



Communication

Design and synthesis of the ring-opened derivative of 3-*n*-butylphthalide-ferulic acid-glucose trihybrids as potential anti-ischemic agents

Jianbing Wu^a, Wei Yin^a, Yinqiu Zhang^a, Hui Ye^a, Yunman Li^{b,*}, Jide Tian^c, Zhangjian Huang^{a,*}, Yihua Zhang^{a,*}

^a State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China

^b State Key Laboratory of Natural Medicines, Department of Physiology, China Pharmaceutical University, Nanjing 210009, China

^c Department of Molecular and Medical Pharmacology, University of California, Los Angeles, CA 90095, United States



ARTICLE INFO

Article history:

Received 22 December 2019

Received in revised form 15 February 2020

Accepted 17 February 2020

Available online 18 February 2020

Keywords:

3-*n*-Butylphthalide

Ferulic acid

Glucose

Hybrids

Ischemic

Brain-blood barrier

ABSTRACT

To improve aqueous solubility and anti-ischemic activity of 3-*n*-butylphthalide (NBP), we designed and synthesized the ring-opened derivative of NBP-ferulic acid-glucose trihybrids (**S1–S8**). These hybrids inhibited adenosine diphosphate (ADP)- or arachidonic acid (AA)-induced platelet aggregation, among them, **S2** was 30-fold more water-soluble, and over 10-fold more potent in inhibition of platelet aggregation, as well as reduced ROS generation and protected primary neuronal cells from OGD/R-induced damage, in comparison with NBP. Additionally, **S2** was more active than its three moieties alone or in combination, suggesting that the activity of **S2** may be attributed to the synergistic effects of these moieties. Importantly, *in vivo* studies indicated that **S2** not only possessed good pharmacokinetic profile, but also improved NBP distribution in rodent brain, suggesting that the glucose moiety in **S2** may be recognized by glucose transporter 1 (GLUT1) on blood-brain barrier (BBB), promoting it to penetrate through BBB. Our findings suggest that **S2** may be a promising candidate for the intervention of ischemic stroke, warranting further study.

© 2020 Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences. Published by Elsevier B.V. All rights reserved.

Ischemic stroke is characterized by high morbidity, mortality, disability and recurrence rates with serious hazard for human life and health. To search for novel drugs for safe and effective treatment of ischemic stroke, great efforts have been made; however, to date there is still a lack of ideal small molecule drug(s) [1–3].

3-*n*-Butylphthalide (NBP) was approved in China for treatment of ischemic stroke in 2004 [4]. NBP can reduce the infarct size and improve the energy metabolism after cerebral ischemia [5,6]. However, the therapeutic effects of NBP are moderate and it is often administered together with other drug(s) [7]. The relatively low efficacy of NBP is partly attributed to its poor aqueous solubility [8]. It has been reported that the potassium salt of 2-(1-hydroxy-*n*-pentyl)benzoate (PHPB), which derives from ring-opening of NBP (HPBA, Fig. 1A), significantly improves aqueous

solubility of NBP [9]. In addition, hybrids of the HPBA derivative with other drug/agent, such as edaravone [10], isosorbide [11], telmisartan [12], nitric oxide donors [13], *etc.*, strengthen the therapeutic effects on acute cerebral ischemia, thus providing a principle concept of ring-opened NBP derivatives for the intervention of ischemic stroke.

It has been reported that oxidative stress plays an important role in cerebral ischemia/reperfusion (I/R) injury [14]. High levels of reactive oxygen species (ROS) can destroy cellular macromolecules, leading to autophagy, apoptosis and necrosis [15]. Therefore, inhibition of ROS production and/or up-regulation of endogenous antioxidant systems *in vivo* is one of the important strategies for designing anti-ischemic drugs. Ferulic acid (FA) is one of the main active ingredients of Chinese herbal medicines such as *Angelica*, *Chuanxiong* and *Awei*, and possesses a large variety of pharmacological activities, including inhibition of ROS, anti-oxidation, anti-inflammatory, and anti-platelet aggregation, *etc.* [16]. Importantly, FA can be used alone or as a linker between two active molecules to enhance the therapeutic effects of cardiovascular diseases [17,18].

* Corresponding authors.

E-mail addresses: liyunman@cpu.edu.cn (Y. Li), zhangjianhuang@cpu.edu.cn (Z. Huang), zyhtgd@163.com (Y. Zhang).

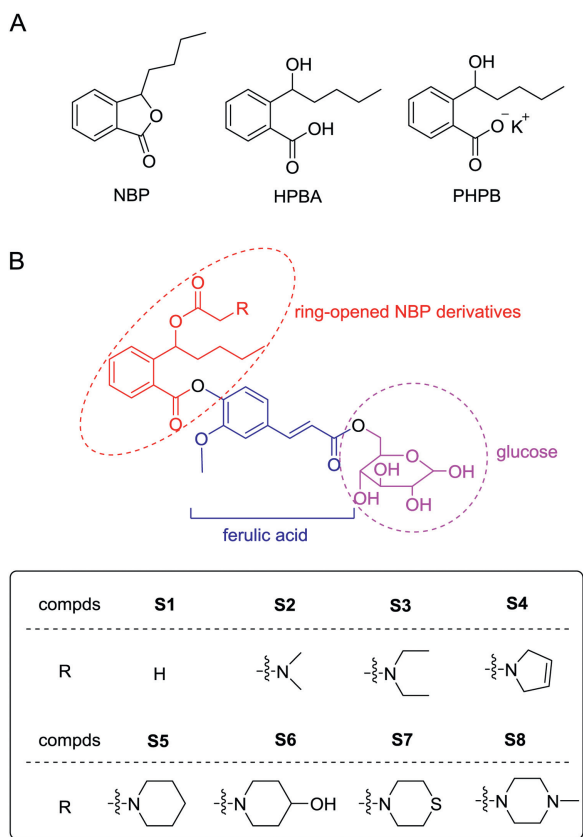


Fig. 1. (A) The chemical structures of NBP, HPBA and PHPB. (B) The design and structures of target compounds **S1-S8**.

Glucose transporters 1 (GLUT1) is the most abundant transporter in blood-brain barrier (BBB) and able to deliver large amounts of aqueous glucose from peripheral circulatory system into brain, maintaining energy metabolism in the brain [19]. In recent years, GLUT1 is frequently utilized for design of brain-targeted drugs [20,21]. Fernandez *et al.* conjugated dopamine with glucose-1, -3 and -6 hydroxyl, respectively, to yield a series of glucose/dopamine hybrids [22]. It was found that the dopamine/glucose-6-hydroxyl hybrid displayed the strongest BBB penetrating and antiparkinsonian activities. Mueckler *et al.* observed that in contrast to other the glucose hydroxyls, the 6-hydroxyl does not form a hydrogen bond that is crucial for affinity of glucose at the binding site of GLUT1, which indicates that the structural modification of the 6-hydroxyl group can retain affinity of glucose to GLUT1 [23]. In addition, the expression of GLUT1 on BBB is reportedly elevated when brain tissue is under ischemic and hypoxic conditions [24]. These investigations led us to hypothesize that glucose-6-hydroxyl derivatives of HPBA may not only improve the aqueous solubility, but also enhance the brain distribution via GLUT1-mediated BBB penetration.

To test the hypothesis, we designed and synthesized new compounds **S1-S8**, in which ring-opened derivatives of NBP were conjugated with glucose-6-hydroxyl by using FA as a linker (Fig. 1B). It is expected that these trihybrids may not only improve aqueous solubility owing to the hydrophilic property of glucose, but also can be recognized by GLUT1 to pass through BBB and increase concentrations of active compound(s) in the brain to exert more potent anti-ischemic activity than NBP.

The synthetic routes of target compounds **S1-S8** were depicted in Scheme 1A. Given that the *R*- and *S*-NBP show almost comparable activities and NBP in market is racemic, for simplicity, we used (*R/S*)-NBP as starting material. (*R/S*)-NBP was ring-opened

under basic conditions to generate hydroxyl intermediate **1**, which was directly treated with acetyl chloride or chloroacetyl chloride to give esters (*R/S*)-**2a** or **-2b**. The two esters were then condensed with *tert*-butyl ferulate **10** in the presence of *N,N'*-dicyclohexyl carbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to form conjugates (*R/S*)-**3a** and **-3b**, followed by removal of the *tert*-butyl group with trifluoroacetic acid (TFA) to afford acids (*R/S*)-**4a** and **-4b**. Subsequently, they were condensed with trimethylsilyl (TMS)-protected *D*-(+)-glucose **8** to offer *D*-(+)-glucose-6-OH esters **5a** and **5b** (each as epimeric mixture). The TMS groups in **5a** were removed by using TFA to yield the target compound **S1** as epimeric mixture. Compound **5b** was reacted with dimethylamine, diethylamine, 2,5-dihydro-1*H*-pyrrole, piperidine, 4-hydroxypiperidine, thiomorpholine and 1-methylpiperazine, respectively, under alkaline conditions to generate amines **6a-g**. The TMS groups in **6a-g** were then removed with TFA to provide the target compounds **S2-S8** (each as epimeric mixture).

The intermediates **8** and **10** for synthesis of target compounds were prepared as shown in Scheme 1B. All the hydroxyl groups of *D*-(+)-glucose were protected by trimethylchlorosilane (TMSCl) furnishing trimethylsilyl ether **7**, followed by selective deprotection in acetic acid to give 6-OH derivative **8**. Moreover, Meldrum's acid (MA) was condensed with *tert*-butyl alcohol to form ester **9**, which was treated with vanillin in the presence of a catalytic amount of pyridine and piperidine to produce *tert*-butyl ferulate **10**.

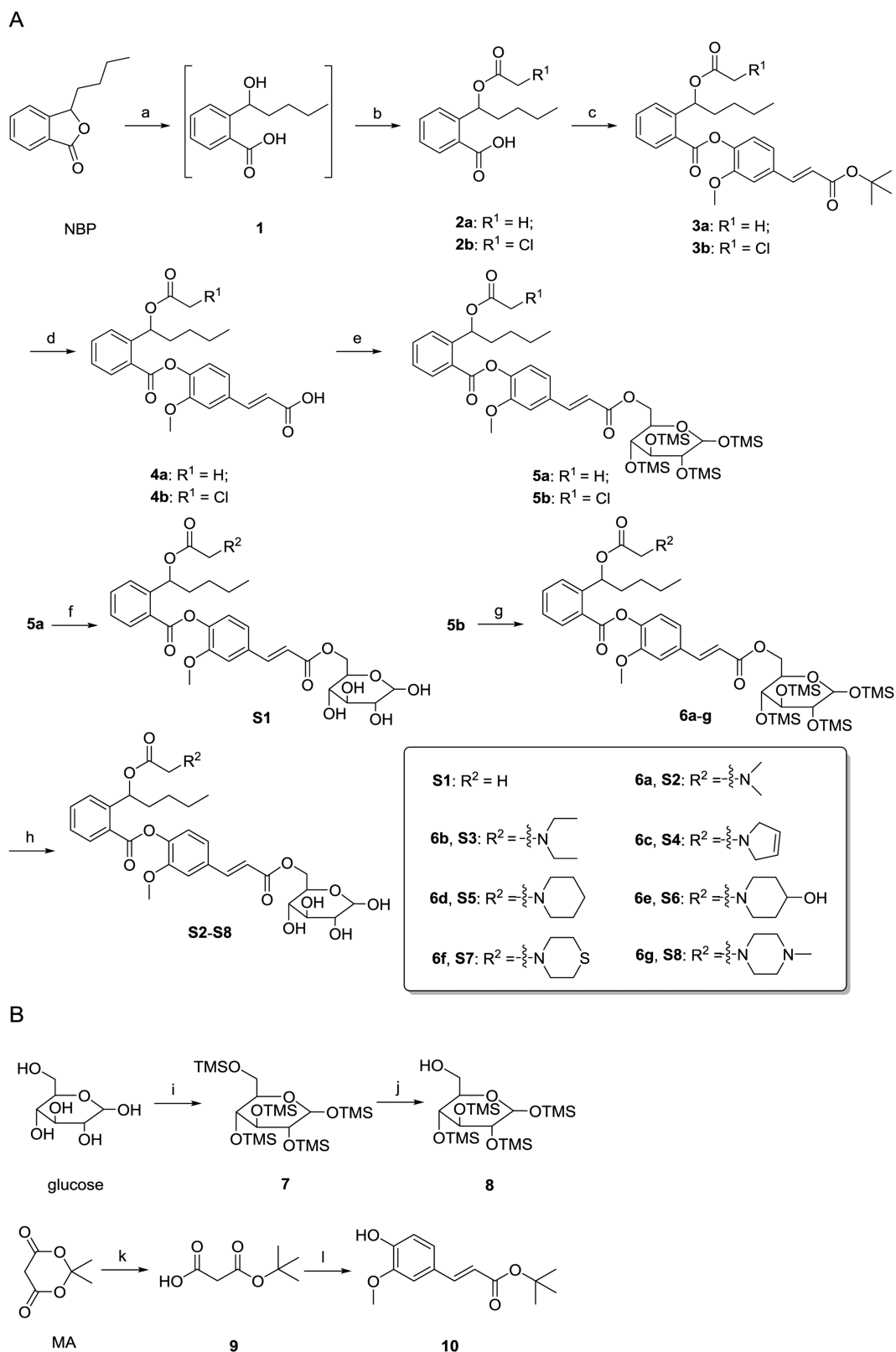
The ratio of two epimers of the target compounds was determined by HPLC using a chiral column (CHIRALPAK[®] AD-H, Lot No. 19325, Daicel Chiral Technologies Co., Ltd.), exemplified by representative compound **S2**, and found to be approximately 1:1 as shown in Supporting information.

All target compounds with a purity of >96%, determined by high performance liquid chromatography (HPLC) analysis, were used for following experiments. The detailed synthetic procedure, *in vitro* and *in vivo* biological evaluations, and structure characterization data of the target compounds **S1-S8**, including ¹H NMR, ¹³C NMR and high-resolution mass spectrometry are shown in Supporting information.

All animal experiments and animal cares were conducted in accordance with the guidelines of the Provision and General Recommendation of Chinese Experimental Animals in China. The experimental protocols were approved by the Animal Research and Care Committee of China Pharmaceutical University (SYKK (SU) 2016-0011).

To investigate the anti-platelet aggregation activity *in vitro* of the target compounds, we firstly evaluated the inhibitory effects of **S1-S8** on the adenosine diphosphate (ADP)- and arachidonic acid (AA)-induced platelet aggregation in rabbit platelet rich plasma (PRP) by using Born's turbidimetric method [25]. NBP and aspirin (ASP) were used as positive controls. As shown in Table 1, all the target compounds displayed inhibitory effects to some extent. Notably, **S2** was the most active in inhibition of ADP-induced platelet aggregation (IC_{50} = 0.08 mmol/L), 12.9-fold more potent than NBP (IC_{50} = 1.03 mmol/L) as well as in inhibition of AA-induced platelet aggregation (IC_{50} = 0.04 mmol/L), 14.3- and 3.5-fold more potent than NBP (IC_{50} = 0.57 mmol/L) and ASP (IC_{50} = 0.14 mmol/L), respectively.

Preliminary analysis of structure-activity relationship suggests that the R^2 substituted acetyl group (Scheme 1) is important to the activity of the target compounds. The amine substituted acetyls are generally more potent than the unsubstituted acetyl (**S2** and **S4-S6** vs. **S1**). Among them, the smallest substituent is the most potent (**S2** vs. **S3-S6**). One nitrogen-containing heterocyclic substituents exert moderate activity (**S4** and **S5**), and introduction of one hydroxyl group to the heterocyclic ring enhances the activity (**S6**). Surprisingly, two heteroatom-containing substituents diminish



Scheme 1. The synthetic route of target compounds **S1-S8**. (A) Reagents and conditions: (a) (1) NaOH, CH₃OH, H₂O, 50 °C, 0.5 h; (2) 5% HCl (aq.), -10 °C to 0 °C; (b) acetyl chloride or chloroacetyl chloride, Et₃N, DMAP, DCM, -10 °C, 5 h; (c) DCC, DMAP, DCM, 0 °C to r.t., 6 h; (d) CF₃COOH, DCM, r.t., 3 h; (e) **8**, DCC, DMAP, DCM, 0 °C to r.t., 8 h; (f) CF₃COOH, DCM, r.t., overnight; (g) corresponding amines, Et₃N, DCM, 24 h; (h) CF₃COOH, DCM, r.t., overnight. (B) Reagents and conditions: (i) TMSCl, HMDS, pyridine, r.t., overnight; (j) acetone, methanol, acetic acid, 40 °C, 4 h; (k) *tert*-butanol, toluene, 100 °C; (l) vanillin, piperidine, pyridine, 10 h.

Table 1
The IC₅₀ values of compounds **S1–S8** against platelet aggregation *in vitro*.^a

Compound	IC ₅₀ (mmol/L)	
	ADP (10 μmol/L)	AA (1 mmol/L)
NBP	1.03 ± 0.12	0.57 ± 0.23
ASP	–	0.14 ± 0.01
S1	0.87 ± 0.08	0.80 ± 0.19
S2	0.08 ± 0.02	0.04 ± 0.07
S3	0.93 ± 0.15	0.74 ± 0.26
S4	0.47 ± 0.09	0.32 ± 0.10
S5	0.30 ± 0.07	0.24 ± 0.05
S6	0.16 ± 0.04	0.09 ± 0.02
S7	1.70 ± 0.24	0.83 ± 0.19
S8	1.65 ± 0.31	1.17 ± 0.22

^a IC₅₀ values are expressed as mean ± SD (*n* = 6) and analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test.

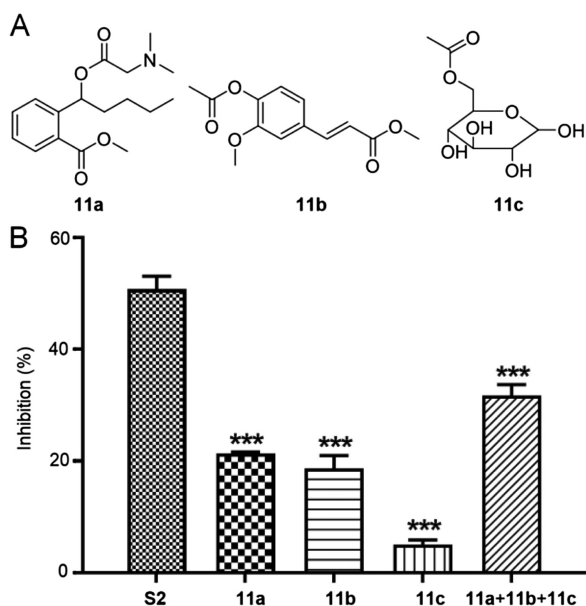


Fig. 2. (A) The structures of compounds **11a–c**. (B) Inhibition of ADP-induced rabbit platelet aggregation by **11a–c** alone or in combination *in vitro*. Rabbit platelet suspensions were pre-incubated with tested compounds (0.1 mmol/L) at 37 °C for 5 min, followed by addition of ADP (10 μmol/L). Data are expressed as the mean ± SD of each compound from six independent experiments. ****P* < 0.001 vs. **S2**.

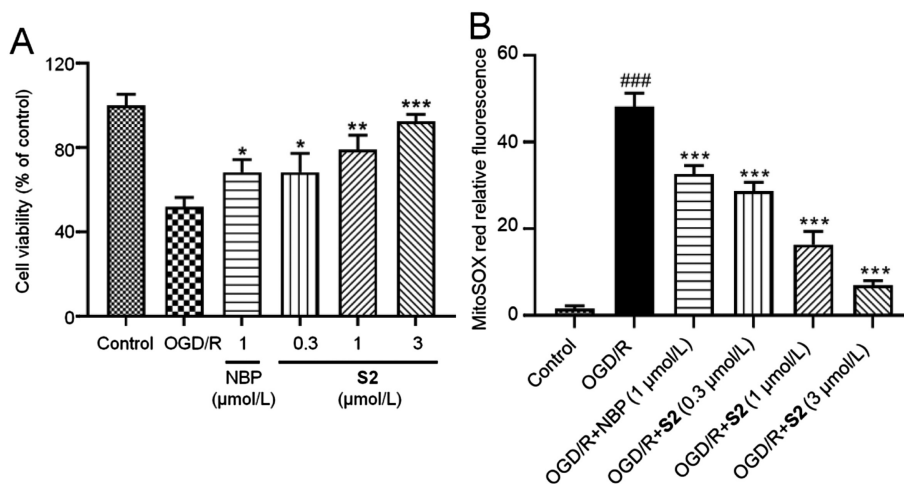


Fig. 3. **S2** protected primary cultured cortical neurons against OGD/R-induced injury. (A) Cell viability determined by MTT assay. (B) Mitochondrion ROS generation determined by staining with MitoSOX Red. Data are presented as mean ± SD from three independent experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. OGD/R group; ###*P* < 0.001 vs. control group.

the activity (**S7** and **S8**). Maybe they are not easy to be recognized by GLUT1 to pass through BBB or difficult to penetrate the cells' membrane. The precise structure-activity relationship should be further investigated in due course. Since **S2** displayed the best inhibitory activity against ADP- or AA-induced platelet aggregation, it was selected for the following investigations.

To examine each contribution of three moieties of **S2** to the anti-platelet aggregation activity, we synthesized **11a–c**, which are structurally related to the three moieties (Fig. 2A and Scheme S1 in Supporting information) and examined their inhibitory activity against ADP-induced platelet aggregation. As shown in Fig. 2B, the inhibition rates of **11a**, **11b**, and **11c** (21.32%, 18.67%, and 5.01%, respectively) were far less than that of **S2** (50.76%). Next, we investigated the inhibitory activity of these compounds in combination. The inhibition rate of the three compounds in combination (31.72%) was still less than that of **S2**. These results suggest that the three moieties may have synergistic inhibitory effects on ADP- or AA-induced platelet aggregation.

The oxygen-glucose deprivation/reoxygenation (OGD/R) model as an *in vitro* model for simulating I/R is commonly used for the evaluation of anti-ischemic stroke activity and mechanism investigation [26]. Thus, a primary cortical neuron cell OGD/R model (hypoxia for 2 h/reoxygenation for 24 h) was established for examining the *in vitro* activities of **S2**. As shown in Fig. 3A, compared with the control group, the neuronal cells survival rate of the OGD/R model group was significantly reduced, and **S2** dose-dependently increased the survival rate of neurons relative to the model group. Moreover, **S2** at 1 μmol/L was more potent than NBP at the same dose. Additionally, treatment of OGD/R primary cortical neuron cells with **S2** dose-dependently reduced the generation of ROS in mitochondrion (Fig. 3B). These results indicated that **S2** had a significant protective effect on OGD/R-induced neuronal damage.

We then assessed the aqueous solubility of **S2** at 25 °C by HPLC and found that **S2** (16.70 ± 1.33 mg/mL) was approximately 30-fold more soluble than NBP (0.54 ± 0.21 mg/mL) in water, which is probably attributable to the water-soluble glucose and amine moieties in **S2**.

It is well known that BBB is a dynamic interface between the blood and brain/spinal cord with low permeability and selective ability to protect the brain from toxins and viruses in the blood circulation, but it also hinders more than 98% of small molecule agents and 100% of macromolecular agents to enter the brain tissue

Table 2

The plasma pharmacokinetic parameters determined after intravenous administration of **S2** at a dose of 15 mg/kg to rats.^a

PK Parameters	Mean \pm SD
Dose (mg/kg)	15.0
Kel (h ⁻¹)	0.271 \pm 0.081
<i>t</i> _{1/2} (h)	2.75 \pm 0.98
C _{max} (ng/mL)	6824 \pm 928
C ₀ (ng/mL)	14192 \pm 2126
AUC _{0-t} (h ng mL ⁻¹)	2379 \pm 353
AUC _{0-∞} (h ng mL ⁻¹)	2425 \pm 348
AUMC _{0-t} (h h ng mL ⁻¹)	1929 \pm 1170
AUMC _{0-∞} (h h ng mL ⁻¹)	2679 \pm 1533
CL (mL min ⁻¹ kg ⁻¹)	105 \pm 15
MRT _{IV} (h)	1.07 \pm 0.46
Vd _{SS} (L/kg)	6.46 \pm 1.9

^a Data are expressed as mean \pm SD of three independent experiments.

[27,28]. Therefore, it is crucial for anti-cerebral ischemic drugs to penetrate BBB into brain.

The pharmacokinetics (PK) behavior of **S2** in rats was firstly evaluated. After intravenous administration of **S2** at 15 mg/kg to rats (*n* = 3), blood samples were taken from the suborbital vein at 0, 5, 15, 30 min and 1, 2, 4, 6, 8, 24 h and then analyzed by LC–MS/MS. The corresponding pharmacokinetic parameters of **S2** are shown in Table 2. In brief, AUC_{0-∞} (area under concentration-time curve from time zero to infinite), *t*_{1/2} (half-life), and C_{max} (maximum plasma concentration) of **S2** were 2379 \pm 353 h ng mL⁻¹, 2.75 \pm 0.98 h, and 6824 \pm 928 ng/mL, respectively. And the CL (clearance rates) and Vd_{SS} (distribution volumes) of **S2** were 105 \pm 15 mL min⁻¹ kg⁻¹ and 6.46 \pm 1.9 L/kg, respectively. Moreover, the corresponding pharmacokinetic parameters of active metabolite NBP are shown in Table S1 (Supporting information), the *t*_{1/2} of NBP was up to 9.46 h, indicating that **S2** possessed an improved PK behavior as compared with NBP (*t*_{1/2} = 44 min) [29].

Furthermore, we studied the brain distribution of **S2** in rats. After intravenous administration of **S2** at 15 mg/kg, the rats were sacrificed at 0.5, 1 and 6 h (*n* = 3) and the post-processed blood and brain samples were then analyzed by LC–MS/MS. In view of that NBP could be a main active metabolite of **S2**, the concentrations of both NBP and **S2** were determined to calculate the brain/plasma (B/P) ratios. As shown in Table S2 (Supporting information), after intravenous administration of **S2**, the B/P ratios of **S2** at 0.5 h and 1 h were 0.107 and 0.135, respectively. The concentration of **S2** in brain at 6 h after administration was under detection limit. Interestingly, after intravenous administration of **S2**, the B/P ratios of NBP were significantly improved (1.99, 1.27 and 4.8 at 0.5, 1 and 6 h, respectively). Collectively, these data clearly demonstrated that **S2** possessed prolonged *t*_{1/2} with higher NBP brain distributions, probably thanks to glucose-based hybridization strategy.

To compare the brain distribution between **S2** and NBP, we conducted another assay in mice. After intravenous administration of **S2** or NBP (25 mg/kg), the mice were sacrificed at 5, 10, 30, 60, 90, 120 and 240 min (*n* = 3), and the brain samples were then analyzed to determine the concentrations of NBP. As shown in Fig. 4, the concentrations of NBP in brain produced by **S2**-treatment or NBP-treatment gradually increased and then declined. And the amounts of NBP in the brain from **S2** were greater than that from NBP alone in all time points except at 30 min, suggesting that **S2** may more smoothly pass through BBB to release active NBP in the brain.

We next conducted the *in vitro* glucose competition uptake assay [30] to examine if **S2** could interact with GLUT1 and be ingested into cells *via* it. The competitive effect of **S2** with D-(+)-glucose was determined by using a cell-based assay with CellTiter-Glo[®] readout for ATP production in COS-7 cells in which GLUT1 is overexpressed. With the rotenone co-incubation, the cells

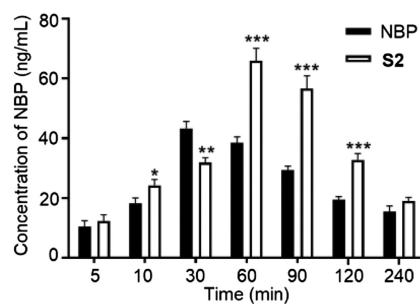


Fig. 4. The concentrations of NBP in mice brain produced by treatment with **S2** or NBP alone at different time. Data are expressed as mean \pm SD of each group from three separate experiments. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. NBP group.

could only produce ATP *via* glycolysis, thus the amount of ATP measured could be positively associated with that of glucose taken up. As shown in Fig. S1 (Supporting information), in comparison with the control group, the ATP levels was significantly reduced in a dose-dependent manner in **S2** groups (10, 30, 100 μ mol/L), while the NBP group (30 μ mol/L) did not significantly affect the levels of ATP under the same conditions. These results suggest that **S2** may be ingested *via* the mediation of GLUT1 to increase the NBP distribution in brain.

In summary, the trihybrids **S1–S8** were designed and synthesized, in which the carboxylic group of a ring-opened NBP derivative hybridized with glucose 6-hydroxyl using FA as a linker. Compound **S2** significantly improved aqueous solubility than NBP and displayed over 10-fold stronger inhibitory activity than NBP against ADP or AA-induced platelet aggregation. In addition, **S2** was more potent than its three structural moieties alone or in combination in inhibition of platelet aggregation, suggesting that the activity of **S2** may be attributed to their synergistic effects. Moreover, **S2** significantly improved the survival rate and reduced ROS generation of OGD/R primary neuron cells. Importantly, **S2** not only possessed an improved PK profile, but also enhanced NBP distribution in rat brain, suggesting that the glucose moiety in **S2** may be recognized by GLUT1, promoting **S2** to pass through BBB. Collectively, our findings suggest that **S2** may be a promising anti-ischemia stroke agent, which deserves further *in vivo* investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. 81773573, 81822041, 21977116 and 81673305), National Science & Technology Major Project “Key New Drug Creation and Manufacturing Program” (No. 2018ZX09711002-006-013), the open project of State Key Laboratory of Natural Medicines (No. SKLNMZCX201824) and State Key Laboratory of Pathogenesis, Prevention and Treatment of High Incidence Diseases in Central Asia Fund (No. SKL-HIDCA-2018-1). Part of the work was supported by Postgraduate Research & Practice Innovation Program of Jiangsu Province (No. KYCX18_0795).

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ccl.2020.02.031.

References

- [1] V.L. Feigin, M.H. Forouzanfar, R. Krishnamurthi, et al., *Lancet* 383 (2014) 245–254.
- [2] C.J. Sommer, *Acta Neuropathol.* 133 (2017) 245–261.
- [3] Q.W. Zhang, L. Jiang, G. Wang, J.Q. Li, *Chin. Chem. Lett.* 28 (2017) 1505–1508.
- [4] X. Zhu, X. Li, J. Liu, *Eur. J. Pharmacol.* 500 (2004) 221–230.
- [5] C. Qin, P. Zhou, L. Wang, et al., *J. Cereb. Blood Flow Metab.* 39 (2018) 2011–2021.
- [6] J. Jia, C. Wei, J. Liang, et al., *Alzheimer's Dementia* 12 (2016) 89–99.
- [7] Y. Zhu, S. Tan, *China J. Pract. Nerv. Dis.* 13 (2010) 10–12.
- [8] X. Wang, Y. Li, Q. Zhao, et al., *Org. Biomol. Chem.* 9 (2011) 5670–5681.
- [9] J. Li, S. Xu, Y. Peng, et al., *Acta Pharmacol. Sin.* 39 (2018) 275–285.
- [10] X. Sheng, K. Hua, C. Yang, et al., *Bioorg. Med. Chem. Lett.* 25 (2015) 3535–3540.
- [11] X. Wang, L. Wang, T. Li, et al., *J. Med. Chem.* 56 (2013) 3078–3089.
- [12] Y. Wang, Y. Huang, Y. Xu, et al., *Antioxid. Redox Signal.* 28 (2018) 141–163.
- [13] X. Wang, L. Wang, Z. Huang, et al., *Bioorg. Med. Chem. Lett.* 23 (2013) 1985–1988.
- [14] A. Chamorro, U. Dirnagl, X. Urra, A.M. Planas, *Lancet Neurol.* 15 (2016) 869–881.
- [15] R. Rodrigo, R. Fernandez-Gajardo, R. Gutierrez, et al., *CNS Neurol. Disord. Drug Targets* 12 (2013) 698–714.
- [16] C. Cheng, T. Ho, E. Lee, et al., *Am. J. Chin. Med.* 36 (2008) 1105–1119.
- [17] J. Li, Y. Zhao, G. Zhong, et al., *Acta Pharm. Sin.* 46 (2011) 305–310.
- [18] Z. Ren, R. Zhang, Y. Li, et al., *Int. J. Mol. Med.* 40 (2017) 1444–1456.
- [19] W.M. Pardridge, *Physiol. Rev.* 63 (1983) 1481–1535.
- [20] F. Lei, W. Fan, X.K. Li, et al., *Chin. Chem. Lett.* 22 (2011) 831–834.
- [21] X.Y. Wu, X.C. Li, J. Mi, et al., *Chin. Chem. Lett.* 24 (2013) 117–119.
- [22] C. Fernandez, O. Nieto, E. Rivas, et al., *Carbohydr. Res.* 327 (2000) 353–365.
- [23] M. Mueckler, C. Makepeace, *J. Biol. Chem.* 283 (2008) 11550–11555.
- [24] S.J. Vannucci, L.B. Seaman, R.C. Vannucci, *J. Cereb. Blood Flow Metab.* 16 (1996) 77–81.
- [25] G.V. Born, M.J. Cross, *J. Physiol.* 168 (1963) 178–195.
- [26] M. Agrawal, V. Kumar, A.K. Singh, et al., *ACS Chem. Neurosci.* 4 (2013) 285–294.
- [27] W.M. Pardridge, *NeuroRx* 2 (2005) 3–14.
- [28] W.M. Pardridge, *Mol. Interv.* 3 (2003) 90–105.
- [29] J. Li, S.F. Xu, Y. Peng, et al., *Acta Pharmacol. Sin.* 39 (2018) 275–285.
- [30] H. Siebeneicher, A. Cleve, H. Rehwinkel, et al., *ChemMedChem* 11 (2016) 2261–2271.