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## **TCP-FA4: A DERIVATIVE OF TRANLYCYPROMINE SHOWING IMPROVED BLOOD-BRAIN PERMEABILITY**

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Authors: Kelly E. Desino, Rosario Pignatello, Salvatore  
Guccione, Livia Basile, Sabah Ansar, Mary Lou Michaelis,  
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## TCP-FA4: A DERIVATIVE OF TRANLYCYPROMINE SHOWING IMPROVED BLOOD-BRAIN PERMEABILITY

Kelly E. Desino<sup>a\*§¥</sup>, Rosario Pignatello<sup>b</sup>, Salvatore Guccione<sup>b\*</sup>, Livia Basile<sup>b</sup>, Sabah Ansar<sup>c</sup>, Mary Lou Michaelis<sup>c</sup>, Rona R. Ramsay<sup>d</sup> and Kenneth L. Audus<sup>a</sup>

<sup>a</sup>The University of Kansas, Department of Pharmaceutical Chemistry, 2095 Constant Ave, Lawrence, KS 66047 (USA). <sup>b</sup>Dipartimento di Scienze Farmaceutiche, University of Catania V.le A. Doria 6, Ed. 2 Città Universitaria, I-95125 Catania (Italy). <sup>c</sup>The University of Kansas, Department of Pharmacology & Toxicology, 1251 Wescoe Hall Drive, Lawrence, KS, 66045 (USA). <sup>d</sup>Centre for Biomolecular Sciences, University of St Andrews, North Haugh, St Andrews KY169ST (UK).

\*Corresponding author: § (Present address) 100 Abbott Park Rd. Abbott Park, IL 60064. Phone: +1 (847) 938-7114; fax: +1 (847) 937-6614; E-mail: [Kelly.Desino@abbott.com](mailto:Kelly.Desino@abbott.com) (K. Desino); Dipartimento di Scienze Farmaceutiche, University of Catania, V.le A. Doria 6, Ed. 2 Città Universitaria, I-95125 Catania (Italy). Phone: +39 095 738-4020; fax: +39 095 504578; E-mail: [guccione@unict.it](mailto:guccione@unict.it) (S. Guccione).

¥ This work is part of K.E. D. PhD thesis.

**A variety of approaches have been taken to improve the brain penetration of pharmaceutical agents. The amphipathic character of a compound can improve its interaction with the lipid bilayer within cell membranes, and as a result improve permeability. Fatty acid chains or lipoamino acids of various lengths were attached to tranlycypromine (TCP), in an attempt to improve the blood-brain barrier (BBB) permeability by increasing the lipophilicity as well as the amphipathic character of the drug. TCP-FA4, one of the derivatives containing a four carbon alkyl acid chain, showed the greatest improvement in permeability. This molecule was slightly neuroprotective in a  $\beta$ -amyloid induced neurodegeneration assay and may also be capable of upregulating brain derived neurotrophic factor (BDNF), as indicated by cell culture assays using human umbilical vein endothelial cells. Since decreased levels of BDNF are observed in many CNS disorders, and direct injection of BDNF is not a viable option due to its poor permeability across the BBB, small molecules capable of regulating BDNF that also cross the BBB may be an interesting treatment option.**

Keywords: Fatty acid chains; lipoamino acids; blood-brain barrier (BBB); neuroprotection;  $\beta$ -amyloid; monoamine oxidase inhibition.

### 1. Introduction

Delivery of pharmaceutical agents to the brain is one of the most challenging areas of drug delivery. Tight junctions, minimized surface area, electrostatic interactions and increased metabolism as well as active efflux systems all work together to create the blood-brain barrier (BBB) [1-4]. Different approaches have been taken to improve the brain penetration of pharmaceutical agents. The objective of this specific study was to investigate the effect of the attachment of lipoamino acids (LAA) or fatty acid (FA) moieties on the BBB permeability of a model compound, tranlycypromine (*trans*-(+)-2-phenylcyclopropanamine, TCP). Although the conjugation of FAs increase the lipophilicity, the addition of LAAs has the added advantage of giving an amphipathic character to the drug molecules, which in turn can facilitate their interaction with cell membranes [5]. This approach to improve the permeability across

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4 biological barriers, including the BBB, has been taken before with some success [6-8]. Various  
5 approaches, both computational and experimental, were taken to predict the interaction of TCP  
6 derivatives with biomembranes (data not included) [9, 10]. The rationale behind this study was  
7 that by increasing the amphiphilic character of a compound, its interaction with the lipid bilayer  
8 within cell membranes can also be enhanced and as a result improve permeability. The proof of  
9 concept was attempted using the irreversible monoamine oxidase inhibitor (MAOI) TCP.  
10 Various derivatives of TCP were previously synthesized in which FAs or LAAs of various alkyl  
11 chain length were conjugated to the TCP amine group [10] (Fig. 1). These TCP derivatives were  
12 then tested in an *in vitro* BBB model using bovine brain microvessel endothelial cells  
13 (BBMECs). The studies showed that although the TCP derivatives containing LAA were  
14 predicted to have better permeability than their FA counterparts, on the basis of computational  
15 and experimental models [9], TCP-FA4, one of the derivatives containing a four carbon alkanolic  
16 acid residue showed the greatest improvement in permeability. The transport properties of this  
17 derivative were thoroughly characterized and are described herein.

18 Furthermore, we briefly investigated the neuroprotective properties of TCP-FA4. Although this  
19 compound was originally synthesized as a model compound to demonstrate a proof of concept,  
20 the literature suggested that TCP could have neuroprotective properties [11]. Since TCP-FA4 is  
21 predicted to have good BBB permeability, it could prove to be an important discovery if it is  
22 neuroprotective. TCP (and potentially its derivatives) could be neuroprotective as a result of their  
23 ability to induce synthesis of brain derived neurotrophic factor (BDNF) [11]. BDNF is a 27 kDa  
24 protein found within the brain and periphery that supports the survival of existing neurons as  
25 well as aids in the growth and differentiation of new neurons [12]. In recent years, BDNF has  
26 become a drug target for many CNS disorders including Alzheimer's, Parkinson's, epilepsy [12],  
27 as well as cerebral ischemia [11]. Since neurotrophins possess less than optimal pharmacological  
28 properties including poor stability and minimal BBB permeation, the discovery of small  
29 molecules that can regulate BDNF expression could become beneficial [13].

## 36 37 **2. Material and Methods**

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39 All chemicals and cell culture reagents were purchased from Sigma-Aldrich (St. Louis, MO)  
40 unless otherwise specified.

### 41 42 **2.1 Isolation and maintenance of BBMECs**

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44 Bovine brain microvessel endothelial cells (BBMECs) were isolated from the gray matter of  
45 bovine cerebral cortices by enzymatic digestion followed by centrifugation and then seeded as  
46 primary cultures following methods described by Audus *et al.* [14]. Isolated BBMECs were  
47 seeded at a density of approximately 50,000 cells/cm<sup>2</sup> onto 24-well culture plates or 100 mm  
48 culture dishes (Corning Costar, Acton, MA) containing Nucleopore® polycarbonate membranes  
49 (pore size 0.4 µm) (Whatman, Florham Park, NJ) pretreated with rat-tail collagen prepared in  
50 house and fibronectin. BBMECs were grown at 37 °C in an atmosphere of 5% CO<sub>2</sub> and 95%  
51 relative humidity. The cells were grown in 50% Minimal Essential Medium and 50% Ham's  
52 F12 supplemented with 100 µg/mL streptomycin, 100 µg/mL penicillin G, 13 mM sodium  
53 bicarbonate, 10% platelet poor horse serum, 0.5% endothelial cell growth supplement (ECGS),  
54 and adjusted to pH 7.4 with 10 mM HEPES.

## 2.2 Maintenance of HUVECs

Human umbilical vein endothelial cells (HUVECs) were obtained from American Type Culture Collection (ATCC) (Manassas, VA) and cultured in Dulbecco's modified eagle's media containing 10% fetal bovine serum, 100 units/mL of penicillin G sodium salt, 100 µg/mL of streptomycin sulfate, 2.0 g/L NaHCO<sub>3</sub>, 1.42 g/L HEPES-Na, 1% glutamine, and 1% nonessential amino acids. Cell culture media was replaced every other day after seeding until cells had grown to confluency. Cells were grown at 37 °C under 5% CO<sub>2</sub> and 95% relative humidity.

## 2.3 Rhodamine 123 uptake assay

BMECs were seeded onto 24-well plates and culture media was replaced every other day until cells formed a confluent monolayer. At the start of the experiment, BBMECs were rinsed with warm PBSA (PBS containing CaCl<sub>2</sub>, MgSO<sub>4</sub>, glucose and L-ascorbic acid) and allowed to acclimate to the warm PBSA for 10 min. The PBSA was removed and solutions of TCP derivatives (10 µM) were added to the wells and allowed to incubate for 30 min at 37 °C. Rhodamine 123 was then added to all wells at a concentration of 5 µM and cells were incubated for an additional 2 h. After the incubation, cells were quickly rinsed with ice cold PBS several times. Lysis buffer (0.5% v/v Triton X-100 in 0.2N NaOH) was then added to all wells and allowed to solubilize cells for at least 30 min. Aliquots (200 µL) were taken from all wells to determine rhodamine 123 concentration and 10 µL aliquots were taken from all wells to determine protein concentration. Rhodamine 123 concentrations were determined on a Biotek FL600 Microplate Fluorescence Reader (excitationλ: 485 nm, emissionλ: 530 nm). Protein determination was performed using a Pierce BCA Protein Assay Kit (Pierce, Rockford, IL) and samples were read on an EIA reader at 540 nm.

## 2.4 BBMEC Permeability Assay

BBMECs were grown on 0.4 µm Nucleopore® polycarbonate membranes in a 100 mm culture dish coated with rat tail collagen and fibronectin. Once cells had formed a confluent monolayer as determined by light microscopy, the membranes were transferred to Side-bi-Side™ diffusion chambers as previously described by Audus *et al.* [15, 16]. Briefly, each chamber was filled with 3 mL of PBSA and the luminal donor chamber contained 10 µM of the TCP derivative. The temperature was maintained at 37°C within the chamber with an external circulating water bath and chamber contents were stirred with Teflon® coated magnetic stir bars driven by an external console. At the various time points up to 90 min, 200 µL aliquots were removed from the receiver side and 20 µL aliquots of the donor solution were taken at time zero. For the TCP-FA4 derivative, permeability was extremely rapid, so in order to monitor permeability within a linear range, earlier time points had to be taken. For all studies with TCP-FA4, time points were instead taken at 2, 5, 10, 15, 20 and 30 min. All samples were analyzed for concentration using LC/MS/MS analysis. The integrity of the cell monolayer was tested post experiment by monitoring the permeability of [<sup>14</sup>C]-sucrose, a low permeability paracellular marker that should not readily cross the cell monolayer. All sucrose samples were analyzed for concentration by liquid scintillation counting.

Additionally, this same experimental setup was used to investigate whether there were any directional differences in transport due to membrane transporters. In these bi-directional

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4 permeability studies, compound was added to the abluminal side and the luminal side was  
5 monitored over time.

6 For temperature dependent permeability studies, the same experimental setup was used and  
7 permeation of TCP-FA4 was calculated at a range of temperatures from 4 to 37°C in order to  
8 determine an activation energy.  
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## 10 11 **2.5 TCP-FA4: Inhibition studies**

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14 Inhibition studies were performed to determine if permeation of TCP-FA4 was an active process.  
15 Inhibitors used included: 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) an anion-  
16 exchange inhibitor, ouabain, a sodium pump blocker (Na-K-ATPase), sodium azide, a metabolic  
17 inhibitor capable of depleting ATP and carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone  
18 (FCCP), an ionophore inhibitor. Inhibitor concentrations and treatment conditions were decided  
19 upon based on prior studies performed in the laboratory [17]. Permeability studies in the  
20 presence of inhibitors were conducted as described in the previous section.  
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## 23 24 **2.6 Neuronal Cell Cultures**

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26 Dissociated cortical cell cultures were established from embryonic day 18 rat fetuses recovered  
27 from pregnant Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN) as described  
28 previously [18]. After the final precipitation step, neurons were suspended in fresh Dulbecco's  
29 modified Eagle's medium/F-12 with 10% fetal bovine serum (Atlanta Biologicals,  
30 Lawrenceville, GA) and plated at a density of  $2.5 \times 10^5$  cells in 35 mm glass bottom microwell  
31 dishes (MatTek Co., Ashland, MA), coated with poly-D-lysine. Serum-containing medium was  
32 removed after 24 h, and the cells were maintained in serum-free Dulbecco's modified Eagle's  
33 medium/F-12 containing the N2 supplements. Cultures were grown at 37 °C in 5% CO<sub>2</sub> and 97%  
34 humidity as previously described [18].  
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## 38 39 **2.7 Measurement of Cell Viability**

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41 The effects of the  $\beta$ -amyloid peptide (A $\beta_{25-35}$ ) (10  $\mu$ M) and TCP and TCP-FA4 (0.1 nM to 200  
42 nM concentrations) were primarily determined by monitoring neuronal cell survival using the  
43 Live/Dead assay as previously described [19]. The A $\beta_{25-35}$  was synthesized and purified in the  
44 Biochemical Research Services Lab at The University of Kansas. We used A $\beta_{25-35}$  at a  
45 concentration of 10  $\mu$ M in the present studies based on earlier results. In previous studies we  
46 have shown that at this concentration we see A $\beta$  induced loss of cortical cells by approximately  
47 50% [20]. This level thus enables us to test drug effects that would not be possible if high A $\beta$   
48 concentration were used to destroy all cells. Following exposure to the peptides and/or test  
49 compounds, cells were labeled with 20  $\mu$ M propidium iodide (PI) and 150 nM calcein acetoxy-  
50 methylester (Molecular Probes, Eugene, OR) for 30 min at 37 °C. After incubation with the dyes,  
51 the dishes were rinsed with phosphate-buffered saline (PBS) and placed on the stage of a Nikon  
52 inverted microscope (Nikon Eclipse TE200; Nikon, Tokyo, Japan) with filters for fluorescein  
53 isothiocyanate and Texas Red. Digital images were captured and the number of viable (green)  
54 and dead (red) neurons was determined by counting the cells in 6-12 microscopic fields per  
55 culture dish in duplicate dishes for each treatment. All experimental treatments were carried out  
56 on at least two separate embryonic neuronal preparations with approximately 1500 neurons  
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4 scored under each treatment condition. The fraction of viable cells in each field was calculated  
5 based on the total number of cells counted in each field. Raw data from each experiment were  
6 combined and the significance of differences between cultures exposed to various treatments was  
7 determined using Student's *t* test.  
8  
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## 10 **2.8 Measurement of BDNF with enzyme-linked immunosorbent assay (ELISA)**

### 11

12 To determine if TCP-FA4 can increase the production of brain derived neurotrophic factor  
13 (BDNF), analysis of cell culture media post treatment with TCP-FA4 was performed. Initially,  
14 BBMECs were used for these studies; however, there was no existing literature confirming that  
15 BBMECs grown in culture synthesize or secrete BDNF. Our studies showed that in fact  
16 BBMECs do not secrete detectable levels of BDNF (detection limit: 15.6 pg/mL). Therefore,  
17 another cell culture system had to be used and based on studies by Nakahashi *et al.* [21] it was  
18 seen that human umbilical vein endothelial cells (HUVECs) synthesize and secrete BDNF [21].  
19 Ideally a brain-derived cell line would have been used for these studies, but it has been shown  
20 that neurotrophin derived from peripheral tissues is available to some peripheral neurons [12].  
21 HUVECs were grown to confluency in a 6 well tissue culture plate (approximately 5 days) then  
22 washed three times with phosphate buffered saline warmed to 37°C. Solutions of 5 µM TCP and  
23 5 µM TCP-FA4 were prepared in culture media and added to the wells (n=2). A lower  
24 concentration of TCP and TCP-FA4 was used relative to the BBMEC permeability studies to  
25 more closely reflect *in vivo* concentrations. Therapeutic plasma concentrations of  
26 tranylcypromine are reported to be approximately 1µM [22]. A slightly higher concentration was  
27 used to ensure we could detect synthesized BDNF. The remaining two wells contained blank  
28 media and served as a control. Aliquots of 200 µL were taken from the wells at 0, 24, 31 and 48  
29 h and stored at -20°C until analysis. Prior to analysis samples were centrifuged at 4°C at 1500 x  
30 g to remove any particulates. Concentrations of BDNF were measured using a commercial  
31 ELISA kit (Promega, Madison, WI). For optimal results, all samples were diluted 1:1 with the  
32 provided Block & Sample Buffer. A standard curve was created and linearity ( $R^2 = 0.99$ ) was  
33 observed from 7 to 250 pg/mL. According to the manufacturer's brochure, the assay provides  
34 specific detection of BDNF with typically less than 3% cross reactivity with other related  
35 neurotrophic factors and provides a lower detection limit of 15.6 pg/mL.  
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## 43 **2.9 Sample analysis of TCP and derivatives by LC/MS/MS**

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45 Samples generated by the permeability studies using BBMECs were analyzed by LC/MS/MS.  
46 The chromatography system consisted of a Waters 2690 system and a Phenomenex Onyx  
47 monolithic C18 column (50 x 4.6 mm). After chromatographic separation, samples were directed  
48 into the mass spectrometer (Micromass™ triple quadrupole) and detected by tandem mass  
49 spectrometry in positive ion mode using electrospray ionization. There was no evidence of any  
50 degradation of TCP nor its derivatives upon permeation across the BBMEC monolayer.  
51

52 An isocratic HPLC method using a mobile phase of acetonitrile/5mM acetic acid, pH 3.9 with  
53 ammonia was used. The ratio of aqueous to organic was modified slightly based on the  
54 lipophilicity of the derivative and ranged from 10:90 (v/v) acetonitrile: 5 mM acetic acid to  
55 50:50 (v/v) acetonitrile: 5 mM acetic acid. A diversion of the HPLC eluent between 0 and 2 min  
56 reduced the exposure of the mass spectrometer to possible salts and cellular contaminants in the  
57 sample. Standards of all derivatives showed good linearity over a calibration range of 10-1000  
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4 ng/mL. Standard curves for TCP-FA4 were extended to include concentrations up to 5000  
5 ng/mL, and good linearity was still maintained.  
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### 8 **2.10 Assay for monoamine oxidase inhibition**

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10 Inhibition of monoamine oxidase was assessed by addition of TCP-FA4 (in DMSO) to the assay  
11 for MAO. MAO A was purified and assayed spectrophotometrically as in past work [23] in 50  
12 mM potassium phosphate pH 7.4 containing 0.05% Triton X-100 and 0.3 mM kynuramine  
13 (Sigma Aldrich, UK) (varied, Km 0.15 mM). Data were analyzed in Prism, using global analysis  
14 to fit the data to the equation for competitive inhibition. Purified MAO B was assayed with 0.6  
15 mM benzylamine (2 x Km) either spectrophotometrically or polarographically because of the  
16 high absorbance of TCP-FA4 at 250 nm. All assays were performed at 30°C. Pre-incubations  
17 with inhibitor to assess the time-dependence of inhibition and spectral collections were  
18 performed on ice or at 30°C.  
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## 24 **3. RESULTS**

### 25 **3.1 Rhodamine 123 uptake assay**

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29 Although TCP is not known to be a substrate for any efflux transporters expressed at the brain  
30 endothelium, the TCP derivatives were tested for potential interaction with P-glycoprotein (P-  
31 gp), one of the most prevalent of the efflux transporters that is responsible for limiting drug entry  
32 into the brain [24]. Since these molecules have increased lipophilicity, there is a chance they  
33 could be substrates, as P-gp substrates are often lipophilic in nature [25]. The rhodamine 123  
34 assay is a rather facile and reliable way to predict potential P-gp interaction [26]. The effect of  
35 the test compound on rhodamine 123 is determined by monitoring intracellular fluorescence. If  
36 the test compound is a substrate for P-gp, then addition of the compound will increase rhodamine  
37 123 uptake relative to the negative control. Results from the rhodamine 123 uptake assay suggest  
38 that none of the derivatives interact with P-gp (data not shown). All compounds were tested at a  
39 concentration of 10 µM and only the positive control cyclosporin A showed any effect on  
40 intracellular rhodamine 123 uptake.  
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### 45 **3.2 BBMEC Permeability Studies**

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47 The permeability of TCP and its derivatives was measured using BBMEC monolayers mounted  
48 in Side-bi-Side™ chambers. The apparent permeability values were calculated using the  
49 following equation:

$$50 P_{app} = (\Delta Q/\Delta t) / A \times C_0$$

51 where  $\Delta Q/\Delta t$  is the linear appearance of the test compound in the receiver chamber, A is the  
52 cross sectional area of the cell monolayer (0.636 cm<sup>2</sup>) and C<sub>0</sub> is the initial concentration of the  
53 test compound in the donor chamber at t = 0. TCP had a permeability of approximately 2.9 x 10<sup>-4</sup>  
54 cm/sec and TCP-FA4, a derivative containing a butanoic side chain, showed an improved  
55 permeability of 3.8 x 10<sup>-4</sup> cm/sec. The derivatives containing LAA moieties did not show an  
56 improvement in permeability relative to TCP and those derivatives with the longest FA and LAA  
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4 groups (TCP-FA12 and TCP-LAA12) were below the limit of detection (Fig. 2). Sucrose values  
5 for these studies ranged from  $2.0 \times 10^{-5}$  cm/sec to  $7 \times 10^{-5}$  cm/sec.  
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### 7 8 **3.3 Bi-directional permeability of TCP-FA4**

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10 To determine if the improved permeability of TCP-FA4 was due to an uptake transporter, the  
11 percentage of TCP-FA4 transported over time across a BBMEC monolayer was monitored in  
12 both the luminal to abluminal and abluminal to luminal directions. Studies indicated that there  
13 was a slight decrease in permeability going from the abluminal to luminal side (Fig. 3). The  
14 apparent permeability of TCP-FA4 in the luminal to abluminal direction had an average  $P_{app} =$   
15  $5.47 \times 10^{-4}$  cm/sec (std dev:  $2.4 \times 10^{-5}$  cm/sec) and in the abluminal to luminal direction a  $P_{app} =$   
16  $4.38 \times 10^{-4}$  cm/sec (std dev:  $1.4 \times 10^{-5}$  cm/sec). To determine if this difference was in fact due to  
17 some sort of uptake or facilitative process, additional temperature dependent and inhibition  
18 studies were performed.  
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### 22 23 **3.4 Temperature dependent permeability studies of TCP-FA4**

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25 The permeability of TCP-FA4 was monitored in a range of temperatures and an activation  
26 energy of transport was calculated. Studies were performed using BBMEC monolayers mounted  
27 in Side-bi-Side™ chambers. The activation energy calculated from the slope of the Arrhenius  
28 plot was 28.0 kJ/mol (Fig. 4) which is suggestive of a passive process since carrier-mediated  
29 processes typically fall within a range of 29-105 kJ/mol. [27]  
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### 32 33 **3.5 TCP-FA4: Inhibition studies**

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35 The effect of various inhibitors on the permeability of TCP-FA4 was investigated (data not  
36 shown). For all inhibition studies, BBMECs were pretreated with agents for 15 min prior to  
37 performing the permeability study. Ouabain octahydrate, a sodium pump blocker, was used to  
38 determine if the permeability of TCP-FA4 was a sodium coupled process. Permeability of TCP-  
39 FA4 was monitored in the presence of 100  $\mu$ M ouabain, but no significant changes in  
40 permeability were noted. Sodium azide, a respiratory chain inhibitor, was used as a metabolic  
41 inhibitor to investigate if the transport of TCP-FA4 was via a process that required cell-  
42 dependent energy expenditure. However, the permeability of TCP-FA4 did not decrease in the  
43 presence of 10 mM sodium azide. FCCP, a protonophore, was used to determine if the  
44 permeability of TCP-FA4 would be altered upon disruption of the proton gradient, but at a final  
45 concentration of 50  $\mu$ M FCCP no changes in TCP-FA4 permeability were noted. Furthermore,  
46 in the presence of 50  $\mu$ M DIDS, an anion-exchange inhibitor, no changes in TCP-FA4  
47 permeability were observed. These studies suggest that the permeation of TCP-FA4 is most  
48 likely a passive process and is neither an active nor facilitative process.  
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### 53 54 **3.6 BDNF production by HUVECs in the presence of TCP-FA4**

55 The effect of TCP-FA4 on the production of BDNF in HUVECs was investigated. HUVECs  
56 were treated with 5  $\mu$ M TCP-FA4 to determine if the derivative was capable of increasing  
57 production of BDNF relative to TCP. Results showed an increase in BDNF production relative to  
58 the control (blank media) as well as TCP. Levels of secreted BDNF increased over time and  
59 were highest when treated with TCP-FA4. At 48 h post treatment with TCP-FA4, BDNF was  
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4 detected at  $122.6 \pm 1.9$  pg/mL, compared to  $101.8 \pm 4.6$  and  $92.3 \pm 2.1$  pg/mL for TCP and blank  
5 media, respectively (n = 4) (Fig. 5)  
6

### 7 8 **3.7 Inhibition of MAO by TCP-FA4** 9

10 The inhibition of MAO A and MAO B by TCP-FA4 was assessed *in vitro*. TCP-FA4 (10  $\mu$ M)  
11 gave only 10% inhibition when added to an assay with 0.75 mM substrate (5x  $K_m$ ) but the  
12 inhibition increased to 70% after pre-incubation with the enzyme for 10 min at 30°C. This  
13 inhibition was fully reversible on dilution. Without pre-incubation, the inhibition was  
14 competitive with a  $K_i$  value of  $2.0 \pm 0.4$   $\mu$ M. Thus TCP-FA4 is a reversible inhibitor of MAO A.  
15 When TCP-FA4 was added to purified MAO A there was a very small spectral change indicating  
16 binding but no reduction of the enzyme was observed when MAO A and TCP-FA4 (30  $\mu$ M)  
17 were incubated together at 20°C overnight in contrast to the full reduction expected with the  
18 parent compound. The spectrum of TCP-FA4 also did not change (data not shown). Thus TCP-  
19 FA4 does not act as a suicide substrate of MAO A. No inhibition of human MAO B was  
20 observed, even at 100  $\mu$ M.  
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## 26 **4. Discussion**

27 Derivatives of TCP containing FA or LAA moieties of varying side alkyl chain length were  
28 synthesized in an attempt to improve BBB permeability, by increasing the lipophilicity as well as  
29 amphiphatic character of the drug molecule. We previously investigated the modified  
30 lipophilicity of these derivatives by calculating the logP as well as the partition coefficient  
31 between blood and brain (logBB) by means of the software packages ACD logP 5.15, Pallas 3.0,  
32 Osiris Property Explorer and KOWIN 1.57 [9]. Results suggested that LAA promoiety  
33 containing medium to long side alkyl chains would be most useful in improving the permeability  
34 and membrane interaction of these compounds [9]. Additionally, experimental data confirmed  
35 that conjugation of TCP to LAA led to a deeper and more complex interaction with  
36 biomembrane models consisting of dimyristoylphosphatidylcholine (DMPC) multilamellar  
37 vesicles and monolayers [9]. However, when using BBMECs as a model for the BBB, the FA  
38 derivative TCP-FA4 showed the greatest improvement in permeability. This derivative contains  
39 a four carbon alkyl chain and has an approximate 2-fold increase in lipophilicity compared to the  
40 parent drug [9, 10]. Computational studies predicted that the large increase in lipophilicity for  
41 compounds like TCP-LAA10 and TCP-LAA12 would improve permeability, however in reality  
42 in an *in vitro* system, these compounds most likely partition nicely into the cell monolayer and  
43 never actually permeate across. They are caught up within the cell but are not released, which  
44 therefore results in a low experimental permeability. Additionally, this increase in lipophilicity  
45 decreased the compounds aqueous solubility, which inherently affects permeability. The  
46 permeability results for TCP-FA4 indicate that it has good passive permeability and is not a  
47 substrate for any active transport systems expressed in BBMECs. Furthermore, the utility as a  
48 pharmaceutical agent of a TCP derivative possessing improved permeability was investigated.  
49 Although it was found that TCP-FA4 does exhibit inhibitory activity against MAO A enzyme, its  
50 affinity was reduced compared to the parent compound, TCP [28] and the inhibition was  
51 reversible. However, our studies indicate that TCP-FA4 may possess neuroprotective properties.  
52 In a  $\beta$ -amyloid-induced neurodegeneration assay, neurons exposed to  $\beta$ -amyloid in the presence  
53 of 200 nM TCP-FA4 showed a 20% improvement in survival rate (Fig. 6). Also TCP and TCP-  
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4 FA4 showed no toxicity to cortical neurons at concentrations up to 200 nM. Although the results  
5 of our experiments taken together suggest that TCP-A4 has neuroprotective activities, the  
6 cellular mechanisms underlying enhanced neuronal survival have yet to be delineated, primarily  
7 due to the fact that the molecular basis for  $\beta$ -amyloid toxicity has still not been fully elucidated.  
8 Additionally, using HUVECs grown in culture, increased levels of the neurotrophic factor BDNF  
9 were observed in the presence of TCP-FA4. Since BDNF is instrumental in the survival and  
10 differentiation of neurons and BDNF expression is altered in many brain disorders, the discovery  
11 of small molecules capable of regulating BDNF may be beneficial.  
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## 15 16 **Acknowledgments**

17  
18  
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23 cedex, France and ETNALEAD).  
24

25  
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27 Kansas, Department of Pharmaceutical Chemistry. Technical assistance was provided by Dr.  
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29

30  
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32 gratefully acknowledged for the NMR analysis.  
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## 37 **Figure legends**

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39 Fig. 1 TCP conjugates with lipoamino acids (LAA) or fatty acids (FA) bearing a side alkyl chain  
40 of varying length.  
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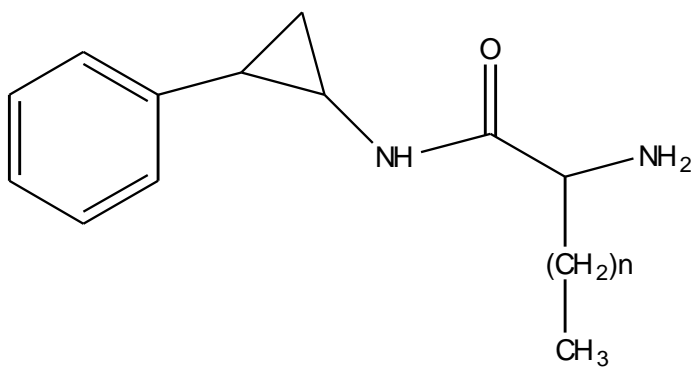
42  
43 Fig. 2 Permeability of TCP and derivatives in BBMECs. (\*\* Denotes  $p < 0.01$  compared to TCP  
44 as determined by ANOVA, ; Data  $\pm$  SD, TCP and TCP-FA4  $n=8$ , TCP-LAA6 and TCP LAA10  
45  $n=4$ ).  
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48 Fig. 3 Percentage of TCP-FA4 transported across BBMEC monolayers over time in the luminal  
49 to abluminal and abluminal to luminal directions. (Data  $\pm$  SD,  $n=8$ ).  
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51  
52 Fig. 4 Arrhenius plot generated by the temperature dependent permeability of TCP-FA4 in  
53 BBMECs. Permeability values were calculated at a variety of temperatures ranging from 4°C to  
54 37 °C. (Data  $\pm$  SD,  $n=4$ , except 4 and 37°C,  $n=8$ ).  
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57 Fig. 5 BDNF secretion by cultured HUVECs in the presence of TCP and TCP-FA4. (\*\* Denotes  
58  $p < 0.01$  compared to control as determined by ANOVA, Data  $\pm$  SD,  $n=4$ ).  
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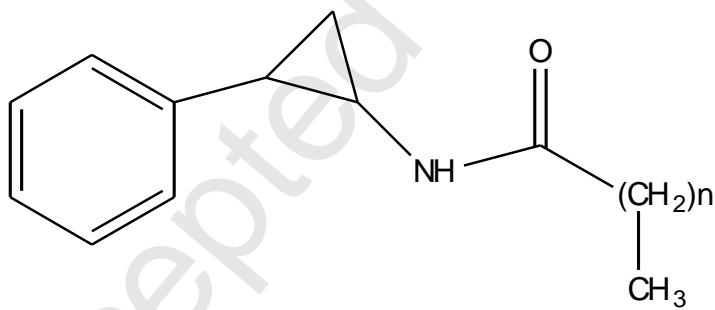
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4 Fig. 6 Effects of A $\beta$ <sub>25-35</sub>, TCP and TCP-FA4 on neuronal survival. Neuronal cells were treated  
5 with vehicle only, TCP, and TCP-FA4. The indicated concentrations of the compound were  
6 added 2 h before A $\beta$ . Cell viability was determined 48 h later. Data represent mean survival  $\pm$  SE  
7 for three separate experiments with ~1500 cells per treatment condition.  
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Compound	n
TCP-LAA6	3
TCP-LAA10	7
TCP-LAA12	9

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Compound	n
TCP-FA4	2
TCP-FA12	10

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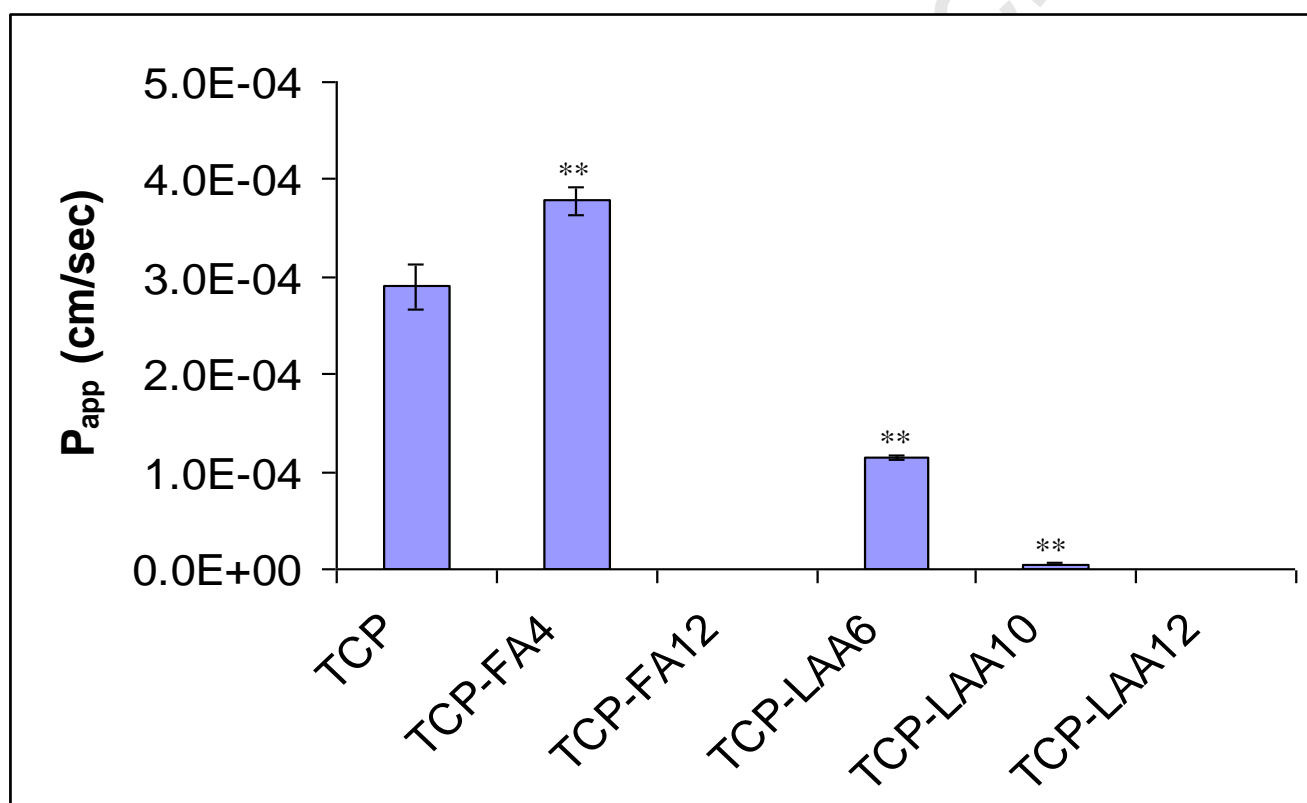


Figure 2

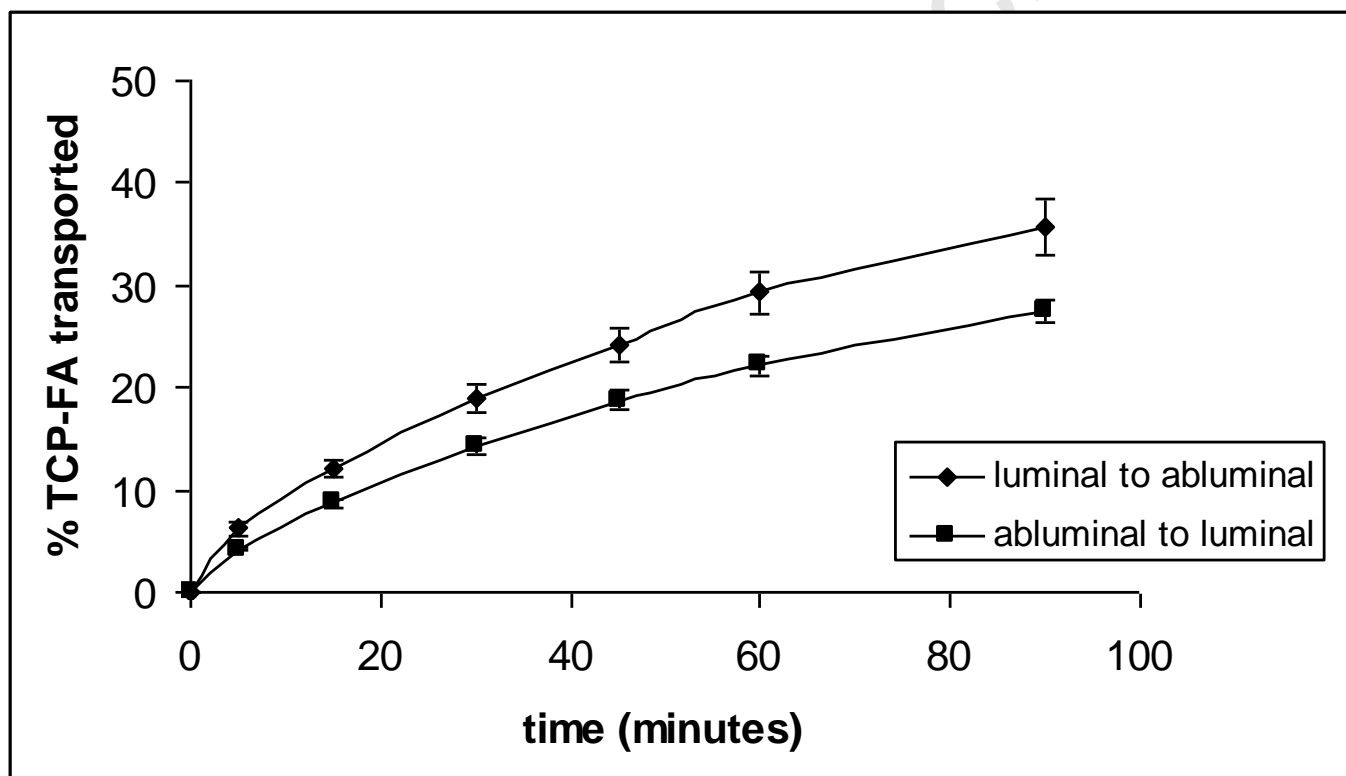


Figure 3

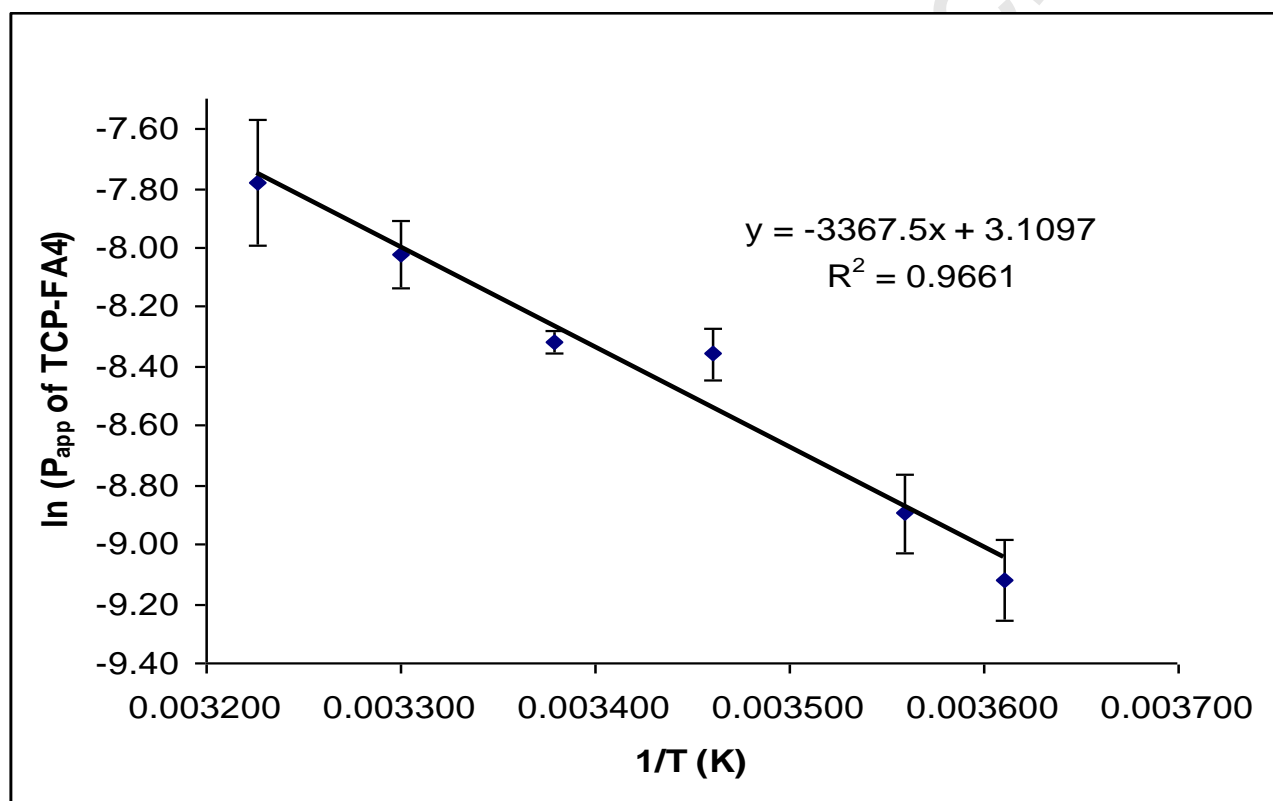


Figure 4



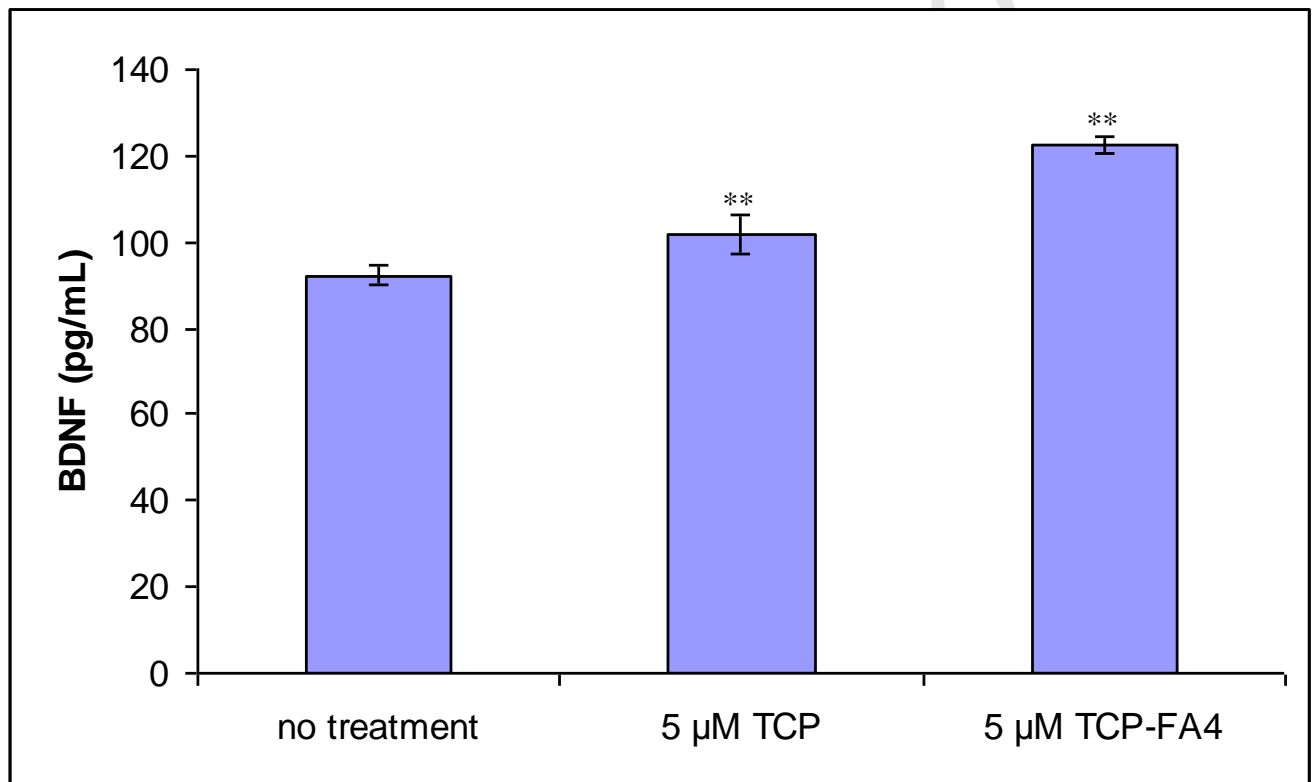


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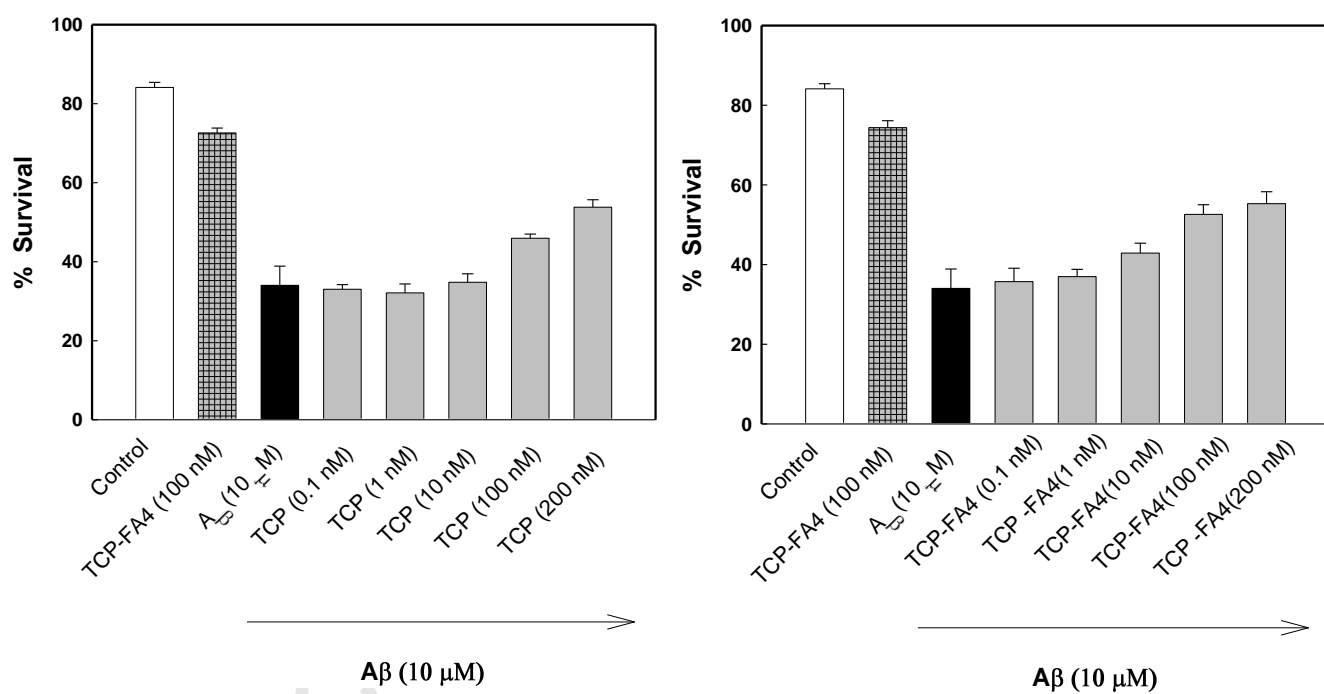
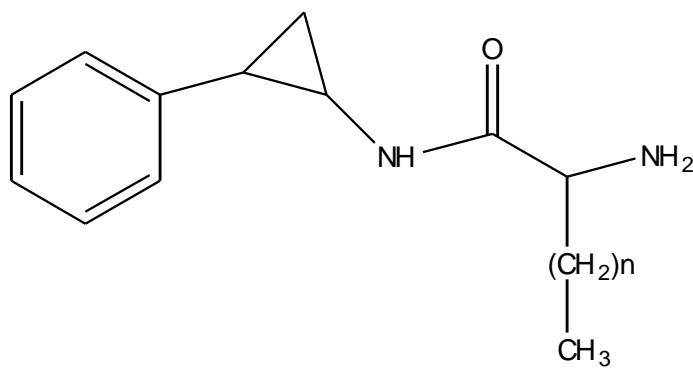


Figure 6

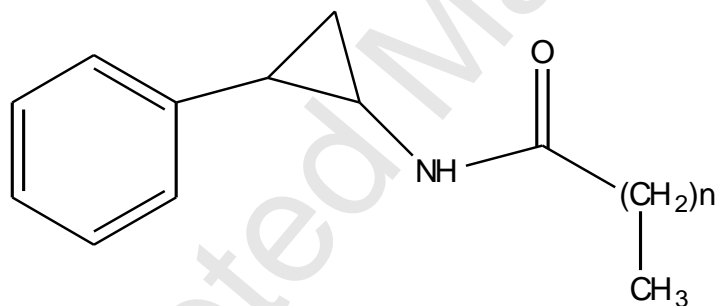
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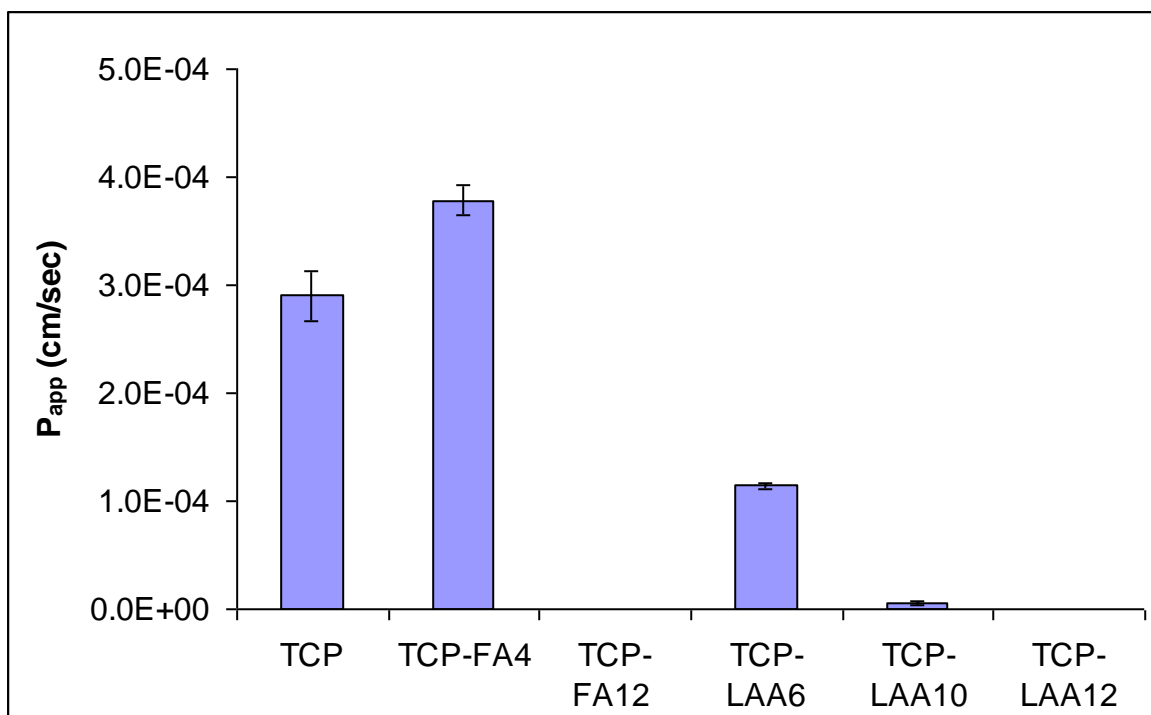


Compound	n
TCP-LAA6	3
TCP-LAA10	7
TCP-LAA12	9

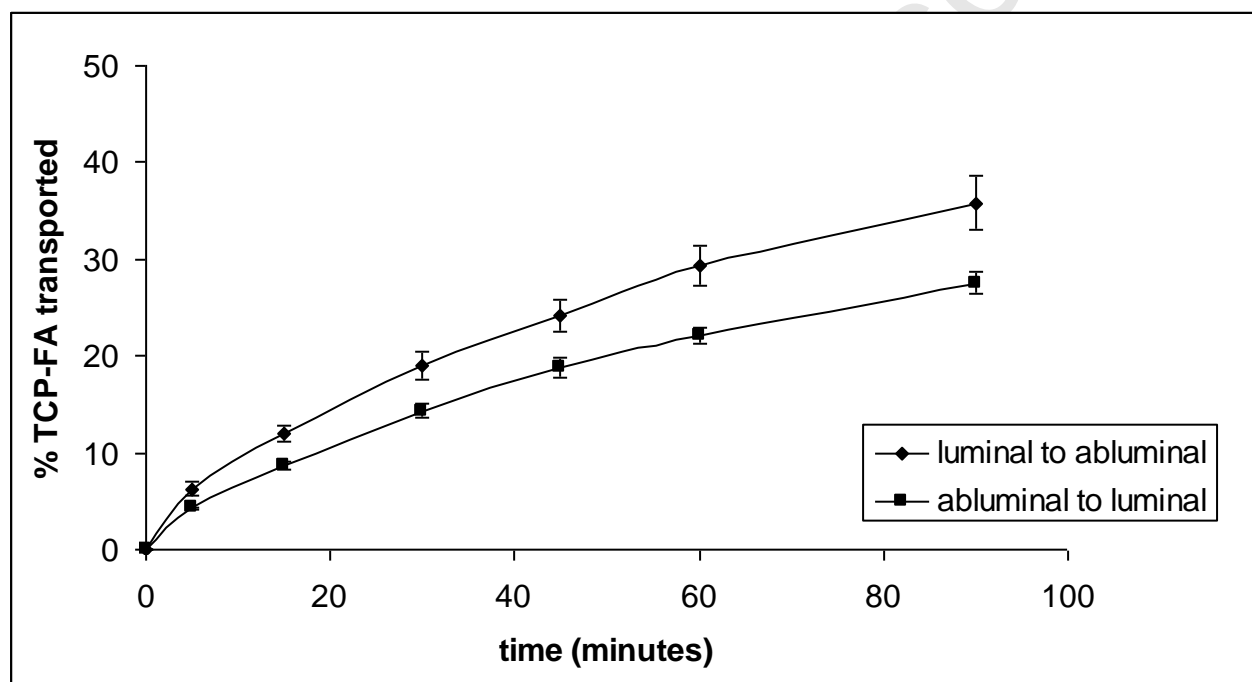


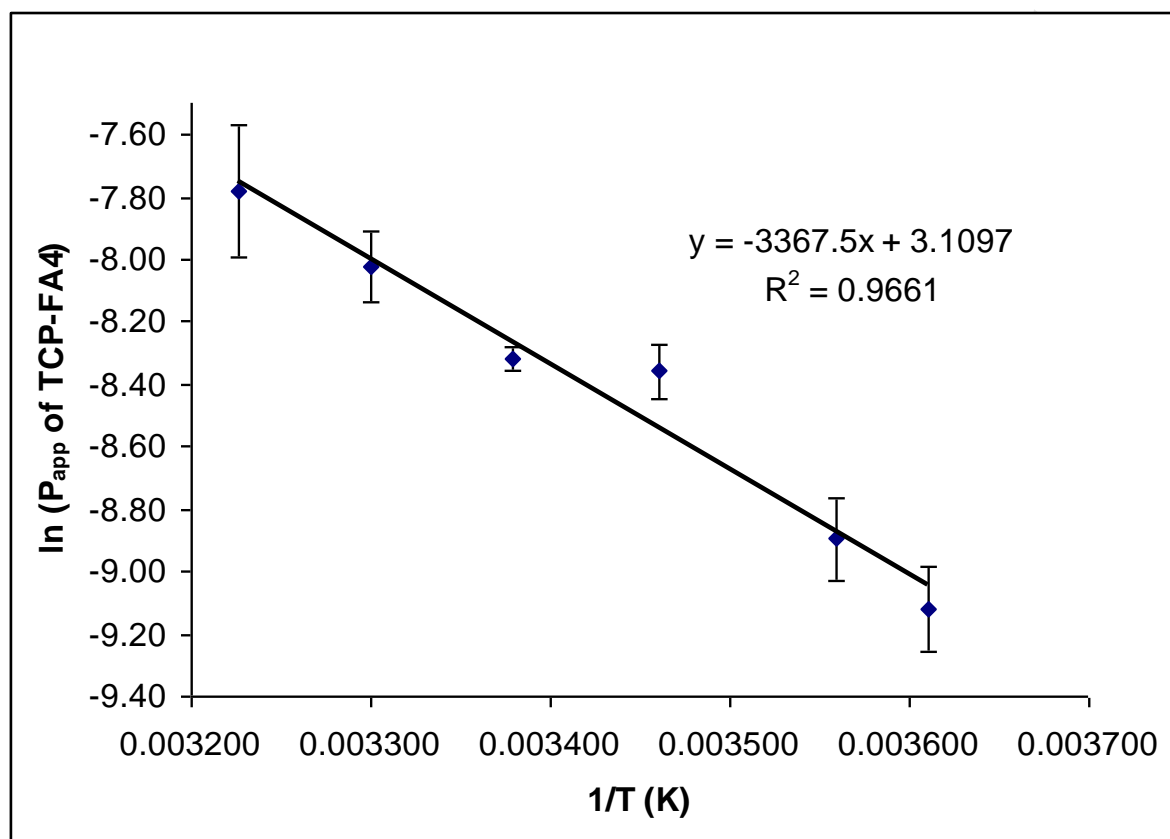
Compound	n
TCP-FA4	2
TCP-FA12	10

Figure 1



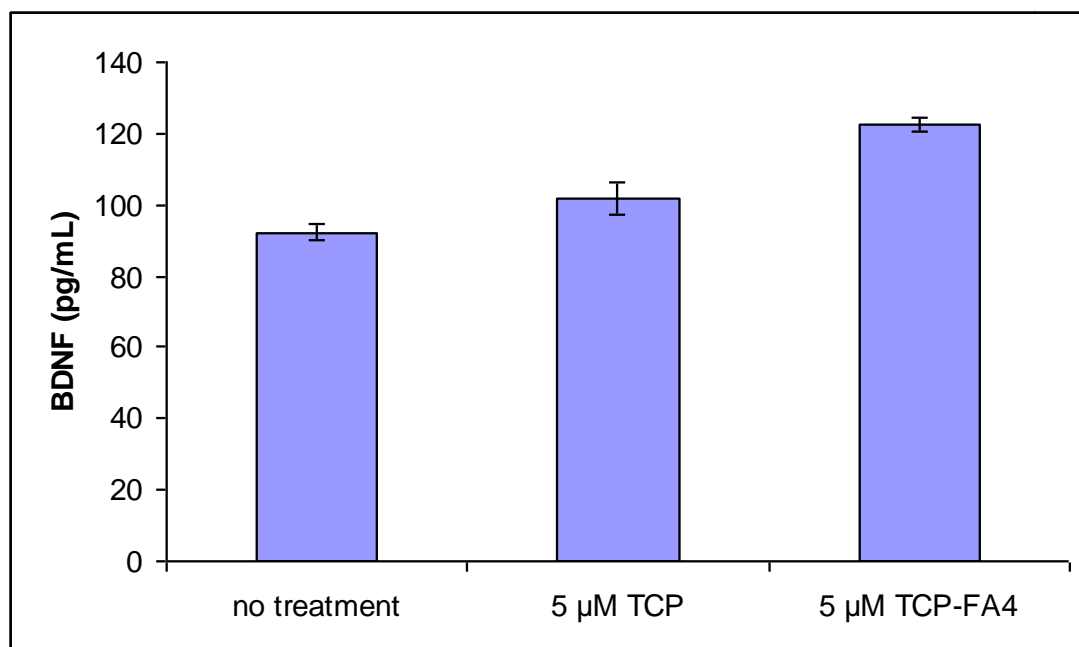
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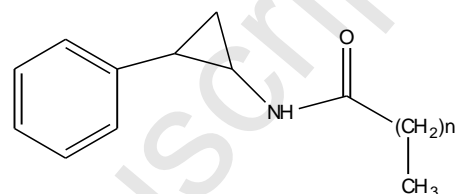
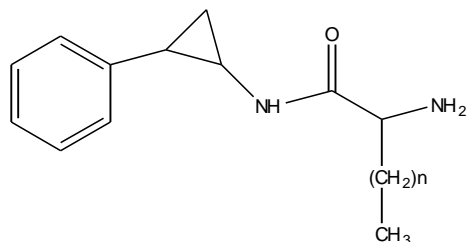




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### TCP-FA4: A DERIVATIVE OF TRANYLCPROMINE SHOWING IMPROVED BLOOD-BRAIN PERMEABILITY

Derivatives of TCP containing FA or LAA moieties of varying side alkyl chain length were synthesized in an attempt to improve BBB permeability, by increasing the lipophilicity as well as amphiphatic character of the drug molecule. Results suggested that LAA promoieties containing medium to long side alkyl chains would be most useful in improving the permeability and membrane interaction of these compounds. TCP-FA4 may possess neuroprotective properties.



Compound	n
TCP-FA4	2
TCP-FA12	10

Compound	n
TCP-LAA6	3
TCP-LAA10	7
TCP-LAA12	9