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Drug Discovery—Development Interface

Assessment of the Blood-Brain Barrier Permeability of Potential Neuroprotective Aurones in Parallel Artificial Membrane Permeability Assay and Porcine Brain Endothelial Cell Models

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ABSTRACT

Previously, several aurone derivatives were identified with promising neuroprotective activities. In developing these compounds to target the central nervous system (CNS), an assessment of their blood-brain barrier (BBB) permeability was performed using *in vitro* BBB models: parallel artificial membrane permeability assay-BBB which measures passive permeability and primary porcine brain endothelial cell model which enables determination of the involvement of active transport mechanism. Parallel artificial membrane permeability assay-BBB identified most compounds with high passive permeability, with 3 aurones having exceptional P_e values highlighting the importance of basic amine moieties and optimal lipophilicity for good passive permeability. Bidirectional permeability assays with porcine brain endothelial cell showed a significant net influx permeation of the aurones indicating a facilitated uptake mechanism in contrast to donepezil, a CNS drug included in the evaluation which only displayed passive permeation. From pH-dependent permeability assay coupled with data analysis using *p*CEL-X software, intrinsic transcellular permeability (P_o) of a representative aurone **4-3** was determined, considering factors such as the aqueous boundary layer that may hinder accurate *in vitro* to *in vivo* correlation. The P_o value determined supported the *in vivo* feasibility of the aurone as a CNS-active compound.

Introduction

The ability to permeate the blood-brain barrier (BBB) is an important requirement for drugs that target the central nervous system (CNS). The BBB comprises brain endothelial cells connected by extensive tight junctions that line the cerebral microvessels which play a major role in regulating the molecular traffic between the blood and the CNS. Comprehensive evaluation of the BBB permeability of a compound of interest requires determining the rate of both passive transcellular permeation across the BBB as well as the active uptake and efflux involving transporter proteins such as the P-glycoprotein (P-gp) and cation and anion transporters.[1,2] Passive BBB permeation can be assessed by *in vitro* models such as the parallel artificial membrane permeability assay (PAMPA) and a cell-based model. The study to determine the involvement of active transport and mechanism of transport however specifically requires a cell-based model which shows highly restrictive tight junctions, reflected by high transendothelial electrical resistance (TEER), and functional polarized expression of transporter proteins to mimic the *in vivo* condition.[3,4]

Recently, we have identified several aurone derivatives (Table 1) that showed anticholinesterase, monoamine oxidase inhibitory, as well as amyloid-beta aggregation inhibitory activities (K. F. Liew et al., unpublished data).[5] One aurone (**4-3**) in particular also displayed moderate metabolic stability in rat liver microsome incubation comparable to donepezil, a drug used in the treatment of Alzheimer's disease.[5] These promising attributes, some indication of drug-likeness, and the aim of developing these small molecules into potential CNS neuroprotective agents have spurred us to conduct an assessment of their BBB permeability. In this study, for BBB models, we employed PAMPA[6] and porcine brain endothelial cell (PBEC) model[7,8] which shows restrictive tight junctions, low paracellular permeability to sucrose, and functional expression of polarized uptake and efflux transporters. The PAMPA model was used to evaluate passive permeability of the aurones, while the

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0022-3549/

Table 1
Structures of Aurone Derivatives With Promising Neuroprotective-Related Activities Investigated for Their BBB Permeability in This Study

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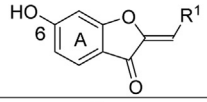
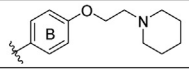
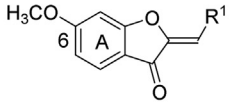
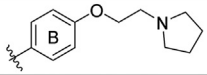
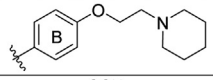
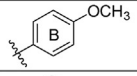
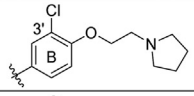
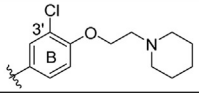
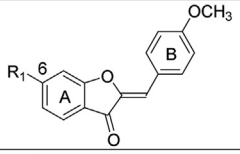
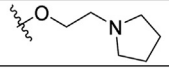
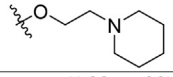
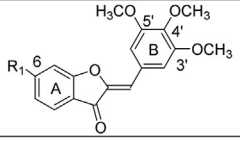
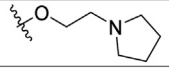
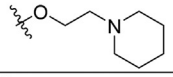
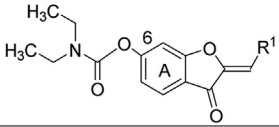
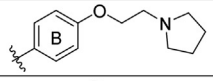
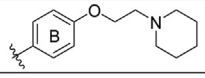
	
1-3	
	
2-2	
2-3	
2-8	
6-2	
6-3	
	
3-2	
3-3	
	
4-2	
4-3	
	
5-2	
5-3	

PBEC model was used to gain additional indications regarding the mechanism(s) of permeability to predict *in vivo* BBB permeability of the aurones.

The aqueous boundary layer (ABL), a region of poorly stirred solution adjacent to the cell layer, which is inherent in *in vitro* cell monolayer models including the PBEC model,[9] was also taken into account in this study. The ABL which is not present in BBB *in vivo* due to efficient stirring of the blood is a rate-limiting factor in *in vitro* permeability assays (Fig. 1) especially for permeability of lipophilic compounds. This factor limits prediction of *in vivo* permeability and gives rise to underestimated apparent permeability (P_{app}) measurement.[11,12] A correction for ABL thus would lead to an improved *in vitro* to *in vivo* correlation in assessing the BBB permeability of the aurones. Such correction was enabled by using the pK_a^{FLUX} method, a pH-dependent permeability assay coupled with *p*CEL-X software[12] for detailed analysis of the *in vitro* permeability data. Using this method, apparent pK_a value is determined from log permeability-pH plot. For lipophilic ionizable compounds, the appearance of a pK_a shift (to a lower value for a basic compound while a shift to a higher value for an acidic compound), termed pK_a^{FLUX}, would indicate ABL-limited permeability.[12,13] The difference between the true pK_a of a compound and its pK_a^{FLUX} enables the determination of intrinsic transcellular permeability. In this report, using the PAMPA and the PBEC models, we demonstrate the BBB permeability of the aurones and deduce the possible mechanism of their permeation.

Materials and Methods

General Details

HPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Deionized water for HPLC analysis was prepared in-house using Maxima Ultrapure water purifier system (Elgastat, Bucks, UK). Waters HPLC 600 system (Waters Corporation, Milford, MA) operated with Empower 2 workstation (Waters Corporation), comprising of Waters 474 scanning fluorescence detector (Waters Corporation) and Waters 2996 photodiode array detector (Waters Corporation), connected to Rheodyne 7725i (Cotati, CA) and a 20 μL sample loop was used for the HPLC method development and validation. Donepezil hydrochloride was purchased from Santa-Cruz Biotechnology, Inc. (Santa Cruz, CA) and theophylline and caffeine were obtained from Sigma-Aldrich (St. Louis, MO). Aurone derivatives selected for this study were synthesized as reported previously.[5]

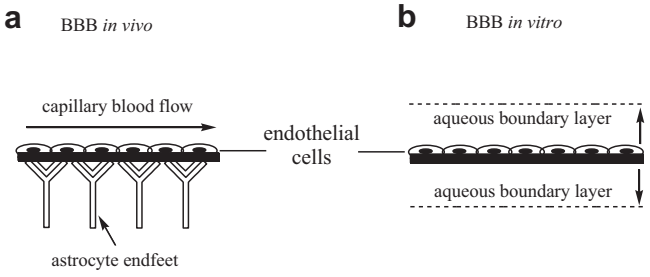


Figure 1. Comparison of the BBB *in vivo* and *in vitro*. (a) ABL is minimal due to a high-velocity capillary blood flow. (b) Presence of ABL adjacent to the cell membrane due to inefficient stirring during permeability assay (based on Youdim et al.[10]).