CNS Drug Development – Lost in Translation?

A. Talevi*[,1,2], C.L. Bellera[1,2], M. Di Ianni[1,2], M. Gantner[1], L.E. Bruno-Blanch[1] and E.A. Castro*[,3]

[1]*Medicinal Chemistry, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP), 47 & 115, La Plata (B1900AJI), Buenos Aires, Argentina;* [2]*Argentinean National Council of Scientific and Technical Research (CONICET) – CCT La Plata;* [3]*Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas INIFTA (Universidad Nacional de La Plata, CCT La Plata-CONICET), Diag. 113 y 64, C.C. 16, Suc. 4, B1900 La Plata, Argentina*

Abstract: CNS drug development is characterized by an especially high attrition rate, despite clear unmet medical needs in the field of neuro-pharmacology and significant investment in R&D of novel CNS drug treatments. Here, we overview the issues underlying the intrinsic difficulty of CNS drugs development, including obstacles of pharmacokinetic nature and lack of predictivity of preclinical tests. We highlight current efforts to overcome these limitations, with an emphasis on modeling opportunities towards early recognition of CNS candidates (stressing the possibilities of multi-target directed ligands or "magic shotguns") and different approaches to improve CNS bioavailability.

Keywords: ADMET models, biomarkers, CNS drugs, *in vitro* models, in silico models, multi-target directed ligands.

1. INTRODUCTION

Drug development is a costly, risky and time-demanding activity. Recent analysis reports an out-of-pocket cost of more than USD 850 million, a capitalized cost of USD 1.8 billion [1], and around 13.5 years in average from patent filing to market launch [2, 3]. Despite investment in drug development has been growing steadily (averaging 12% per year) between 1970 and 2007 [4], the number of new drug applications (NDA) has been generally declining after 1999 and most of them represented variations of existing drugs instead of new molecular entities (i.e. truly innovative drugs) [5]. Current estimates indicate that around 1 out of every 10,000 chemicals tested as potential new medicines makes it to the market [5]. Only 9-11% of candidates that make it to clinical stage survive to lunch; however, the proportion of candidates which become marketed therapeutics is even lower among central nervous system (CNS) agents, reaching only 3-7% [6, 7].

In other words, we are experiencing a translational problem (i.e. to transform drug discovery in animal models into drug development in human patients) [6], which is a general issue in the drug discovery environment (as well as in many others fields of knowledge) but poses a particular challenge in drug development for certain therapeutic categories. Noteworthy, CNS disorders include a considerable number of unmet medical needs and have been the target of substantial research efforts [8]. It should be added that due to growing aging population, CNS may tomorrow be to improvement of life quality and life expectancy what cardiovascular drugs were in the second half of the XXth Century.

Many possible reasons are invoked to explain this drop of innovation. One determinant seems to be the increased regulatory scrutiny and the necessity of demonstrating a greater benefit-to-risk ratio for new medicines [5, 9-11], with some actors and experts suggesting unbalanced benefit-risk assessments. On the other hand, drug developers have been increasingly focusing on "difficult" targets, such as CNS disorders or oncology [12]. There are many factors which contribute to make the development of novel therapies for brain disorders a difficult task. To begin with, CNS disorders encompass both neurological and psychiatric disorders. The etiology of psychiatric and some neurological disorders includes a complex combination of multiple genes, environmental and neuro-developmental factors, which may demand either single drugs capable of modulating multiple targets (selectively non-selective drugs or multi-target-directed-ligands) [7, 13-14] or, alternatively, many highly selective ("magic bullet") drugs simultaneously attacking different aspects of a given disease [7]. On the other hand, CNS drugs are likely to present safety (e.g. CNS-mediated side-effects) and pharmacokinetic issues. Unbound brain concentration is a critical parameter for a compound to elicit its effect on its CNS target [8, 15]; however, the ability of a drug to achieve adequate unbound concentrations in the brain is greatly challenged by the physical and biochemical barrier posed by the blood-brain barrier (BBB). Finally, the development of CNS agents has been hindered by the difficulties to develop appropriate, validated biomarkers and surrogate endpoints to estimate receptor-occupancy and efficacy [6, 7] and the lack of predictive power of preclinical (*in vitro* and *in vivo*) models [6].

In this mini-review we will analyze the aforementioned issues inherent to the development of CNS drugs. We will focus on the ADMET-related issues and the limitations of

*Address correspondence to this author at the Medicinal Chemistry, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP), 47 & 115, La Plata (B1900AJI), Buenos Aires, Argentina; Tel: 542214235333 Ext. 41;
E-mail: alantalevi@hotmail.com

1875-5607/12 $58.00+.00

preclinical models. We will also analyze emerging solutions, with a focus on the potential role that refined and integrated *in silico* and *in vitro* models may play in limiting the ADMET-related attrition.

2. ADMET-RELATED ISSUES

The BBB is situated at the interface between blood and the brain, i.e. at the endothelial cells that constitute the micro-vessels which form brain capillary bed. It protects the brain from fluctuations in plasma concentrations of physiological compounds and xenobiotics (e.g. drugs), it provides mechanisms for the exchange of nutrients, signaling molecules and ions and for the elimination of waste products [16]. Essentially, it comprises two basic elements [17-19]: a) a physical barrier defined by particularly tight tight junctions (TJ) with no fenestrations, which eliminates the ultra-filtrate characteristic of peripheral capillary beds, posing a size- and charge-selective barrier that limits the para-cellular transport and; b) a biochemical barrier, composed by a number of efflux transporters that actively limit the entrance of potentially toxic compounds and facilitate the elimination of waste material, plus the metabolic enzymes that are present at the endothelial cells [20]. Remarkably, the BBB is a dynamic structure highly dependent on environmental factors and health condition. What is more, its composition is regulated by other elements of the neurovascular unit, such as neighboring glial cells and neurons [16, 21]. The dynamic nature of the BBB plus the incidence of neighboring elements on its barrier properties are important challenges to the development of suitable *in vitro* models to assist CNS drug development campaigns, as will be discussed later.

In the past, the ratio between brain and blood or plasma concentrations in steady state (Cb:Cp) and BBB permeability per surface area have been used as criteria to guide lead selection in CNS drug discovery settings [8, 15]. Nevertheless, it is now accepted that these parameters may well be insufficient to guide the development of new therapies for brain disorders. For instance, a large variability has been observed among the Cb:Cp ratios of structurally heterogeneous CNS marketed drugs [22, 23]. There is a simple explanation to this result: total brain concentration of a given drug includes both the amounts of bound and unbound drug and, due to their physicochemical properties, some CNS agents tend to present extensive non-specific tissue binding (in particular, basic drugs) [15]. It has been stated that Cb:Cp only represents the inert partitioning process of drug into lipid material [24], while the free drug concentration is responsible for drug action. Consistently, the concentration ratio of free drug in the brain to free drug in blood seems to be far less variable for CNS agents and should replace Cb:Cp as lead selection criteria [22]. What is more, measuring the unbound brain concentration of the drug may increase the probability of conclusively testing pharmacological hypothesis in clinical studies [8].

A number of processes have a role in drug delivery and clearance within the various compartments of the CNS, among them: passive transport, uptake and efflux transport, bulk flow of brain interstitial and cerebrospinal fluid (CSF), metabolism and tissue binding [15, 25]. Passive membrane permeability, facilitated transport and tissue-binding have been referred at the major determinants of drug disposition in the brain [15].

TJs block the para-cellular diffusion pathway, abolishing leakage of water-soluble non-electrolytes, ions and plasma proteins, which are likely to permeate through leaky interendothelial spaces at peripheral capillaries. Instead, passive diffusion at the BBB level takes place through the transcellular pathway; therefore, CNS drugs which are delivered passively into the brain are frequently small, lipophilic compounds. Thus, some molecular properties and features such as octanol-water partition coefficients, polar surface area (PSA), number of hydrogen-bond donors (HBD) and molecular mass (MM) are important predictors of passive permeability through the BBB [8]. For instance, a recent analysis of the distribution of six ADMET-related properties among 119 marketed CNS drugs and 108 Pfizer's candidate CNS drugs reveals median values of ClogP = 2.8, topological PSA = 44.8 $Å^2$, molecular mass = 305 Da and HBD = 1 for the marketed drugs Fig. (**1**) [26]. It is worth mentioning that the physicochemical properties analyzed by these authors have a simultaneous impact on a variety of end points (passive permeability, Pgp-mediated efflux, metabolic stability, safety) [26]. For example, the same features that improve passive permeability and thus drug delivery to the CNS raise potential safety issues. There is, for instance, a well-established relationship between logP and toxicity. Log P correlates with carcinogenicity and mutagenicity [27, 28]. It also influences metabolic fate: most metabolic pathways tend to convert hydrophobic compounds into hydrophilic species; occasionally, this detoxification attempt leads to highly reactive electrophilic products that can form adducts with DNA and proteins [29]. Compounds with high log P value tend to bio-accumulate and cause long-term adverse effects and to induce cytochrome P450 enzymes, leading to potential drug interactions [30]. Therefore, a clever strategy in the design of novel CNS agents would be to explore those regions of chemical space linked to a better safety profile. For example, we have mentioned that basic drugs tend to bind unspecifically to brain tissue, thus leading to a high distribution volume, which should be compensated by administration of higher doses to achieve the desired free drug concentration, and which in turn may also lead to drug-drug interactions whenever concomitantly administered drugs compete for a given tissue. A possible strategy to reduce these effects may be to focus on neutral and acidic drug candidates. In this sense, Wager *et al*. have proposed an elegant CNS multiparameter optimization approach to balance multiple variables influencing CNS drug-likeness (holistic alignment of attributes) without the penalty of strict cutoffs for single properties: since multiple parameters are considered concurrently, many possible ways to achieve the same result (a desired score of the multiparameter algorithm) may be visualized [31]. A series of novel anticonvulsants was discovered by Talevi et. al by joint application of *in silico* models and rule-based ADME filters in VS campaigns Fig. (**2**) [32-34]. Note that all of them occupy the optimal chemical space for CNS drugs recently defined by Wager *et al*., having a CNS desirability score above 4. All of them but abietic acid survived a recently developed ensemble of topological models to identify Pgp-substrates defined by our group [35].

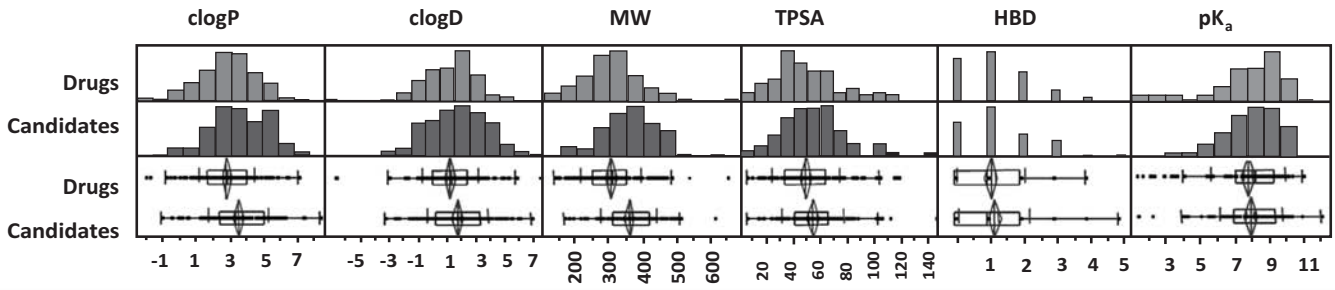

Fig. (1). Distribution of physicochemical properties and features related to distribution, safety and efficacy of CNS drugs. 119 marketed CNS drugs are compared to 108 CNS candidates. Extracted from Reference [26]. Reproduced under permission of the American Chemical Society.

	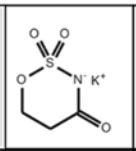	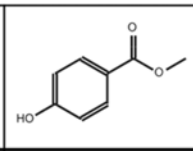	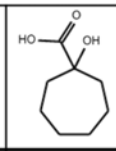	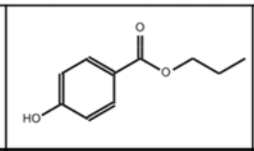	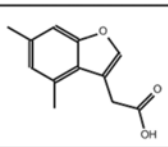	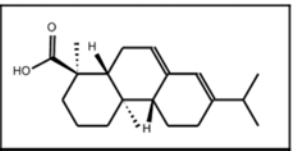
MW	151.16	152.16	158.22	180.22	204.22	274.44
pKa	3.03	8.50	4.15	8.50	4.84	4.54
clogP	-1.07	1.70	1.76	2.62	2.61	3.93
clogD	-1.84	1.66	-1.29	2.58	0.09	1.16
TPSA	69.67	46.53	60.36	46.53	53.27	37.30
nHDon	1	1	2	1	1	1

Fig. (2). Relevant properties to define CNS drug-likeness, for a set of novel anticonvulsants identified through virtual screening. Note that all of them have a CNS desirability score above 4, as defined by the recent work of Wager *et al.*

Another central aspect to be taken into account in modern CNS drug discovery is the interaction of CNS candidates with uptake and efflux transporters expressed in the BBB and CNS cells. Regarding uptake transporters, since they are involved in the facilitated penetration of physiological compounds (e.g. aminoacids and sugars) into the brain, they can be exploited by designing drugs which mimic their natural substrates, as it has already been proved by the experience with L-dopa and gabapentin, which take advantage of the large neutral aminoacid transporter 1 [25]. In this respect, the preparation of esters of antiepileptic agent valproic acid and myo-inositol has been reported Fig. (**3**) [36-38], aiming to capitalize the active influx of inositol stereoisomers, which conducts to brain levels of these sugars 100-fold greater than those found in the periphery [39].

In relation to efflux transporter expression, recent studies demonstrate that Breast Cancer Resistance Protein (BCRP) is surprisingly the most expressed ABC transporter at the BBB, at both protein and RNA levels, followed by P-glycoprotein (Pgp) (previously, it was believed that Pgp was the most expressed efflux transporter at the BBB) [40-42]. ABC efflux transporters are not only an obstacle in achieving therapeutic concentrations of free drug in the brain, but also a source of potential drug to drug interactions whenever two good Pgp-substrates are administered together. In fact, regulatory agencies have recognized the need of more complex pre-clinical trials to predict potential drug interactions, with an emphasis on *in vitro* assessment of Pgp- and CYP450-mediated interactions [43-45], while some researchers have recently begun to address the necessity of *in vitro* studies to assess potential interactions comprising other important drug transporters and support regulatory submissions and drug registration [46]. On the other hand, cumulative evidence indicates the inter-relation between the expression levels of different ABC transporters. Cisternino *et al.* reported that the expression of abcg2 mRNA was three times higher in Pgp-deficient mice than in wild-type mice [47]. Bark *et al.* proved that BCRP expression was significantly down-regulated (in drug-free state) in a doxorubicin-resistant lung cancer cell line which over-expresses Pgp (compared to the wild-type); what is more, the BCRP expression was restored in the presence of Pgp inhibitors and ABCB1 siRNA, and down-regulation was not observed in the presence of doxorubicin [48]. Similar observations between the expression levels of Pgp and members of the ABCC family had been previously observed in acute myelogenus leukemia sublines and childhood neuroblastoma [49, 50]. These data suggest that the altered expression of a given ABC transporter can be compensated by other ABC transporters [51], and that co-expression patterns depend on environmental factors (e.g. drug exposure). A second source of complexity comes from the fact that the effect of ABC transporters is synergic rather than additive when a given compounds is transported by more than one transporter [52]. Polli *et al*. observed that

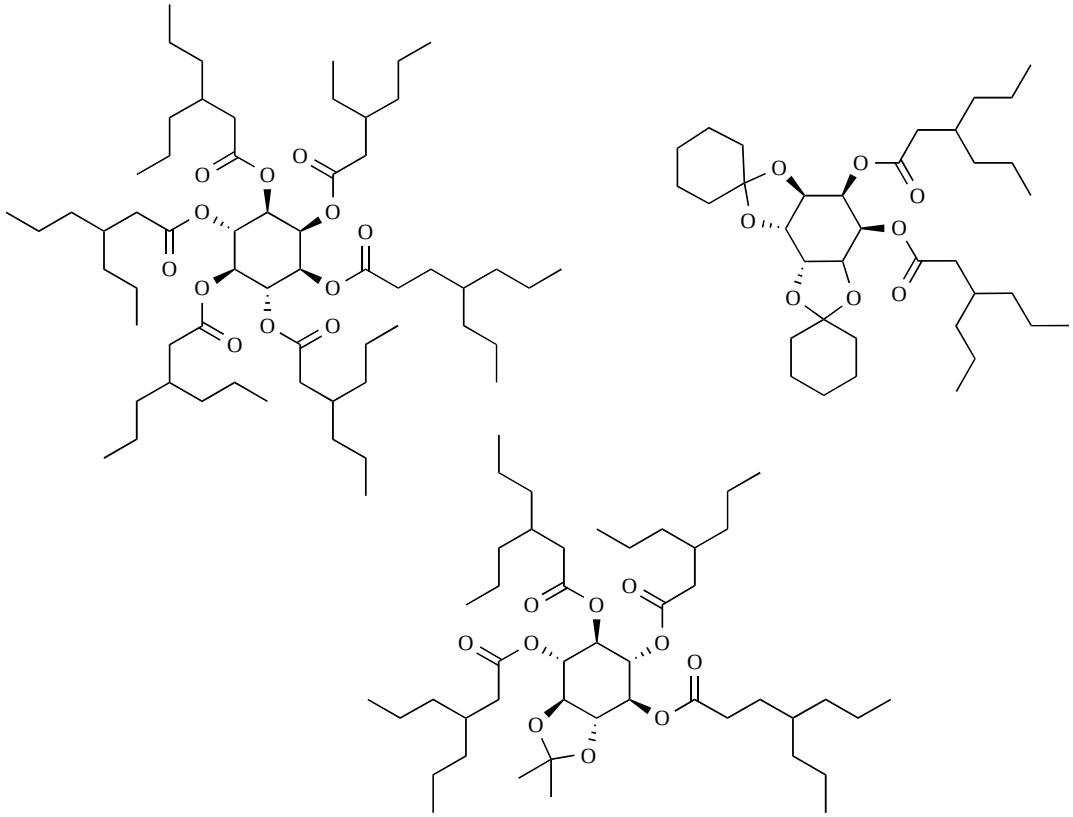

Fig. (3). Prodrugs of valproic acid and myo-inositol.

lapatinib brain-to-plasma ratio was 3- to 4- times higher in Mdr1a/b knockout mice compared to wild type mice; however, although Bcrp1 knockout mice showed no difference in the lapatinib brain-to-plasma ratio, triple knockout mice ($Mdr1a/b^{-/-}Bcrp^{-/-}$) presented a surprising 40-fold higher brain-to-plasma ratio than wild-type [53]. Similar results were obtained with topotecan by de Vries *et al*. [54]. The general strategies explored in the last fifteen years aiming to circumvent ABC efflux transporters can be synthesized in the following three approaches: a) modulation of ABC transporters function or expression; b) design of novel drugs which are not recognized by ABC transporters (disregarding early good Pgp- and BCRP- substrates in drug discovery screens) and; c) carrier-mediated transport (a "Trojan horse" approach, using different vehicles, e.g. nanosystems, to hide a substrate from its transporter).

Regarding transporters modulation, the most explored approach points to the development of transporter inhibitors. Although pre-clinical and initial clinical results of first- and second-generation Pgp-inhibitors as adjunct anticancer therapies have been encouraging, some trials stopped at phase III due to serious side effects [55-57], even though trials continue in order to find more effective and safe inhibitors for Pgp and other transporters [55, 58]. It should be remembered that substrates of ABC transporters do not only include drugs but also endogenous compounds (e.g. waste products) and general toxins; furthermore, the ubiquitous presence of Pgp and BCRP in the body should be taken into consideration: it is unlikely that inhibition of these transporters will be CNS-specific. Inhibition of these transporters would result in alteration of the whole body pharmacokinetics. Pgp also seems to play an important role in the inflammatory response to several stress and harmful stimuli, and, apparently, in neurodegenerative diseases such as Alzheimer's and Parkinson's disease [59]. Permanent impairment or disruption of the BBB biochemical barrier and exchange functions is likely to result in severe side effects (especially thinking of chronic brain disorders which demand long-term treatment). What is more, direct transporter inhibition is a one-way road, and apparently, certain conditions (e.g. Alzheimer's disease) may improve through the improvement (and not impairment) of transporter activity. Recent research has then focused on elucidating intracellular signaling pathways that control ABC transporters (their expression, intra-cellular trafficking, activation and inactivation). It is proposed that finding the molecular switches of these transporters will allow selective modulation of their activity and/or expression for therapeutic purposes in different clinical scenarios [59], which includes turning the efflux mechanisms off for short, controlled periods of time or increase the efflux activity when needed.

In contrast, the virtual screening or computer-aided design of novel drugs which are not recognized by ABC transporters may provide delivery of a drug to the brain without the toxic issues associated to the impairment of the biochemical barrier component of the BBB [60, 61]. It was not until lately that a 3.8 A° crystal structure of Pgp was reported [62]. Most of the computational models to recognize Pgp substrates are thus ligand-based models, with recent exceptions based on homology modeling [63]. Most

of them are descriptor-based 3D QSAR models or pharmacophoric hypothesis [64-75] which can be used in computer-assisted drug design but might pose efficiency problems to explore large chemical databases through virtual screening. However, more simple models capable of fast exploration of large chemical repositories, such as "simple-rule"-based methods and conformational-independent models have also been developed [35, 76, 77]. Remarkably, the modeling efforts aimed to early identification of BCRP substrates and non-substrates remain sparse.

Finally, different carrier systems aimed to brain delivery have been tested, among them nanosystems (nanoparticulate systems, nanogels, lipid nanocapsules, microgels, hydrogels, liposomes, prodrugs and inclusion complexes) [78-80]. Given the complexity of the CNS, a conservative choice of materials must be performed when targeting brain diseases, especially those which require long therapies [81]. Carriers must then be particularly safe and fully biodegradable, producing well-characterized, harmless degradation products. An exhaustive review of all the carriers that have been tested in brain drug targeting would deserve at least an entire article, so we will include some examples just to illustrate the concept, although many more can be found in literature. Using solid lipid nanoparticles, Chattopadhyay *et al*. improved cellular accumulation of atazanavir and rhodamine-123 (a well-established Pgp-substrate) in a human endothelial cell line, demonstrating that Pgp activity can be bypassed by formulations based on solid lipid nanoparticles [82]. Pluronic micelles increased drug permeability in bovine brain microvessel endothelial cells and Caco-2 cells of a series of diverse compounds, among them well-known Pgp substrates such as rhodamine-123, doxorubicin, etoposide and taxol [83, 84]. The effect of pluronics in drug disposition was more pronounced in Pgp-substrates. It was also shown that, while pluronics increased the drug delivery to the brain in wild-type mice, this benefit was not observed in mdr1a/b knockout mice [85]. It should be highlighted that in the last few years several studies show that, besides helping bypassing Pgp, many pharmaceutical excipients which are usually incorporated into carrier-systems can inhibit or modulate Pgp function by different mechanisms [78]. For example, it has been proposed that surfactans such as Span 80, Tween 20 and Tween 80 can disrupt the lipid arrangement of the cellular membrane, which could explain their modulatory effect on Pgp activity [86]. Pluronics effects seem to be related to ATP depletion [87]. This kind of modulation is interesting since it may transiently increase drug permeability without the undesired effects of direct inhibition.

3. LIMITATIONS OF CURRENT PRECLINICAL MODELS

The Innovative Medicines Initiative strategic research agenda draws attention to brain disorders as one of the areas which currently needs better predictivity in efficacy evaluation [88]. Current treatments for brain disorders are largely symptomatic and there is an urgent need of disease-modifying therapies and to increase efficacy and tolerability of symptomatic treatments. The agenda underlines the development of model systems that translate to human pathology as one of the priority research areas and, as a matter of fact, it recommends using tissue of human origin whenever possible.

One of the biggest concerns within CNS drug discovery environment is that *in vitro* studies are frequently conducted using healthy tissues [15], while dysfunctional or modified BBB features are implicated (either as determinants or consequence) in brain disorders and in multi-drug resistance issues linked to brain pathologies. There are many examples of such alterations throughout different conditions. Cumulative evidence shows, for instance, that P-gp. BCRP and members of the ABCC family are over-expressed in brain microvessels and brain cells from epileptic patients with refractory epilepsy [89-94]. Alzheimer's disease (AD) is characterized by neuronal loss, senile plaques and cerebrovascular deposits. Compromised BBB has been described in AD brain, transgenic AD animal models and cellular cultures [95]. Marco and Skaper showed that the amyloid-β peptide (the major component of senile plaques and cerebrovascular deposits) alters the expression patterns of TJ proteins in endothelial cells isolated from rat brain microvessels [96]. Alterations in the BBB amyloid-β transporters may also contribute to AD pathogenesis [97]. Gonzalez-Velásquez *et al*. demonstrated that small soluble amyloid-β aggregates are potent stimulators of endothelial permeability [95]. Up-regulation of several ABC transporters at brain micro-vessels has been found in different models of stroke [98-100] and altered expression and localization of TJs proteins has also been observed in both *in vitro* and *in vivo* models after hypoxia [101-103].

A similar point could be raised regarding the limitations of animal models of chronic or degenerative brain conditions which are based on mimicking symptoms of the disease in healthy animals. Antiepileptic drugs are again a good example of this subject. Most marketed anticonvulsants have been identified in animal models of epileptic seizures rather than animal models of epilepsy (which is defined by spontaneous, recurrent seizures) [104-106]. Thus, it should not be surprising that current pharmacological therapies fail to modify the progression of the disease, and only provide symptomatic control. The use of chronic animal models of epilepsy (e.g. kindling) in the early stages of antiepileptic drugs screening has been suggested to develop disease-modifying therapies [104]. It has also been commented that definition of experimental seizures has focused on specific types of motor seizures, ignoring short non-convulsive seizures which resemble some types of human epilepsy [105].

We may finally mention two additional reasons that may limit the predictivity of animal models of CNS disorders. The first of them relates to the inability of animals to communicate, i.e. the difficulty to accurately translate certain animal behaviors into symptoms or side-effects. Symptoms such as guilt or suicidality are practically impossible to model [7], as well as subjective neurological toxic reactions such as headache, dizziness and hallucinations [107]. The other cause of lack of predictivity relates to inter-species variability. For example, a quantitative proteomic analysis revealed that Pgp is the most expressed efflux transporter at mouse brain microvessels, with expression levels 3-fold above those of bcrp [108]. Nevertheless, as we have already

Table 1. Comparison Between Expressed ABC Transporters (at Protein level) in Human- and Mouse-Isolated Brain microvessels (Taken from data from Kamiie *et al.* and Uchida *et al.*)

Transporter	mol/μg Protein		Fold Difference Human/Mouse
	Human	Mouse	
ABCA8/abca8b	1.21	< 0.0324	> 37.40
ABCA8/abca8a	1.21	< 0.144	> 8.40
ABCA8/abca9	1.21	< 0.752	> 1.61
BCRP/bcrp	8.14*	4.41	1.85
MDR1/mdr1a	6.06**	14.1	0.43
MRP4/mrp4	0.195**	1.59	0.12

Note that human homologs are written in capital letters, whereas mouse homologs are written in lower-case letters. The data represent the mean of 5-7 human donors and 6 mice. *Indicates a significant difference between protein expression in humans and mice, at a 0.01 level. **Indicates significant difference at a 0.001 level. Extracted from the original Table published by Uchida *et al*. With permission from Wiley.

mentioned, a similar study reported that BCRP was the most expressed ABC transporter at human BBB (Table **1**) [41, 42]. Another good example of inter-species variability is the pregnane X receptor (PXR). This nuclear receptor is a transcription factor activated by a wide diversity of endogenous and exogenous compounds, including steroids, anticonvulsants, HIV protease inhibitors, dietary compounds, antibiotics and many others [51]. PXR targets genes that are responsible for phase I and phase II metabolic enzymes and efflux transporters, being considered a master regulator of defenses against xenobiotics [51, 109]. Although the DNA binding domain of PXR is highly conserved across species, the ligand binding domain is not, resulting in substantial species differences in ligand affinities for rodent vs. human PXR [51, 110]. Moreover, inter-species variability may not only occur at the expression level but also at the activity level. Even though, in general, a good correlation was observed between the Pgp-mediated transport in MDCK cells expressing human and mouse mdr1 [111], some compounds such as diltiazem exhibit important differences across species [112]. Using various models, animal ages, multiple efficacy end-points and diverse species has been suggested as a strategy to minimize the effect of inter-species variability [7]; the use of non-human primates might be useful to reproduce neuropathological and subjective responses.

4. BIOMARKERS

An important note should be made regarding the development of biomarkers in brain diseases [88], with special interest in pre-symptomatic and surrogate markers of disease progression which –by replacing or anticipating clinical outcome- would be especially helpful to establish early proof-of-concept in those diseases with long natural history and silent, asymptomatic periods. Biomarkers of receptor occupancy are also important in the case of CNS disorders, where the molecular targets are located in one of the most unreachable compartments in the body due to the multifaceted barrier created by BBB. The most direct (although expensive and laborious) estimate of receptor occupancy is the use of positron emission tomography (PET) scanning using high-specific radio-labeled ligands [6, 113], which can be used whenever suitable PET tracers are available. CSF sampling constitutes a less direct, but also less expensive approach [6]. Examples of efficacy markers are the use of magnetic resonance imaging (MRI) to evaluate the periodic appearance of demyelinating lesions during relapsing-remitting multiple sclerosis [114], quantification of brain amyloid burden by PET imaging and volumetric MRI in AD [115, 116], among many others. A much more detailed analysis on CNS biomarkers perspectives can be found in a number of recent reviews [6, 117, 119-121]. Table **2** presents a summary of advantages and disadvantages of some biomarker technologies as well as examples of some applications. Most of the information in the table has been gathered from refs 6 and 117, and from Tarawneh & Holtzman and Bieck & Potter [121, 122].

5. VIRTUAL SCREENING AND COMPUTER-ASSISTED DESIGN OF MULTI-TARGET-DIRECTED-CNS DRUGS

As it has been already mentioned, the multi-target-directed ligands have been regarded as magic shotguns [14], in contrast to the previously dominant paradigm of “magic bullets”. Another way of conceiving this strategy is to resort to the traditional key and lock paradigm and think of a “master key” capable of eliciting the correspondent response in a number of different locks. The use of this strategy makes sense in those therapeutic categories where the target may be subject to compensatory or neutralizing actions or when the target disorder emerges from a complex combination of multiple factors. We may mention several examples of therapeutic categories where multi-target drugs have proven or might prove successful, among them antiinfectives, anticancerigens and, interestingly to the scope of this review, several compounds for the treatment of CNS disorders of complex pathophysiology, such as Alzheimer and Parkinson diseases [122-125]; epilepsy [126]; depression, psychosis and bipolar disorder [7, 127-130]. In epilepsy, for instance, it has been observed that therapies combining multiple drugs acting through different mechanisms may be beneficial [131]. Development of multi-target antiepileptic drugs may

Table 2. Summary of Advantages and Disadvantages of Some CNS Biomarkers

Biomarker	Advantages	Disadvantages	Examples of Applications
Electroencephalography	Non-invasive, safe, cost-effective	Prone to artifacts, not fully standardized, difficult to establish associations to specific mechanisms (though rencently developed acquisition and processing tools raise interesting possibilities such as source-localization analysis and topographical images)	Increased delta-power of the centrotemporal and posterior fields has been observed in patients with mild cognitive impariment; increased theta activity is observed in very early dementia; decreased beta power has been dsecribed in individuals with mild AD
Measurement of concentrations in CSF	Biochemical changes in the brain extracellular fluid are reflected in the CSF, assessment of drug concentrations in central compartment provides valuable pharmacokinetical data and allows differentiation of pharmacokinetic and pharmacodynamic issues. Inexpensive	Invasive, sampling requires lumbar puncture	AD patients with dementia show increases in P- and T-tau and decrease in Abeta-42.
Functional and structural MRI	Safe, generally available, non-invasive	Expensive. Motion artifacts in agitated patients	Evaluation of cerebral atrophy, cortical thinning and mapping of cerebral perfusion in neurodegenerative disorders
PET imaging	Non-invasive, direct measure of availability of a drug in its site of action and receptor occupancy, high sensitivity,	Exposure to significant radiation levels, very expensive, limited number of radiotracers	High retention of amyloid PET ligand was observed in patients with mild cognitive impairment that evolves into AD

then prove to be a good alternative to polypharmacy, covering diverse targets through monotherapy.

Polivalent drugs can be directed to different binding sites of a single protein, to different targets belonging to the same family or even to different targets from different protein families [132]. Most of the multi-target drugs approved or in advanced stage of development aim to members of a single family: there are of course better chances of finding or designing a multi-target agent when binding sites are conserved through the intended targets, which is often observed among members of a protein family. Stephenson *et al*., however, have searched for common peptide motifs among key components of multiple biochemical pathways involved in AD pathophysiology, identifying common potential binding sites among more than 40 of those proteins, which may then (in theory) be targeted by a single agent [133]. Whenever multiple members of a single family are being targeted, it may be convenient to check the selectivity of the drug in relation to other non-targeted family members [132].

In silico approaches to develop multifunctional agents can be classified in two strategies [132]. On the one hand, the combinatorial approach, in which parallel VS searches against each target of interest are conducted, retaining those hits that simultaneously gather all the structural requisites needed to interact with each individual target. In other words, the common hits from parallel VS searches (one for every model associated to a particular target) are retained. In the background of multi-target drug discovery, the virtual screening for ligands for each individual target must be highly-sensitive (i.e., a reduced number of false negatives should be observed) since the collective retrieval rate for multiple targets will tend to be relatively low than when aiming to individual targets (one might speculate that, naturally, it is more difficult to find compounds that selectively interact with different targets without being excessively promiscuous). In contrast, when drugs that selectively interact with a single target are being searched, in some contexts one might sacrifice sensitivity in order to gain specificity. The second strategy is the fragment-based approach. Here, multiple elements or scaffolds that bind to each of the targeted targets are combined (usually through a linker) into a single, often larger molecule. The main drawback of this later approach relates to the poorer pharmacokinetic and toxicological profile of the final drug. Unless small, highly specific blocks/fragments are combined, it is unlikely that a given compound will gathered the already discussed features for a CNS drug-like drug (which can be translated into a CNS desirability score above 4). While the application of VS seems suitable in the case of the combinatorial approach, computer-aided design based on

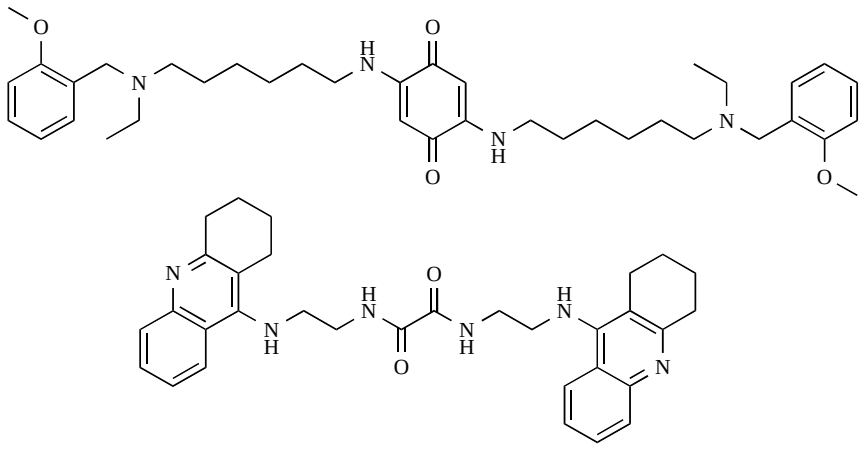

Fig. (4). Trifunctional drug candidates for the tratment of Alzheimer´s disease.

docking and/or pharmacophores may be more adequate in the case of the fragment-based approach.

For example, memoquin is a multi-target directed ligand intended to treat AD", designed on the basis of caproctamine [134]. Caproctamine can act, simultaneously, as an acetylcholinesterase inhibitor (AChEI) and a muscarinic M_2 receptor antagonist. It therefore elevates acetylcholine (ACh) levels by inhibiting its catabolism and inducing their release in the synapse. What is more, molecular modeling studies suggested that due to its dimeric flexible structure it can interact with both active and peripheral AChEI sites [135]. Since it has been observed that AChE promotes formation of the senile plaque [136] and that the interaction between the enzyme and amyloid β seems to occur at a peripheral site of the enzyme [137], caproctamine may then alleviate the cognitive impairment and also alter the progression of the disease. Memoquin was obtained by the replacement of polymehtylene chain by a benzoquinone nucleous, with the intention of conferring antioxidative properties to the drug (see Fig. **4**). A similar strategy has been used to generate trifunctional bis-tacrines capable of inhibiting AChE, reversing AChE-induced amyloid fibrillogenesis and chelating metals [138] Fig. (**4**), whose homeostasis is altered in neurodegenerative disorders and may play a role in the aetiology of the AD [139].

6. CONCLUSIONS

CNS drug discovery is characterized by a particularly high attrition rate compared to drugs targeting peripheral disorders. A number of explanations to this fact may be presented, among them the particular complexity of neurological and psychiatric disorders (whose etiology often involves the concerted action of multiple interrelated factors, both intrinsic and environmental). The highly selective barrier posed by the BBB, which restricts the rate and amount of drug reaching its molecular target/s, is also a critical matter that should be and is being considered at early stages of development (i.e. lead selection), with emphasis on passive permeability of the drug, interaction with influx and efflux transporters and propensity to unspecific tissue-binding. Interestingly, a natural association exists between certain physicochemical features and different aspects of an ideal CNS drug. For instance, some properties linked to recognition by the most expressed transporters at the BBB (high lipophilicity, high molecular weight) are also associated to unfavorable safety events: long residence time within the body, adduct formation following phase I metabolic reactions, mutagenicity and carcinogenicity. The recent work from Wager *et al*. defines an optimal region of the chemical space for CNS compounds. This region can be reached in different ways by simultaneously optimizing some of the physicochemical properties associated to CNS drug issues.

Back in the 1990s, around one third of the attrition causes (considering both CNS and non-CNS candidates) were linked to pharmacokinetic issues. By applying *in vitro* and *in silico* ADME filters (e.g. Lipinski's rules), the ADME-related project terminations were reduced to only 10%. At present, more specific rules and models for the selection of CNS candidates can be applied to reduce CNS candidates' late-attrition rates. These include cell cultures of human brain endothelial tissue that include other elements from the neurovascular unit, transport assays to assess BCRP- and Pgp-mediated transport, *in silico* models to recognize Pgp-substrates and the aforementioned Wager CNS desirability score. Recent studies underline the importance of the BCRP at the BBB and the interrelation between expression levels of different ABC transporters. Therefore, prediction and evaluation of Pgp-mediated transport should be always complemented by prediction and evaluation of BCRP-mediated efflux.

Regarding limitations of preclinical models that acquaint for part of the translational issues in CNS development, we may highlight the use of healthy tissue in many *in vitro* assays (whose features are remarkably different from those of pathologic tissues throughout many CNS disorders), inter-species variability and the inability of animals to express certain subjective symptoms and side-effects. The use of human tissue and multiple species at preclinical stage is recommended whenever possible. The development and application of receptor occupancy- and efficacy-biomarkers are fundamental to gather early proof-of-concept, especially for the many CNS diseases which are characterized by long natural history and asymptomatic periods. The joint use of

such biomarkers and chronic animal models may be valuable to shift the marketed drugs from symptomatic drugs to disease-modifying therapies.

At last, the multifactorial aetiology of many CNS disorders (for instance, neurodegenerative and psychiatric conditions) has determined a shift of paradigm from "magic bullet", single-mechanism-drugs to multi-target-directed ligands capable of dealing simultaneously with two or more determinants of the symptoms and progression of CNS diseases, a strategy that has already proven successful in the treatment of psychiatric disorders. When virtual screening approaches are applied in the search of multi-target agents, the different models linked to single mechanisms of action should be highly sensitive. If specific members of a protein family are being targeted, the candidates should be tested against other members of the same family in order to assure the desired selectivity.

ACKNOWLEDGEMENTS

The authors would like to thank the American Chemical Society for the permission to reproduce Fig. (**1**), and Dr. Travis Wager for kindly providing a high-resolution copy of that figure. The authors would also like to thank Wiley Publishers for the permission to reproduce the content of Table **1**.

A. Talevi, C. L. Bellera, M. E. Di Ianni and E. A. Castro thank CONICET and National University of La Plata (UNLP).

M. Gantner thanks CIC for her doctoral fellowship.

A. Talevi and L. E. Bruno-Blanch thank ANPCyT (PICTs 2010-2531 and 2010-1774).

L. E. Bruno-Blanch thanks UNLP (Incentivos X-597, X-510) and CONICET (PIP 11220090100603).

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

PATIENT CONSENT

Declared none.

REFERENCES

[1] Morgan, S.; Grootendorst, P.; Lexchin, J.; Cunningham, C.; Greyson, D. The cost of drug development: A systematic review. *Health Policy*., **2011**, *100*, 4-17.

[2] Paul, S. M.; Mytelka, D. A.; Dunwiddie, C. T.; Pesinger, B. H.; Munos, B. H.; Lindborg, S. R.; Schacht, A. L. How to improve R&D productivity: The pharmaceutical industry´s grand challenge. *Nature Rev. Drug Discov*., **2010**, *9*, 203-214.

[3] Pammolli, F; Magazzini, L; Riccaboni, M. The productivity crisis in pharmaceutical R&D. *Nature Rev. Drug Discov.*, **2011**, *10*, 428-438.

[4] Koenig, J. Does process excellence handcuff drug development? *Drug Discov. Today*, **2011**, *16*, 377-381.

[5] United States Government Accountability Office (GAO) Report to Congressional Requesters. New Drug Development. Science, business, regulatory, and intellectual property issues cited as hampering drug development efforts, **2006**.

[6] Hurko, O.; Ryan, J. L. Translational research in Central Nervous System drug discovery. *NeuroRx*, **2005**, *2*, 671-682.

[7] Pangalos, M. N.; Schechter, L. E.; Hurko, O. Drug development for CNS disorders: strategies for balancing risk and reducing attrition. *Nat. Rev. Drug Discov*., **2007**, *6*, 521-532.

[8] Wager, T. T.; Villalobos, A.; Verhoest, P. R.; Hou, X.; Shaffer, C. L. Strategies to optimize the brain availability of central nervous system drug candidates. *Expert Opin. Drug Discov.*, **2011**, *6*, 371-381.

[9] Tang, Z.-M. New drugs approved by US FDA in 2007 and problems and suggestions. *Chinese J. New Drugs*, **2008**, *17*, 988-992.

[10] Franson, T. R. Has the FDA Amendments Act of 2007 impaired drug development? *Clin. Pharmacol. Ther.*, **2011**, *89*, 169-171.

[11] Corey, G. R.; Stryjewski, M. E. New rules for clinical trials of patients with acute bacterial skin and skin-structure infections: do not let the perfect be the enemy of the good. *Clin. Infect. Dis.*, **2011**, *52*, S469-S476.

[12] Filmore, D.; Thayer, A. M.; Willis, R. C. Pipeline challenges. Major pharmaceutical and biotechnology companies take a variety of approaches to remain productive. *Modern Drug Discov*., **2004**, *7*, 28-34.

[13] Musk, P. Magic shotgun methods for developing drugs for CNS disorders. *Discov. Med.*, **2004**, *4*, 299-302.

[14] Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov*., **2004**, *3*, 353-359.

[15] Jeffrey, P.; Summerfield, S. Assessment of the blood-brain barrier in CNS drug discovery. *Neurobiol. Dis*., **2010**, 33-37.

[16] Redzic, Z. Molecular biology of the blood-brain and the blood-cerebrospinal fluid barriers: similarities and differences. *Fluids Barriers CNS*, **2011**, 8, 3.

[17] Löscher, W.; Potschka, H. Role of drug efflux transporters in the brain for drug disposition and treatment of brain diseases. *Prog. Neurobiol.*, **2005**, *76*, 22-76.

[18] Begley, D. J. In: Blood-spinal cord and brain barriers in health and disease; Sharma H. S. and Westman J., Eds.; Elsevier Academic Press: San Diego, 2004; pp. 83-97.

[19] Hawkins, BT; Davis, TP. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol. Rev.*, **2005**, *75*, 173-185.

[20] Talevi, A.; Bruno-Blanch, L. E. In: The blood-brain barrier: new research. Montenegro P. A. and Juárez S. M., Eds.; Nova publishers, in press.

[21] Cardoso, F. L.; Brites, D.; Brito, M. A. Looking at the blood-brain barrier: Molecular anatomy and possible investigation approaches. *Brain Res. Rev.*, **2010**, *64*, 328-363.

[22] Maurer, T. S.; DeBartolo, D. B.; Tess, D. A.; Scott, D. O. Relationship between exposure and nonspecific binding of thirty-three central nervous system drugs in mice. *Drug Metab. Dispos.*, **2005**, *33*, 175-181.

[23] Doran, A.; Obach, R. S.; Smith,. B. J.; Hosea, N. A.; Becker, S.; Callegari, E.; Chen, C.; Chen, X.; Choo, E.; Cinfrogna, J.; Cox, L. M.; Gibbs, J. P.; Gibbs, M. A.; Hatch, H.; Hop, C. E. C. A. *et al*. *Drug Metab. Dipos.*, **2005**, *33*, 165-174.

[24] Van de Waterbeemd, H.; Smith, D. A.; Jones, B. C. Lipophilicity in PK design: methyl, ethyl, futile. *J. Comput. Aided Mol. Des.*, **2001**, 15, 273-286.

[25] Liu, X.; Chen, C.; Smith, B. J. Progress in brain penetration evaluation in drug discovery. *J. Pharmacol. Exp. Ther.*, **2008**, *325*, 349-356.

[26] Wager, T. T.; Chandrasekaran, R. Y.; Hou, X.; Troutman, M. D.; Verhoest, P. R.; Villalobos, A.; Will, Y. Defining desirable central nervous system drug space through the alignment of molecular properties, *in vitro* ADME, and safety attributes. *ACS Chem. Neurosci*,, **2010**, *1*, 420-434.

[27] Debnath, A. K.; López de Compadre, R. L.; Debnath, G.; Shusterman, A. J.; Hansch, C. Structure-activity relationship of mutagenic aromatic and heteroaromatic nitro compounds. Correlation with molecular orbital energies and hydrophobicity. *J. Med. Chem.*, **1991**, *34*, 786-797.

[28] Debnath, A. K.; Shusterman, A. J.; López de Compadre, R. L.; Hansch, C. International Comission for Protection Against Environmental Mutagens and Carcinogens. The importance of the hydrophobic interaction in the mutagenicity of organic compounds. *Mutat. Res.*, **1994**, *305*, 63-72.

[29] Testa, B.; Krämer, S. D. The biochemistry of drug meta – An introduction. Part 5. Metabolism and bioactivity. *Chem. Biodivers.*, **2009**, *6*, 591-684.

[30] Smith, C. J.; Hansch, C. The relative toxicity of compounds in mainstream cigarette smoke condensate. *Food Chem. Toxicol.*, **2000**, *38*, 637-646.¨

[31] Wager, T. T.; Hou, X; Verhoest, P. R.; Villalobos, A. Moving beyond rules: the development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. *ACS Chem. Neurosci*,, **2010**, *1*, 435-449.

[32] Talevi, A.; Cravero, M. S.; Castro, E. A.; Bruno-Blanch, L. E. Discovery of anticonvulsant activity of abietic acid through application of linear discriminant analysis. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 1684-1690.

[33] Talevi, A.; Bellera, C. L.; Castro, E. A.; Bruno-Blanch, L. E. A successful virtual screening application: prediction of anticonvulsant activity in MES test of widely used pharmaceutical and food preservatives methylparaben and propylparaben. *J. Comput. Aided Mol. Des.*, **2007**, *21*, 527-538.

[34] Gavernet, L.; Talevi, A.; Castro, E. A.; Bruno-Blanch, L. E. A combined virtual screening 2D and 3D QSAR methodology for the selection of new anticonvulsant candidates from a natural product library. *QSAR Comb. Sci.*, **2008**, *27*, 1120-1129.

[35] Di Ianni, M.; Talevi, A.; Castro, E. A.; Bruno-Blanch, L. E. Development of a highly specific ensemble of topological models for early identification of P-glycoprotein substrates. *J. Chemometr*., **2011**, *25*, 313-322.

[36] Bodor, N.; Moon, S. C.; Bruno-Blanch, L. E. Synthesis and pharmacological evaluation of prodrugs of valproic acid. *Pharmazie*, **2000**, *55*, 184-186.

[37] Moon, S. C.; Echeverria, G. A.; Punte, G.; Ellena, J.; Bruno-Blanch, L. E. Crystal structure and anticonvulsant activity of (+/-)-1,2:4,5-di-O-isopropylidene-3,6-di-O-(2-propylpentanoyl)-myo-inositol. *Carbohydr. Res.*, **2007**, *342*, 1456-1461.

[38] Bruno-Blanch, L.; Moon, S. C. U.S. Patent 7,763,650, **2010.**

[39] Fenili, D.; Weng, Y-Q.; Aubert, I.; Nitz, M.; McLaurin, J. Sodium/myo-Inositol transporters: substrate transport requeriments and regional brain expression in the TgCRND8 mouse model of amyloid pathology. *PloS One*, **2011**, *6*, e24032.

[40] Dauchy, S.; Dutheil, F.; Weaver, R. J.; Chassoux, F.; Daumas-Duport, C.; Couraud, P. O.; Schermann, J. M.; De Waziers, I.; Declèves, X. ABC transporters, cytochromes P450 and their main transcription factors: Expression at the human blood-brain barrier. *J. Neurochem.*, **2008**, *107*, 1518-1528

[41] Uchida, Y.; Ohtsuki, S.; Katsukura, Y.; Ikeda, C;. Suzuki, T.; Kamiie, J.; Terasaki, T. Quantitative targeted absolute proteomics of human blood-brain barrier transporters and receptors. *J. Neurochem.*, **2011**, *117*, 333-345.

[42] Shawahna, R.; Uchida, Y.; Decleves, X.; Ohtsuki, S.; Yousif, S.; Dauchy, S.; Jacob, A.; Chassoux, F.; Daumas-Duport, C.; Couraud, P.-O.; Terasaki, T.; Scherrmann, J.-M. Transcriptomic and quantitative proteomic analysis of transporters and drug metabolizing enzymes in freshly isolated human brain microvessels. *Mol. Pharm.*, **2011**, *8*, 1332-1341.

[43] US Food and Drug Administration. Draft Guidance for Industry: Drug Interaction Studies – Study Design, Data Analysis, and Implications for Dosing and Labeling, **2006**.

[44] European Medicines Agenc. Guideline on the Investigation of Drug Interactions, **2010**.

[45] Zhang, l:; Zhang, Y.; Strong, J. M.; Reynolds, K. S.; Huang, S. –M. A regulatory viewpoint on transporter-based drug interactions. *Xenobiotica*, **2008**, *38*, 709-724.

[46] Elsby, R.; Fox, L.; Stresser, D.; Layton, M.; Butters, C.; Sharma, P.; Smith, V.; Surry, D. *In vitro*risk assessment of AZD9056 perpetrating transporter-mediated drug-drug interaction with methotrexate. *Eur. J. Pharm. Sci.*, **2011**, *43*, 41-49.

[47] Cisternino, S.; Mercier, C.; Bourasset, F.; Roux, F.; Scherrmann, J. M. Expression, up-regulation, and transport activity of the multidrug-resistance protein abcg2 at the mouse blood-brain barrier. *Cancer Res.*, **2004**, *64*, 3296-3301.

[48] Bark, H.; Xu, H. D.; Kim, S. H.; Yun, J.; Choi, C. H. P-glycoprotein down-regulates expression of breast cancer resistance protein in a drug-free state. *FEBS Lett.*, **2008**, *582*, 2595-2600.

[49] Choi, C. H.; Kim, S. H.; Rha, H. S.; Jeong, J. H.; Park, Y. H; Min, Y. D.; Kee, K. H.; Lim, D. Y. Drug concentration-dependent expression of multidrug resistance-associated protein and P-glycoprotein in the doxorubicin-resistant acute myelogenous leukemia sublines. *Mol. Cell*., **1999**, *9*, 314-319.

[50] Bordow, S. B.; Haber, M.; Madafiglio, J.; Cheung, B.; Marshall, G. M.; Norris, M. D. Expression of the multidrug resistance-associated protein (MRP) gene correlates with amplification and overexpression of the N-myc oncogene in childhood neuroblastoma. *Cancer Res.*, **1994**, *54*, 5036-5040.

[51] Miller, D. S.; Bauer, B.; Hartz, A. M. S. Modulation of P-glycoprotein at the blood-brain barrier: opportunities to improve CNS pharmacotherapy. *Pharmacol. Rev.*, **2008**, *60*, 196-209.

[52] Miller, D. S. Regulation of P-glycoprotein and other ABC drug transporters at the blood-brain barrier. *Trends Pharmacol. Sci*., **2010**, 31, 246-254.

[53] Polli, J. W.; Olson, K. L.; Chism, J. P.; St. John Williams, L.; Yeager, R. L.; Wooddard, S. M.; Otto, V.; Castellino, S.; Demby, V. E. An unexpected synergist role of P-Glycoprotein and Breast Cancer Resistance Protein on the central nervous system penetration of the tyrosine kinase inhibitor lapatinib (N-{3-Chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furyl]-4-quinazolinamine; GW572016). *Drug Metab. Dispos.*, **2009**, *37*, 439-442.

[54] De Vries, N. A.; Zhao, J.; Kroon, E.; Buckle, T.; Beijnen, J. H.; van Tellingen, O. P-glycoprotein and Breast Cancer Resistance Protein: Two dominant transporters working together in limiting the brain penetration of topotecan. *Clin. Cancer Res.*, **2007**, *13*, 6440-6449.

[55] Deeken, J. F.; Löscher, W. The blood-brain barrier and cancer: Transporters, treatment and Trojan horses. *Clin. Cancer. Res.*, **2007**, *13*, 1663-1674.

[56] Lhommé, C.; Joly, F.; Walker, J. L.; Lissoni, A. A.; Nicoletto, M. O.; Manikhas, G. M.; Baekelandt, M. M.; Gordon, A. N.; Fracasso, P. M.; Mietlowski, W. L.; Jones, G. J.; Dugan, M. H. Phase III study of valspodar (PSC 833) combined with paclitaxel and carboplatin compared with paclitaxel and carboplatin alone in patients with stage IV or suboptimally debulked stage III epithelial ovarian cancer or primary peritoneal cancer. *J. Clin. Oncol.* **2008**, *26*, 2674-2682.

[57] Tiwari, A.; Sodani, K.; Dai, C. L.;: Ashby, C. R.; Chen, Z. S. Revisiting the ABCs of multidrug resistance in cancer chemotherapy. *Curr. Pharm. Biotechnol*., **2011**, *12*, 570-594.

[58] Akhtar, N.; Ahad, A.; Khar, R. K.; Jaggi, M.; Ágil, M.; Igbal, Z.; Ahmad, F. J.; Talegaonkar, S. The emerging role of P-glycoprotein inhibitors in drug delivery: a patent review. *Expert. Opin. Ther. Pat.*, **2011**, *21*, 561-576.

[59] Hartz, A. M.; Bauer, B. Regulation of ABC transporters at the blood-brain barrier: new targets for CNS therapy. *Mol. Interv*., **2010**, *10*, 293-304.

[60] Demel, M. A.; Schwha, R.; Krämer, O.; Ettmayer, P.; Haaksma, E. E.; Ecker, G. F. In silico prediction of substrate properties for ABC-mulridrug transporters. *Expert Opin. Drug. Metab. Toxicol*, **2008**, *4*, 1167-1180.

[61] Demel, M. A.; Krämer, O.; Ettmayer, P.; Haaksma, E. E.; Ecker, G. F. Predicting ligand interactions with ABC tansporters in ADME. *Chem. Biodivers.*, **2009**, *6*, 1960-1969.

[62] Aller, S.; Ward, A.; Weng, Y.; Chittaboina, S.; Zhuo, R.; Harrell, P.; Trinh, Y.; Zhang, Q.; Urbatsch, I.; Chang, G. Structure of P-glycoprotein reveals a molecular basis for poly-specific drug binding. *Science*, **2009**, *323*, 1718–1722.

[63] Tarcsay, A.; Keseru, G. M. Homology modeling and binding site assessment of the human P-glycoprotein. *Future Med. Chem*., **2011**, *3*, 297-307.

[64] Penzotti, J.; Lamb, M.; Eyensen, E.; Grootenhuis, P. A computational ensemble pharmacophore model for indetifying substrates of P-glycoprotein. *J. Med. Chem.*, **2002**, *45*, 1737-1740.

[65] Xue, Y.; Yap, C.; Sun, L.; Cao, Z.; Wang, J.; Chen, Y. Prediction of P-glycoprotein substrates by a support vector machine approach. *J. Chem. Inf. Comput. Sci*., **2004**, *44*, 1497-1505.

[66] Cerqueira Lima, P.; Golbraikh, A.; Oloff, S.; Xiao, Y.; Tropsha, A. Combinatorial QSAR modeling of P-glycoprotein substrates. *J. Chem. Inf. Model*., **2006**, *46*, 1245-1254.

[67] Ciancheta, G.; Singleton, R.; Zhang, M.; Wildgoose, M.; Giesing, D.; Fravolini, A.; Cruciani, G.; Vaz, R. A pharmacophore

hypothesis for P-glycoprotein substrate recognition using GRIND-based 3D-QSAR. *J. Med. Chem.*, **2005**, *48*, 2927-2935.

[68] Zhang, L.; Balimane, P.; Johnson, S.; Chong, S. Development of an i*n silico* model for predicting efflux substrates in Caco-2 cells. *Int. J. Pharm*., **2007**, *343*, 98-105.

[69] Li, W.; Li, L.; Eksterowicz, J.; Ling, X.; Cardozo, M. Significance analysis and multiple pharmacophore models for differentiating P-glycoprotein substrates. *J. Chem. Inf. Model.*, **2007**, *47*, 2429-2438.

[70] Chang, C.; Bahadduri, P.; Polli, J.; Swaan, P.; Ekins, S. Rapid identification of P-glycoprotein substrates and inhibitors. *Drug Metab. Dispos.*, **2006**, *34*, 1976-1984.

[71] Ekins, S.; Kim, R.; Leake, B.; Dantzig, A.; Schuetz, E.; Lan, L.; Yasuda, K.; Shepard, R.; Winter, M.; Schuetz, J.; Wikel, J.; Wrigton, S. Application of three-dimensional quantitative structure-activity relationships of p-glycoprotein inhibitors and substrates. *Mol. Pharmacol.*, **2002**, *61*, 974-981.

[72] Wang, R; Kuo, C; Lien, L; Lien, E. Structure-activity relationship: analysis of P-Glycoprotein substrates and inhibitors. *J. Clin. Pharm. Ther.*, **2003**, *28*, 203-228.

[73] Boccard, J.; Bajot, F.; Di Pietro, A.; Rudaz, S.; Bournendjel, A.; Nicolle, E.; Carript, P. A 3D linear solvatation energy model to quantify the affinity of flavonoid derivatives toward p-glycoprotein. *Eur. J. Pharm. Sci.*, **2009**, *36*, 254-264.

[74] Nakagawa, H.; Saito, H.; Ikegami, Y.; Aida-Hyugaji, S.; Sawasa, S.; Ishikawa, T. Molecular modeling of new camptothecin analogues to curcumvent ABCG2-mediated drug resistance in cancer. *Cancer Lett.*, **2006**, *234*, 81-89.

[75] Wang, Z.; Chen, Y.; Liang, H.; Bender, A.; Glen, R. C.; Yan, A. P-glycoprotein substrate models using support vector machines based on a comprehensive data set. *J. Chem. Inf. Model.*, **2011**, *51*, 1447-1456.

[76] Huang, J.; Ma, G.; Muhammad, I.; Cheng Y. Identifying P-glycoprotein substrates using a support vector machine optimized by a particle swarn. *J. Chem. Inf. Model.*, **2007**, *47*, 1638-1647.

[77] Cabrera, M.; González, I.; Fernández, C.; Navarro, C.; Bermejo, M. A topological substructural approach for the prediction of P-glycoprotein substrates. *J. Pharm. Sci*., **2006**, *95*, 589-606.

[78] Bansal, T.; Akhtar, N.; Jaggi, M.; Khar, R. K.; Talegaonkar, S. Novel formulation approaches for optimizing delivery of anticancer drugs based on P-glycoprotein modulation. *Drug Discov. Today*., **2009**, *14*, 1067-1074.

[79] Gohel, M. C.; Parikh, R. K.; Modi, S. C.; Vyas, K. G.; Sheth, K. M. Novel drug delivery approaches to bypass P-glycoprotein efflux pump. *Pharmaceutical Rev.*, **2011**, 9.

[80] Alam, M. I.; Beg, S.; Samad, A.; Baboota, S.; Kohli, K.; Ali, J.; Ahuja, A.; Akbar, M. Strategy for effective brain drug delivery. *Eur. J. Pharm. Sci.*, **2010**, *40*, 385-403.

[81] Wong, H. L.; Chattopadhyay, N.; Wu, X. Y.; Bendayan, R. Nanotechnology applications for improved delivery of antiretroviral drugs to the brain. *Adv. Drug. Deliv. Rev.*, **2010**, *62*, 503-517.

[82] Chattopadhyay, N.; Zastre, J.; Wong, H.-L.; Wu, X. Y.; Bendayan, R. Solid lipid nanoparticles enhance the delivery of the HIV protease inhibitor, atazanavir, by a human brain endothelial cell line. *Pharm. Res.*, **2008**, *10*, 2262-2271.

[83] Batrakova, E. V.; Li, S.; Alakhov, V. Y.; Miller, D. W.; Kabanov, A. V. Optimal structure requeriments for pluronic block copolymers in modifying P-glycoprotein drug efflux transporter activity in bovine brain microvessel endothelial cells. *J. Pharmacol. Exp. Ther.* , **2003**, *304*, 845-854.

[84] Batrakova, E. V.; Li, S.; Miller, D. W.; Kabanov, A. V. Pluronic P85 increases permeability of a borad spectrum of drugs in polarized BBMEC and Caco-2 cell monolayers. *Pharm. Res*., **1999**, *16*, 1366-1372.

[85] Kabanov, A. V.; Alakhov, V. Y. Pluronic block copolymers in drug delivery from micellar nanocontainers to biological response modifiers. *Crit. Rev. Ther. Drug Carrier Syst.*, **2002**, *19*, 1-72.

[86] Lo, Y. L. Relationships between the hydrophilic-lipophilic balance values of pharmaceutical excipients and their multidrug resistance modulating effect in Caco-2 cells and rat intestines. *J. Control. Release*, **2003**, *90*, 37-48.

[87] Kabanov, A. V.; Batrakova, E. V., Alakhov, V. Y. An essential relationship between ATP depletion and chemosensitizing activity of Pluronic© block copolymers. *J. Control. Release*, **2003**, *91*, 75-81.

[88] European Commission, European Federation of Pharmaceutical Industries and Associations. The Innovative Medicines Initiative (IMI) Strategic Search Agenda, **2006**.

[89] Aronica, E.; Gorter, J. A.; Ramkema, M.; Redeker, S.; Ozbas-Gerçeker, F.; van Vliet, E. A.; Scheffer, G. L.; Scheper, R. J.; van der Valk, P.; Baaven, J. C.; Troost, D. Expression and cellular distribution of multidrug resistance-related proteins in the hippocampus of patients with mesial temporal lobe epilepsy. *Epilepsia*, **2004**, *45*, 441-451.

[90] Dombrowski, S. M.; Desai, S. Y.; Marroni, M.; Cucullo, L.; Goodrich, K.; Bingaman, W.; Mayberg, M. R.; Bengez, L.; Janigro, D. Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. Epilepsia, **2001**, *42*, 1501-1506.

[91] Sisodiya, S. M.; Martinian, L.; Scheffer, G. L.; van der Valk, P.; Scheper, R. J.; Harding, B. N.; Thom, M. Vascular colocalization of P-glycoprotein, multidrug resistance associated protein 1, breast cancer resistance protein and major vault protein in human epileptogenic pathologies. *Neuropathol. Appl. Neurobiol.*, **2006**, *32*, 51-63.

[92] Aronica, E.; Gorter, J. A.; Jansen, G. H.; van Veelen, C. W.; van Rijen, P. C.; Leenstra, S.; Ramkema, M.; Scheffer, G. L.; Scheper, R. J.; Troost, D. Expression and cellular distribution of multidrug transporter proteins in two major causes of medically intractable epilepsy: focal cortical dysplasia and glioneuronal tumors. *Neurosc*., **2003**, *118*, 417-429.

[93] Ak, H.; Ay, B.; Tanrivedi, T.; Sanus, G. Z.; Is, M.; Sar, M.; Oz, B.; Ozkara, C.; Ozyurt, E.; Uzan, M. Expression and cellular distribution of multidrug resistance-related proteins in patients with focal cortical dysplasia. *Seizure*, **2007**, *16*, 493-503.

[94] Aronica, E.; Gorter, J. A.; Redeker, S.; van Vliet, E. A.; Ramkema, M.; Scheffer, G. L.; Cheper, R. J.; van der Valk, P.; Leenstra, S.; Bayeen, J. C.; Spliet, W. G. M.; Troost, D. Localization of breast cancer resistance protein (BCRP) in microvessel endothelium of human control and epileptic brain. *Epilepsia*, **2005**, *46*, 849-857.

[95] Gonzalez-Velasquez, F. J.; Kotarek, J. A.; Moss, M. A. Soluble aggregates of the amyloid-beta protein selectivelt stimulate permeability in human brain microvascular endothelial monolayers. *J. Neuroche*., **2008**, *107*, 466-477.

[96] Marco, S.; Skaper, S. D. Amyloid beta-peptide1-42 alters tight junction protein distribution and expression in brain microvessel endothelial cells. *Neurosci. Lett.*, **2006**, *401*, 219-224.

[97] Donahue, J. E.; Johanson, C. E. Apolipoprotein E, amyloid-beta, and blood-brain barrier permeability in Alzheimer disease. *J. Neuropathol. Exp. Neurol*., **2008**, *67*. 261-270.

[98] Spudich, A.; Kilic, E.; Xing, H.; Kilic, U.; Renstch, K. M.; Wunderl-Allenspach, H.; Bassetti, C. L.; Hermann, D. M. Inhibition of multidrug resistance transporter-1 facilitates neuroprotective therapies after focal cerebral ischemia. *Nature Neurosci.*, **2006**, *9*, 487-488.

[99] Dazert, P.; Suofu, Y.; Grube, M.; Popa-Wagner, A.; Kroemer, H. K.; Jedlitschky, G.; Kessler, C. Differential regulation of transport proteins in the periinfarct region following reversible middle cerebral artery occlusion in rats. *Neurosci.*, **2006**, *142*, 1071-1079.

[100] Ueno, M.; Nakagawa, T.; Huang, C. L.; Ueki, M.; Kusaka, T.; Hosomi, N.; Kanenishi, K.; Onodera, M.; Wu, B.; Sakamoto, H. The expression of P-glycoprotein is increased in vessels with blood-brain barrier impairment on a stroke-prone hypertensive model. *Neuropathol. Appl. Neurobiol.*, **2009**, *35*, 147-155.

[101] Koto, T.; Takubo, K.; Ishida, S.; Shinoda, H.; Inoue, M.; Tsubota, K.; Okada, Y.; Ikeda, E. Hypoxia disrupts the barrier function of neural blood vessels through changes in the expression of claudin-5 in endothelial cells. *Am. J. Pathol.*, **2007**, *170*, 1389-1397.

[102] Kago, T.; Takagi, N.; Date, I.; Takenaga, Y.; Takagi, K.; Takeo. S. Cerebral ischemia enhances tyrosine phosphorylation of occludin in brain capillaries. *Biochem. Biophys. Res. Commun*., **2006**, *339*, 1197-1203.

[103] Mark, K. S.; Davis, T. P. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *Am. J. Physiol. Heart Circ. Physiol.*, **2002**, *282*, H1485 H1494.

[104] Löscher, W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res.*, **2002**, *50*, 105-123.

[105] Löscher, W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. *Seizure*, **2011**, *20*, 359-368.

[106] Löscher, W.; Schmidt, D. Modern antiepileptic drug development has failed to deliver: ways out of the current dilemma. *Epilepsia*, 52, 657-678.

[107] Olson, H.; Betton, G.; Robinson, D.; Thomas, K.; Monro, A.; Kolaja, G.; Lilly, P.; Sanders, J.; Sipes, G.; Bracken, W.; Dorato, M.; Van Deun, K,; Smith, P.; Berger, B.; Heller. A. Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol.*, **2000**, *32*, 56-67.

[108] Kamiie, J.; Ohtsuki, S.; Iwase, R.; Ohmine, K.; Katsukura, Y.; Yanai, K.; Sekine, Y.; Uchida, Y.; Ito, S.; Terasaki, T. Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria. *Pharm. Res*., **2008**, *25*, 1469-1483.

[109] Dussault, I., Forman, B. M. The nuclear receptor PXR: a master regulator of "homeland" defense. *Crit. Rev. Eukaryot. Gene Expr*., **2002**, *12*, 53-64.

[110] Xie, W.; Barwick, J. L.; Downes, M.; Blumberg, B.; Simon, C. M.; Nelson, M. C.; Neuschwander-Tetri, B. A.; Brunt, E. M.; Guzelian, P. S.; Evans, R. M. *Nature*, **2000**, *406*, 435-439.

[111] Feng, B.; Mills, J. B.; Davidson, R. E.; Mireles, R. J.; Janiszewski, J. S.; Troutman, M. D.; de Morais, S. M. *In vitro* P-glycoprotein assays to predict the *in vivo* interactions of P-glycoprotein with drugs in the central nervous system. *Drug Metab. Dipos*., **2008**, *36*, 268-275.

[112] Katoh, M.; Suzuyama, N.; Takeuchi, T.; Yoshitomi, S.; Asahi, S.; Yokoi, T. Kinetic analysis for species differences in P-glycoprotein-mediated drug transport. *J. Pharm. Sci.*, **2006**, *95*, 2673-2683.

[113] Ahmed, S.; Mozley, P. D.; Potter, W. Z. Biomarkers in psychotropic drug development. *Am. J. Geriatr. Psychiatry*, **2002**, *10*, 678-686.

[114] Sormani, M.P.; Bruzzi, P.; Comi, G.; Filippi, M. MRI metrics as surrogate markers for clinical relapse rate in relapsing-remitting MS patients. *Neurology*, **2002**, *58*, 417-421.

[115] Kadir, A.; Nordberg, A. Target-specific PET probes for neurodegenerative disorders related to dementia. *J. Nucl. Med*., **2010**, *51*, 1418-1430.

[116] Nordberg, A. Amyloid imaging in early detection of Alzheimer's disease. *Neurodeger. Dis*., **2010**, *7*, 136-138.

[117] Hampel, H.; Wilcock, G.; Andrieu, S.; Aisen, P.; Blennow, K.; Broich, K.; Carrillo, M.; Fox, N. C.; Frisoni, G. B.; Isaac, M.; Lovestone, S.; Nordberg, A. *et al*. Biomarkers for Alzheimer's disease therapeutic trials. *Prog. Neurobiol.*, in press.

[118] Kantarci, K.; Jack, C. R. Jr. Neuroimaging in Alzheimer disease: an evidence-based review. *Neuroimaging Clin. N. Am*., **2003**, *13*, 197–209.

[119] Hurko, O. Biomarkers in brain disease. *Ann. N. Y. Acad. Sci*., **2009**, 1180, 1-10.

[120] Sherer, T. B. Biomarkers for Parkinson's disease. *Sci. Transl. Med*., **2011**, *3*, 79ps14.

[121] Tarawneh, R.; Holtzman, D. M. Biomarkers in translational research of Alzheimer's disease. *Neuropharmacol.*, **2010**, *59*, 310-322.

[122] Bieck. P. R.; Potter, W. Z. Biomarkers in psychotropic drug development: integration of data across multiple domains. *Annu. Rev. Pharmacol. Toxicol.*, **2005,** *45*, 227-246.

[123] Liu, Z.; Zhang, J.; Zhang, A. Design of multivalent ligand targeting G-protein-coupled receptors. *Curr. Pharm. Des.*, **2009**, *15*, 682-718.

[124] Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem*., **2008**, *51*, 347-372.

[125] Youdim, M. B. H., & Buccadfasco, J. J.. Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends Pharmacol. Sci.*, **2005**, *26*, 27-35.

[126] Bianchi, M. T.; Pathmanathan, J.; Cash, S. S.; From ion channels to complex networks: magic bullets versus magic shotgun approaches to anticonvulsant pharmacotherapy. *Med. Hypoytheses*, **2009**, *72*, 297-305.

[127] Decker, M.; Lehmann, J. Agonistic and antagonistic bivalent ligands for serotonin and dopamine receptors including their transporters. *Curr. Top. Med. Chem*., **2007**, *7*, 347-353.

[128] Wong, E. H. F; Tarazi, F. I.; Shahid, M. The effectiveness of multi-target agents in schizophrenia and mood disorders: relevance of receptor signature to clinical action. *Pharmacol. Therapeut.*, **2010**, *126*, 173-185.

[129] Stahl, S. M. Multifunctional drugs: a novel concept for psychopharmacology. *CNS Spectrums*, **2009**, *14*, 71-73.

[130] Wong, E. H. F., Nikam, S. S., & Shahid, M. Multi- and single-target agents for major psychiatric diseases: therapeutic opportunities and challenges. *Curr. Opin. Investig. Drugs*, **2008**, *9*, 28−36.

[131] Perucca, E. Clinically relevant drug interactions with antiepileptic drugs. *Br. J. Clin. Pharmacol*., **2006**, *61*, 246-255.

[132] Ma, X. H.; Shi, Z.; Tan, C.; Jiang, Y.; Go, M. L.; Low, B. C.; Chen, Y. Z. In-silico approaches to multi-target drug discovery. Computer aided multi-target drug design, multi-target virtual screening. *Pharm. Res*., **2010**, *27*, 739-749.

[133] Stephenson, V. C.; Heyding, R. A.; Weaver, D. F. The ''promiscuous drug concept" with applications to Alzheimer's disease. *FEBS Lett.*, **2005,** *579*, 1338–1342.

[134] Bolognesi, M. L.; Cavalli, A.; Melchiorre, C. Memoquin: a multi-target-directed ligand as an innvative opportunity for Alzheimer's disease. *NeuroRx*, **2009**, *6*, 152-162.

[135] Melchiorre, C.; Andrisano., V.; Bolognesi, M. L.; Budriesi, R.; Cavalli, A.; Cavrini, V; Rosini, M.; Tumiatti, V.; Recanatini, M. Acetylcholinesterase noncovalent inhibitors based on a polyamine backbone for potential use against Alzheimer's disease. *J. Med. Chem*., **1998**, *41*, 4186-4189.

[136] Alvárez, A.; Alarcon, R.; Opazo, C.; Campos, E. O.; Munoz, F. J.; Calderón, F. H.; Dajas, F.; Gentry, M. K.; Doctor, B.P.; De Mello, F. G.; Inestrosa, N. C.Stable complexes involving acetylcholinesterase and amyloid-beta peptide change the biochemical properties of the anzyme and increase the neurotoxicity of Alzheimer's fibrils. *J. Neurosci.*, **1998**, *18*, 3213-3223.

[137] Bourne, Y.; Taylor, P.; Bougis, P. E.; Marchot, P. Crystal structure of mouse acetylcholinesterase. A peripheral site-occluding loop in a tetrameric assembly. *J. Biol. Chem*., **1999**, *274*, 2963-2970.

[138] Bolognesi, M. L.; Cavalli, A.; Valgimigli, L.; Bartolini, M.; Rosini, M.; Andrisano, V. Multi-target-directed drug design strategy: from a dual binding site acetylcholinesterase inhibitor to a trifunctional compound against Alzheimer's disease. *J. Med. Chem*., **2007**, *50*, 6446-6449.

[139] Gaeta, A.; Hider, R. C. The crucial role of metal ions in neurodegeneration: the basis for a promising therapeutic strategy. *Br. J. Pharmacol..*, **2005**, *146*, 1041–1059

Received: September 15, 2011 Revised: September 29, 2011 Accepted: October 01, 2011