inhibits chronic and AML cell proliferation and synergizes with targeted tyrosine kinase inhibitors (Figure 1s).[45]

Dual topoisomerases I and II inhibitors

Aclarubicin is a dual topoisomerase I and II inhibitor.[92] Gliotoxin, a natural mycotoxin with immunosuppressive and antimicrobial activity, is a dual inhibitor of farnesyltransferase and geranylgeranyltransferase I. It has demonstrated pronounced antitumor activity against breast cancer *in vitro* and *in vivo*.[93] The dietary flavonoids myricetin and fisetin are reported as dual inhibitors of DNA topoisomerase I and II in cells.[94] Other dual topoisomerase I and II inhibitors are benzophenanthridine alkaloids, indolocarbazoles and lipophilic bis(naphthalimides), anthraquinones, pyridoindoles, indenoquinolones, acridines, TAS-103 i.e. 6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7H-indeno-[2,1-c] quinolin-7one dihydrchloride (Figure 1t), leptosins F and C (indole derivatives, isolated from a marine fungus, *Leptoshaeria* sp.), tafluposide (F 11782), a novel epipodophylloid, and XR11576, a novel phenazine.[46–50]

Miscellaneous

Roscovitine, a cyclin-dependent kinase (CDK) inhibitor, is in phase II clinical trials as an anticancer agent. Recent studies have reported that it blocks the human ether-a-go-go-related gene (HERG) potassium current, which plays a role in cell proliferation. This suggests the utility of a dual CDK/HERG channel blockade as an adjuvant cancer therapy.[95] NVP-AEE788, indicated in biliary tract cancer, is a dual inhibitor of epidermal growth factor receptor, ErbB-2, and vascular endothelial growth factor receptor-2.[51] Lapatinib ditosylate is an epidermal growth factor receptor and ErbB-2 (Her2/neu) dual tyrosine kinase inhibitor. It is under development as a treatment for solid tumors, such as breast and lung cancer. AS602868 inhibits two different kinases: IkappaB kinase-2 and Fms-like tyrosine kinase 3. These play a crucial role in the pathogenesis of AML, thereby suggesting this as a new therapeutic approach for AML (Table 1).[52]

Conclusion

As described above, some dual inhibitors are already in use and have shown great efficacy. Thus the current focus of research has shifted towards the development of dual inhibitors.

Future Directions

As research is revealing more and more disease targets, a new strategy for drug development lies in the synthesis of drugs capable of acting on multiple components of a disease. Likewise this has generated interest in the development of multifunctional drugs, which can act on multiple targets.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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