MedChemComm

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: S. Manda, A. Wani, S. S. Bharate, R. A. Vishwakarma, A. Kumar and S. B. Bharate, *Med. Chem. Commun.*, 2016, DOI: 10.1039/C6MD00300A.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/medchemcomm

MedChemComm

Dynamic Article Links►
View Article Online
DOI: 10.1039/C6MD00300A

Cite this: DOI: 10.1039/b000000x

www.rsc.org/medchemcomm

Paper

Design, synthesis and P-gp induction activity of aryl phosphonate esters: Identification of tetraethyl-2-phenylethene-1,1-diyldiphosphonate as an orally bioavailable P-gp inducer‡

Sudhakar Manda,[a,b] Abubakar Wani,[b,c] Sonali S. Bharate,[d] Ram A. Vishwakarma,[a,b] Ajay Kumar[c,*] and Sandip B. Bharate[a,b,*]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: XXX

The clearance of amyloid-beta is mediated by P-glycoprotein (P-gp) transporter pump located at the blood brain barrier. Therefore, the induction of P-gp has been considered as a potential therapeutic strategy for treatment of Alzheimer's disease. The expression of P-gp is regulated through a nuclear receptor - pregnane-X-receptor (PXR). Thus, herein we investigated the potential of a known pregnane-X-receptor (PXR) activator diphosphonate ester SR12813 (6a) for P-gp induction activity and further studied its structure-activity relationship. A diphosphonate ester SR12813 along with a three series of analogs *viz.* aryl alkylidene, aryl alkynyl, and aryl α-amino phosphonate esters were synthesized and screened for P-gp induction activity in P-gp overexpressing adenocarcinoma LS180 cells using rhodamine-123 efflux assay. The parent compound SR12813 along with several new analogs displayed P-gp induction activity at 5 µM. The western-blot analysis indicated that tetraethyl-2-phenylethene-1,1-diyldiphosphonate (6c) and tetraethyl-2-(anthracene-10-yl)ethene-1,1-diyldiphosphonate (6s) showed 7-8 fold increase in P-gp expression in LS180 cells. The diphosphonate ester 6c displayed excellent aqueous solubility, no cytochrome P450 inhibition liability and no efflux pump substrate liability. Furthermore, it possesses excellent oral pharmacokinetic profile in BALB/c mice with $AUC_{0-\infty}$ of 2067 ng·h/mL and 37.6% oral bioavailability. The results presented here clearly indicate the potential of this scaffold to increase the clearance of brain Aβ across the BBB and thus its promise for development as potential anti-Alzheimer agents.

Introduction

Deposition of amyloid-beta (Aβ) in the brain has been considered as a major hallmark for progression of the Alzheimer's disease (AD). Studies have demonstrated that the clearance mechanism of amyloid-beta gets hampered in Alzheimer patients.[1, 2] The clearance of Aβ is mediated by the P-gp transporter pump located at the blood brain barrier (BBB).[3-9] Thus, it is evident that drugs which can induce P-gp expression at BBB will have a great potential to emerge as novel AD therapeutics. With this hypothesis, several P-gp inducers have been identified in recent years which showed significant efficacy in Alzheimer's disease in-vivo models.[5] Anti-tubercular drug rifampicin (**1**) is the widely known P-gp inducer which enhances Aβ clearance,[10] and thus in recent years it has been investigated up to the clinical trial stage for its potential in Alzheimer's disease.[11-13] The olive-oil-derived oleocanthal (**2**) enhances amyloid-beta clearance via upregulation of P-gp and LRP1 expression.[5] Recently, our group has identified a natural product colupulone based synthetic analog 3,5-dihydroxy-4,4-bis(3-methylbut-2-enyl)-2,6-dipropionylcyclohexa-2,5-dienone (**3**) as an inducer of P-gp and LRP-1.[14] Fascaplysin (**4**)[15] and a quinoline derivative **5**[16] are other P-gp inducer leads discovered by us (Figure 1).

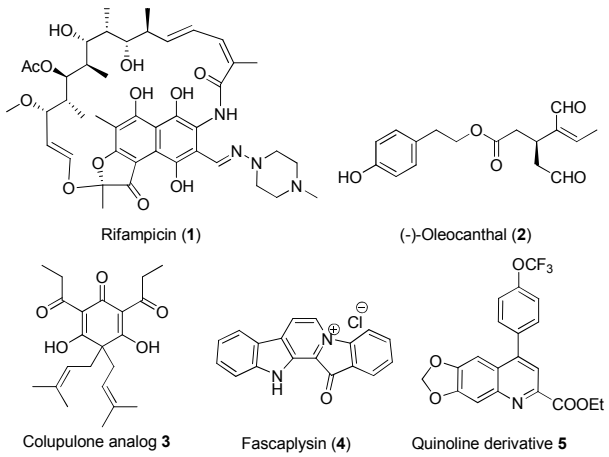


Figure 1. Structures of known P-gp inducers

[a]*Medicinal Chemistry Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India*
[b]*Academy of Scientific & Innovative Research (AcSIR), CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India*
[c]*Cancer Pharmacology Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India*
[d]*Preformulation Laboratory, PKPD Toxicology and Formulation Division, CSIR-Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India*
**E-mail: sbharate@iiim.ac.in (SBB); ajaykmahajan@hotmail.com (AK).*
Fax: +91-191-2589333; Tel: +91-191-2589006
‡ *The authors declare no competing interests.*
Electronic supplementary information (ESI) available for experimental details. See DOI: XXX

Received XXth XXXXXX
DOI: 10.1039/C6MD00300A

The pregnane X receptor (PXR), a member of the nuclear receptor superfamily of proteins regulates the expression of P-gp, located in various tissues such as intestinal epithelium, hepatic canalicular membrane, proximal tubular of kidneys and endothelial cells of the blood-brain barrier (BBB). Thus, it is quite obvious that activators of PXR could be a potential starting point to identify promising P-gp inducer leads. A diphosphonate ester SR12813 (**6a**), apart from its potent anti-hypocholesterolemic activity,[17, 18] is a potent activator of PXR.[19]

In continuation to our efforts on discovery of P-gp inducers as potential anti-Alzheimer therapeutics,[14-16] herein our objective was to investigate the P-gp induction potential, and establish structure-activity relationship of a PXR activator SR12813 (**6a**). Despite of the promising P-gp induction activity of two natural product based P-gp inducer leads **3** and **4** discovered earlier, their oral pharmacokinetic profile was very poor (%F = < 5%). The pharmacophoric features of SR12813 indicated that this scaffold will have better pharmacokinetic properties. Furthermore, literature search indicated that no medicinal chemistry efforts have been made on this unique scaffold for P-gp induction activity. Thus, herein, the synthesis, P-gp induction activity in LS180 cells, structure-activity relationship, physicochemical properties/ drug discovery liabilities (aqueous solubility, solubility in other biological media's, cytochrome P450 inhibition liabilities, Caco-2 permeability) and pharmacokinetic analysis of aryl phosphonates is presented in this paper.

Results and discussion

SR12813 is an alkylidene tetraethyl bisphosphonate ester, containing three main structural components *viz*. substituted aryl (bearing two *tert*-butyl and one hydroxyl group), alkylidene linker, and bisphosphonate ester. As this scaffold has never been studied for P-gp induction activity, here one of the objective was also to establish preliminary structure-activity relationship. For this purpose, modifications were planned on ester portion, aryl moiety as well as on the alkylidene linker, as depicted in Figure 2. Three series of phosphonate esters were synthesized as depicted in Figure 2.

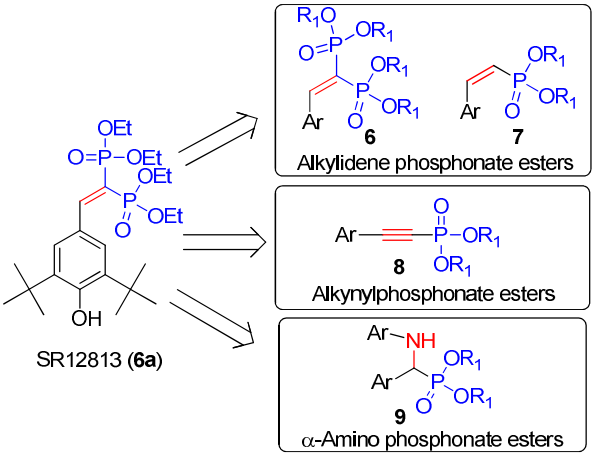
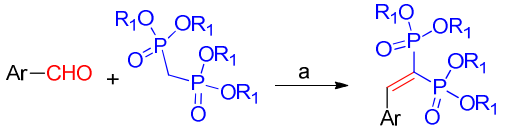


Table 1. Synthesis, P-gp induction activity and cytotoxicity of alkylidene di-phosphonate esters **6a-s**[ab]



10 **11** **6**

Entry	R_1	Ar	% accumulation of Rh123 in LS180 cells (±SD)[cd]	% cell viability of LS180 cells at 30 μM[d]
6a	-Et	-Ph (4-OH, 3,5-di-tert-Bu)	90 ± 6[ns]	100
6b	-$CH(CH_3)_2$	-Ph (4-OH, 3,5-di-tert-Bu)	66.93 ± 3.48***	100
6c	-Et	-Ph	**58.09 ± 4.63*****	100
6d	-$CH(CH_3)_2$	-Ph	73.88 ± 3.49***	92
6e	-Et	-Ph(2-NO_2)	65.69 ± 5.29***	100
6f	-$CH(CH_3)_2$	-Ph(3-Br)	68.08 ± 11***	74
6g	-$CH(CH_3)_2$	-Ph(4-N,N-diMe)	65.32 ± 7.56***	100
6h	-Et	-Ph(3,5-di-OMe)	64.8 ± 4.96***	100
6i	-$CH(CH_3)_2$	-Ph(3,5-di-OMe)	72.45 ± 5.26***	100
6j	-$CH(CH_3)_2$	-Ph(4-F, 5-OMe)	70 ± 4.11***	86
6k	-Et	-Ph(3,5,6-tri-OMe)	68.74 ± 6.23***	100
6l	-$CH(CH_3)_2$	-Ph(3,4,6-tri-OMe)	64.07 ± 2.89***	100
6m	-Et	-5-nitrofuran-2-yl	88.3 ± 3.32[ns]	92
6n	-$CH(CH_3)_2$	-5-nitrofuran-2-yl	67.84 ± 8.61***	41
6o	-$CH(CH_3)_2$	-Ph(3,4-dimethylene-dioxy)	83.27 ± 4.9[ns]	100
6p	-Et	-naphth-1-yl	73.66 ± 8.08***	100
6q	-$CH(CH_3)_2$	-naphth-1-yl	63.71 ± 7.36***	100
6r	-$CH(CH_3)_2$	-quinolin-5-yl	70.48 ± 2.19***	100
6s	-Et	-anthracen-10-yl	**56.6 ± 3.65*****	100
Control	-	-	100	100
Rifampicin	-	-	77.3 ± 7.5***	nd

[a] Reagents and conditions: (a). *N*-methyl morpholine, $TiCl_4$, CCl_4, dry THF, 0 °C to room temp., overnight, 50-65% yield; [b] product yields are mentioned in the experimental section; [c] The P-gp induction activity of compounds tested at 5 μM was measured in terms of the % intracellular accumulation of rhodamine 123 (Rh123)/total protein (μg) in LS180 cells. The decrease in % intracellular accumulation (compared to control) of Rh123 indicates induction of P-gp. Rifampicin (5 μM) was used as a reference P-gp inducer; [d]The statistical comparisons were made between control vs compounds. The p value <0.5 was considered to be significant. P value *< 0.5, **<0.01, ***<0.001. All values are shown as average of three experiments ± SD. nd: not determined.

DOI: 10.1039/C6MD00300A

Figure 2. Chemical structure of SR12813 and general structures of designed series for synthesis.

A series of analogues were synthesized[20] starting from commercially available substituted aldehydes **10**. Aldehydes **10** on treatment with diphosphonate ester **11a-b** in dry THF in presence of titanium tetrachloride produced alkylidene diphosphonate esters **6a-s** and alkylidene monophosphonate esters **7a-d** in 55-65% yield (Table 1 and 2). Next, a series alkynyl phosphonates[21] were synthesized by treatment of substituted phenylacetylenes **12** with diethylchloro phosphate **13** using n-BuLi as a base. Three alkynyl phosphonates **8a-c** were prepared (Table 3). Further, we synthesized α-amino phosphonates[22] by treatment of substituted aldehydes **10** with anilines **14** and diethyl phosphite **15** using silica perchloric acid as a catalyst. Two α-amino phosphonate esters **9a-b** were prepared (Table 4). All synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and MS analysis.

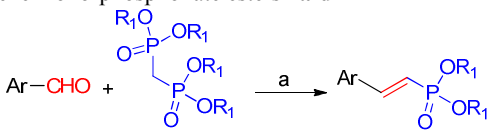
All synthesized compounds were screened for P-gp induction activity using rhodamine 123 based efflux assay in P-gp expressing human colorectal adenocarcinoma LS180 cells. Prior to screening compounds for P-gp induction activity in LS180 cells, the cytotoxic effect of all compounds was investigated in these cells in order to select the non-toxic test concentration. In MTT assay, all compounds were non-toxic to LS180 cells with IC_{50} > 30 μM, except compound **6n** which showed 41% cell viability at 30 μM. Therefore, for P-gp induction assay, we selected 5 μM as a test concentration. LS180 cells treated with 5 μM of each compound, for 48 h, displayed significant induction of P-gp activity, as displayed by the reduced intracellular accumulation of rhodamine-123, compared to control, as a result of increased efflux of rhodamine-123 (Table 1-4).

In alkylidene di-phosphonate esters series (**6a-s**), several newer analogs displayed promising ability to decrease the intracellular accumulation of Rh123 levels in LS180 cells. The SR12813 showed moderate P-gp induction activity at 5 μM. However, several newly synthesized analogs showed promising P-gp induction activity. Particularly, the compound **6c** showed reduction in % accumulation of Rh123 levels up to 58% (control = 100%; positive control rifampicin – 77%). Similarly, another new compound **6s** also showed similar level of potent P-gp induction activity (56% intracellular levels of Rs123).

A precise structure-activity relationship was not observed; however some of the key features noticed were: (a) In case of alkylidene di-phosphonate esters, the unsubstituted aryl moiety was preferred over substituted aryl moiety (e.g. compound **6c** and **6s** *versus* **6e**, **6h**, **6k**, **6m**, **6p**; Table 1). (b) In case of alkylidene mono-phosphonates, the aryl moiety substituted with one halogen atom showed 76% accumulation of Rh123. Substitution of aryl moiety with two halogen atoms, results in reduced P-gp induction activity (e.g. **7b** and **7d** – both contain two halogen atoms – 91 and 97% accumulation of Rh123 levels). However, when aryl moiety containing one halogen atom was substituted with one electron-donating group (e.g. OMe), P-gp induction activity was increased (e.g. **7c** – 70% Rh123 levels; Table 2). (c) The alkynylphosphonate esters, in general displayed good P-gp induction activity (74-77% intracellular accumulation of Rh 123) without any toxicity (Table 3). (d) α-amino phosphonate esters showed weak activity (Table 4). Here, only marginal decrease in the % intracellular accumulation of Rh123 levels was observed (82-90% intracellular accumulation of Rh 123). (e) Both mono- and bis-phosphonate esters showed P-gp induction activity. Similarly, alkynyl mono-phosphonate esters also were active. This indicates that there is no effect of change of two phosphonates to one and change of double bond linker with triple bond linker.

In general, amongst the four series tested, series one (alkylidene di-phosphonate esters) was most promising, wherein the phenyl alkylidene di-phosphonate ethyl ester **6c** and anthracen-10-yl alkylidene di-phosphonate ethyl ester **6s** showed the lowest % of intracellular accumulation of rhodamine-123 (58% and 56%, respectively), in comparison to untreated control cells, suggesting both compounds as the most potent P-gp inducers. Furthermore, the P-gp induction activity of these two compounds was better than the positive control rifampicin. Both these compounds does not showed any toxicity to LS180 cells at 30 μM. Results are shown in Table 1.

Table 2. Synthesis, P-gp induction activity and cytotoxicity of alkylidene mono-phosphonate esters **7a-d**[ab]

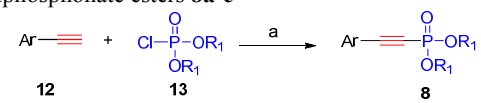


10 **11** **7**

Entry	R_1	Ar	% accumulation of Rh123 in LS180 cells (±SD)[cd]	% cell viability of LS180 cells at 30 μM[d]
7a	-Et	-Ph(4-Cl)	76.38 ± 3.69***	82
7b	-Et	-Ph(3,5-di-F)	91.31 ± 1.95[ns]	100
7c	-Et	-Ph(3-Br, 4-OMe)	70.49 ± 3.86***	100
7d	-Et	-Ph(3-Br, 4-F)	97.7 ± 4.49[ns]	100
Control	-	-	100	100
Rifampicin	-	-	77.3 ± 7.5***	nd

[a] Reagents and conditions: (a). *N*-Methyl morpholine, $TiCl_4$, CCl_4, dry THF, 0 °C to room temp., overnight, 55-65% yield; [b] product yields are mentioned in the experimental section; [c]The P-gp induction activity of compounds tested at 5 μM was measured in terms of the % intracellular accumulation of rhodamine 123 (Rh123)/ total protein (μg) in LS180 cells. The decrease in % intracellular accumulation (compared to control) of Rh123 indicates induction of P-gp. Rifampicin (5 μM) was used as a reference P-gp inducer; [d] The statistical comparisons were made between control *vs* compounds. The p value <0.5 was considered to be significant. *P* value *< 0.5, **<0.01, ***<0.001. All values are shown as average of three experiments ± SD. nd: not determined.

Table 3. Synthesis, P-gp induction activity and cytotoxicity of alkynylphosphonate esters **8a-c**[ab]



12 **13** **8**

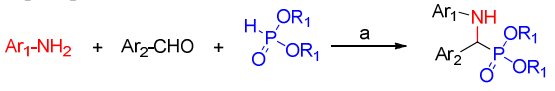
Entry	Ar	% accumulation	% cell viability of LS180 cells at

DOI: 10.1039/C6MD00300A

		of Rh123 in LS180 cells (±SD)[cd]	30 μM[d]
8a	Ph	77.64 ± 4.31*	100
8b	Ph (4-CF_3)	74.63 ± 0.64**	100
8c	Ph (3-CF_3)	75.16 ± 2.61**	100
Control	-	100	100
Rifampicin	-	77.3 ± 7.5***	nd

[a] Reagents and conditions: a). n-BuLi, dry THF, -78 °C, 2 h, 75-85%; [b] product yields are mentioned in the experimental section; [c] The P-gp induction activity of compounds tested at 5 μM was measured in terms of the % intracellular accumulation of rhodamine 123 (Rh123)/total protein (μg) in LS180 cells. The decrease in % intracellular accumulation (compared to control) of Rh123 indicates induction of P-gp. Rifampicin (5 μM) was used as a reference P-gp inducer; [d] The statistical comparisons were made between control vs compounds. The p value <0.5 was considered to be significant. P value *< 0.5, **<0.01, ***<0.001. All values are shown as average of three experiments ± SD. nd: not determined.

Table 4. Synthesis, P-gp induction activity and cytotoxicity of α-amino phosphonate esters **9a-b**[a]

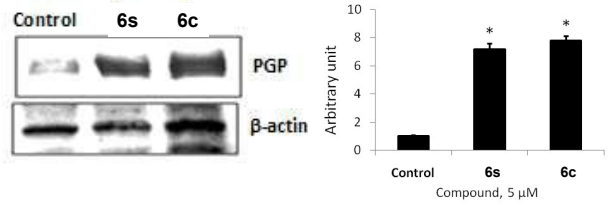


14 **10** **15** **9**

Entry	Ar_1	Ar_2	% accumulation of Rh123 in LS180 cells (±SD)[bc]	% cell viability of LS180 cells at 30 μM[c]
9a	-Ph	-Ph	82.56 ± 5.32[ns]	100
9b	-Ph	-Ph (4-Cl)	90.85 ± 3.21[ns]	100
Control	-		100	100
Rifampicin	-		77.3 ± 7.5***	nd

[a] Reagents and conditions: (a). *n*-BuLi, dry THF, -78 °C, 2 h, 80-85%; [b] product yields are mentioned in the experimental section; [c]The P-gp induction activity of compounds tested at 5 μM was measured in terms of the % intracellular accumulation of rhodamine 123 (Rh123)/total protein (μg) in LS180 cells. The decrease in % intracellular accumulation (compared to control) of Rh123 indicates induction of P-gp. Rifampicin (5 μM) was used as a reference P-gp inducer; [d]The statistical comparisons were made between control vs compounds. The p value <0.5 was considered to be significant. P value *< 0.5, **<0.01, ***<0.001. All values are shown as average of three experiments ± SD. nd: not determined.

The promising P-gp induction activity of these compounds was further confirmed by western-blot (WB) analysis. In the WB analysis, the exposure of LS180 cells with compounds **6c** and **6s** at 5 μM, resulted in 7.2 and 7.8-fold increase in the P-gp expression in LS180 cells (Figure 3).



(a) (b)

Figure 3. The effect of alkylidene bis-phosphonate esters **6c** and **6s** (5 μM) on P-gp expression in LS180 cells. (a) Western-blot (b) quantitation results of P-gp expression.

Next, the EC_{50} for P-gp induction was determined for SR12813 (**6a**) and best new analogs **6c** and **6s** along with rifampicin (Table 5). Both newly synthesized phosphonate esters **6c** and **6s** showed better P-gp induction activity (EC_{50} = 0.27-0.29 μM) than SR12813 (**6a**: EC_{50} = 1.25 μM). The compound **6s** possesses 4.3-4.6-fold higher EC_{50} value than SR12813 (**6a**). The EC_{50} of new analogs **6c** and **6s** was comparable to that of positive control rifampicin.

Table 5. P-gp induction activity in terms of EC_{50} values of **6a**, **6c** and **6s**

Entry	EC_{50} (μM)
SR12813 (**6a**)	1.250
6c	0.271
6s	0.290
Rifampicin	0.226

After obtaining two interesting P-gp inducer leads based on the known PXR activator SR12813 scaffold, next we decided to perform their preclinical characterization. Both compounds **6c** and **6s** displayed excellent thermodynamic aqueous solubility in water, PBS, SGF and SIF (*S* = >1.5 mg/ml) (Table 6).

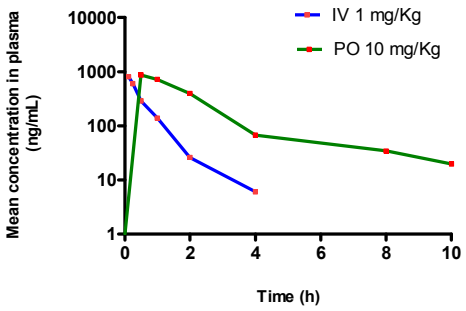
Table 6. Aqueous solubility of SR12813 (**6a**) and its analogs **6c** and **6s**

Media[a]	Solubility (μg/ml)		
	6a	**6c**	**6s**
Water	200	>1500	400
PBS	200	>1500	>1500
SGF	200	>1500	>1500
SIF	200	40	>1500

[a]PBS: phosphate buffer saline (pH 7.4); SGF: simulated gastric fluid (pH 1.2); SIF: simulated intestinal fluid (pH 6.8)

Next, the Caco-2 permeability and CYP liability was determined for compound **6c**. The cytochrome P450 liability was assessed on four key drug metabolizing enzymes CYP3A4, CYP2D6, CYP2C9, CYP2C19 at 10 μM. Compound **6c** showed 19, 20, 0, and 0% inhibition of these enzymes at tested concentration, indicating that this compound does not possess any CYP liability (<50% inhibition at 10 μM). In caco-2 permeability assay, the efflux ratio of 0.9 indicated that it is not a substrate of efflux pumps. The pharmacokinetic behaviour of compound **6c** was then evaluated in BALB/c mice using oral and IV dosing.[23] A single 10 mg/kg oral dose and 1.0 mg/kg intravenous dose was given to the BALB/c mice. Compound showed good plasma exposure on oral administration with AUC_{0-t} and $AUC_{0-\infty}$ of 1968 and 2067 ng·h/mL, respectively. Furthermore, compound has good elimination half-life (3.48 h). The in vivo blood clearance following intravenous dosing was found to be 30.4 mL. $min^{-1}.kg^{-1}$ with a large volume of distribution 1.81 $L.kg^{-1}$. Compound **6c** showed good oral bioavailability (37.6%) (Figure 4).

DOI: 10.1039/C6MD00300A



Parameters	IV	PO
Dose (mg/kg)	1	10
$t_{1/2,\beta}$ (h)	0.69	3.48
$AUC_{0\text{-}t}$ (ng·h/mL)	543	1968
$AUC_{0\text{-}\infty}$ (ng·h/mL)	549	2067
C_{max} (ng/mL)	811	870
C_0 (ng/mL)	1153	-
CL (mL/min/Kg)	30.4	-
V_d (L/Kg)	1.81	-
V_{dss} (L/Kg)	1.10	-
T_{last} (h)	4.0	-
t_{max} (h)	-	0.50
%F	-	**37.6**

Figure 4. Pharmacokinetic analysis of compound **6c** in BALB/c mice. The plasma versus plasma concentration profile of compound in BALB/c mice is shown [$t_{1/2,\beta}$: terminal half life; $AUC_{0\text{-}t}$: the area under the plasma concentration-time curve from 0 to last measurable time point; $AUC_{0\text{-}\infty}$: area under the plasma concentration-time curve from time zero to infinity; C_{max}: maximum observed plasma concentration; C_0: extrapolated concentration at zero time point; CL: clearance; V_d: volume of distribution; V_{dss}: volume of distribution at steady state; T_{last}: time at which last concentration was found; F: bioavailability].

Conclusion

In conclusion, the alkylidene phosphonate scaffold has shown promising P-gp induction activity in LS180 cells. The alkylidene bisphosphonate **6c** strongly induced the expression of P-gp which plays major role in enhancing Aβ efflux across the BBB. Furthermore, this compound **6c** possessed excellent aqueous solubility, no CYP and efflux pump liability; and has excellent pharmacokinetic properties with oral bioavailability of 37%. Thus, the results presented herein, demonstrate the potential of this scaffold for investigation as amyloid-β clearing agent and thereby as a potential anti-Alzheimer treatment.

Acknowledgements

AK is thankful to CSIR, New Delhi for the award of senior research associateship. VK thanks UGC for fellowship. S.S.B. thanks ICMR for research associateship. This research was supported by a grant from the CSIR 12th FYP project (BSC-0205).

Abbreviations

Aβ, amyloid-beta; AD, Alzheimer's disease; BBB, blood-brain barrier; bEnd3, endothelial polyoma middle T antigen transformed cells of cerebral cortex of the brain; DMSO, dimethyl sulfoxide; LS180, intestinal human colon adenocarcinoma cell line; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); P-gp, P-glycoprotein; PXR, pregnane-X-receptor; Rh123, rhodamine 123; SAR, structure-activity relationship.

Notes and references

1. K. G. Mawuenyega, W. Sigurdson, V. Ovod, L. Munsell, T. Kasten, J. C. Morris, K. E. Yrasheski and R. J. Bateman, *Science* 2010, **330**, 1774.
2. D. M. E. van Assema, M. Lubberink, M. Bauer, W. M. van der Flier, R. C. Schuit, A. D. Windhorst, E. F. I. Comans, N. J. Hoetjes, N. Tolboom, O. Langer, M. Müller, P. Scheltens, A. A. Lammertsma and B. N. M. van Berckel, *Brain*, 2012, **125**, 181-189.
3. F. C. Lam, R. Liu, P. Lu, A. B. Shapiro, J. M. Renoir, F. J. Sharom and P. B. Reiner, *J. Neurochem.*, 2001, **76**, 1121-1128.
4. H. Qosa, A. H. Abuznait, R. A. Hill and A. Kaddoumi, *J. Alzheimers Dis.*, 2012, **31**, 151-165.
5. A. H. Abuznait, H. Qosa, B. Busnena, K. A. El Sayed and A. Kaddoumi, *ACS Chem. Neurosci.*, 2013, **19**, 973-982.
6. A. H. Abuznait, C. Cain, D. Ingram, D. Burk and A. Kaddoumi, *J. Pharm. Pharmacol.*, 2011, **63**, 1111–1118.
7. J. R. Cirrito, R. Deane, A. M. Fagan, M. L. Spinner, M. Parsadanian, M. B. Finn, H. Jiang, J. L. Prior, A. Sagare, K. R. Bales, S. M. Paul, B. V. Zlokovic, D. Piwnica-Worms and D. M. Holtzman, *J. Clin. Invest.*, 2005, **115**, 3285-3290.
8. S. Vogelgesang, I. Cascorbi, E. Schroeder, J. Pahnke, H. K. Kroemer, W. Siegmund, C. Kunert-Keil, L. C. Walker and R. W. Warzok, *Pharmacogenetics*, 2002, **12**, 535-541.
9. D. Kuhnke, G. Jedlitschky, M. Grube, M. Krohn, M. Jucker, I. Mosyagin, I. Cascorbi, L. C. Walker, H. K. Kroemer, R. W. Warzok and S. Vogelgesang, *Brain Pathol.*, 2007, **17**, 347-353.
10. H. Qosa, A. H. Abuznait, R. A. Hill and A. Kaddoumi, *J. Alzh. Dis.*, 2012, 151-165.
11. D. W. Molloy, T. I. Standish, Q. Zhou, G. Guyatt and D. S. GROUP, *Int. J. Geriatr. Psychiatry*, 2013, **28**, 463-470.
12. B. Yulug, L. Hanoglu, E. Kilic and W. R. Schabitz, *Brain Res. Bull.*, 2014, **107**, 37-42.
13. M. B. Loeb, D. W. Molloy, M. Smieja, T. Standish, C. H. Goldsmith, J. Mahony, S. Smith, M. Borrie, E. Decoteau, W. Davidson, A. McDougall, J. Gnarpe, M. O'DONNell and M. Chernesky, *J. Am. Geriatr. Soc.*, 2004, **52**, 381-387.
14. J. B. Bharate, Y. S. Batarseh, A. Wani, S. Sharma, R. A. Vishwakarma, A. Kaddoumi, A. Kumar and S. B. Bharate, *Organic & biomolecular chemistry*, 2015, **13**, 5488-5496.
15. S. Manda, S. Sharma, A. Wani, P. Joshi, V. Kumar, S. K. Guru, S. S. Bharate, S. Bhushan, R. A. Vishwakarma, A. Kumar and S. B. Bharate, *Eur. J. Med. Chem.* , 2016, **107**, 1–11.
16. J. B. Bharate, A. Wani, S. Sharma, S. I. Reja, M. Kumar, R. A. Vishwakarma, A. Kumar and S. B. Bharate, *Org. Biomol. Chem.*, 2014, **12**, 6267-6277.
17. T. A. Berkhout, H. M. Simon, D. D. Patel, C. Bentzen, E. Niesor, B. Jackson and K. E. Suckling, *J. Biol. Chem.*, 1996, **271**, 14376-14382.
18. G. B. W. Ryan E. Watkins, Linda B. Moore, Jon L. Collins, Millard H. Lambert, Shawn P. Williams, Timothy M. Willson, Steven A. Kliewer, Matthew R. Redinbo, *Science*, 2001, **292**, 2329-2333.

DOI: 10.1039/C6MD00300A

19. S. A. Jones, L. B. Moore, J. L. Shenk, G. B. Wisely, G. A. Hamilton, D. D. McKee, N. C. O. Tomkinson, E. L. LeCluyse, M. H. Lambert, T. M. Willson, S. A. Kliewer and J. T. Moore, *Mol. Endocrinol.*, 2000, **14**, 27-39.
20. Z.-Y. Xue, Q.-H. Li, H.-Y. Tao and C.-J. Wang, *J. Am. Chem. Soc.*, 2011, **133**, 11757-11765.
21. D. Lecerclé, M. Sawicki and F. Taran, *Org. Lett.*, 2006, **8**, 4283-4285.
22. A. K. Bhattacharya and K. C. Rana, *Tetrahedron Lett.*, 2008, **49**, 2598-2601.
23. The pharmacokinetic study was carried out at Jubilant Biosys Limited Bangalore (India) on a commercial basis. These experiments were approved by the Jubilant Biosys Institutional Animal Ethics Committee, Bangalore, India (IAEC/JDC/2012/27) and were in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Environment, Government of India.

View Article Online
DOI: 10.1039/C6MD00300A

GRAPHICAL ABSTRACT

We report P-gp induction activity of a PXR activator SR12813 and its structure-activity relationship. SR12813 analogs displaying promising P-gp induction have been identified and studied for aqueous solubility, CYP/ efflux liabilities, and pharmacokinetic analysis.