Journal of Neural Transmission
https://doi.org/10.1007/s00702-018-1923-z

PSYCHIATRY AND PRECLINICAL PSYCHIATRIC STUDIES - ORIGINAL ARTICLE

Derivatization of common antidepressant drugs increases inhibition of acid sphingomyelinase and reduces induction of phospholipidosis

Cosima Rhein[1] · Stefan Löber[2] · Peter Gmeiner[2] · Erich Gulbins[3] · Philipp Tripal[1,4] · Johannes Kornhuber[1]

Received: 4 June 2018 / Accepted: 28 August 2018

Abstract

In recent studies, major depressive disorder (MDD) was linked to an increase in acid sphingomyelinase (ASM) activity. Several drugs that are commonly used to treat MDD functionally inhibit the lysosomal enzyme ASM and are called functional inhibitors of ASM (FIASMAs). These drugs are classified as cationic amphiphilic drugs (CADs) that influence the catalytic activities of different lysosomal enzymes. This action results in the side effect of phospholipidosis (PLD), which describes a detrimental increase in the phospholipid content in lysosomes. FIASMAs differ only slightly in their physico-chemical properties, but their effects on ASM activity and induction of the lysosomal phospholipid content vary significantly. In this study, we systematically induced minor chemical modifications to the FIASMAs imipramine, desipramine and fluoxetine. We generated a library of 45 new CADs with slightly different log *P* (logarithmic partition coefficient) and p*K*a (logarithmic acid dissociation constant) values. The effects of the compounds on the ASM activity and lysosomal phospholipid content were assessed in cell culture assays. We identified four compounds with beneficial effects, i.e., increased ASM activity inhibition and reduced PLD induction compared with the original drugs. The compounds HT04, RH272B and RH272D outperformed the original imipramine, whereas RH281A performed better than desipramine. Thus, minor chemical variations of CADs impact lysosomal metabolism in a specific manner and can lead to antidepressant drugs with less deleterious side effects.

Keywords Acid sphingomyelinase · Functional inhibitors of ASM activity (FIASMAs) · Imipramine · Desipramine · Fluoxetine · Phospholipidosis · Major depression

Introduction

Major depressive disorder (MDD) is a psychiatric disease with an unresolved etiology. In recent studies, the enzyme acid sphingomyelinase (ASM, EC 3.1.4.12) was investigated as a potential target for antidepressant action (Gulbins et al. 2013; Kornhuber et al. 2005; Rhein et al. 2017). ASM is a mainly lysosomal located enzyme that optimally functions at a pH of 5. ASM hydrolyzes the abundant membrane lipid sphingomyelin to ceramide, a bioactive lipid messenger that affects downstream signaling in the cell (Gulbins and Grassme 2002; Gulbins and Kolesnick 2002). Interestingly, several antidepressant drugs indirectly inhibit ASM activity (Albouz et al. 1986) and are called 'functional inhibitors of ASM' (FIASMAs) (Kornhuber et al. 2011, 2010, 2008). Therefore, the role of ASM in the etiology of MDD was investigated in human and murine studies. Depressed patients displayed significantly increased levels of ASM activity in their cultivated blood cells compared with a control group. In addition, the severity of depression as indicated by the Hamilton Scale of Depression (HAMD) and ASM activity levels of patients were positively correlated (Kornhuber et al. 2005). In a genetic mouse model overexpressing ASM, the ASM/ceramide system mediates the effects of antidepressant drugs (Gulbins et al. 2013). Thus,

Philipp Tripal and Johannes Kornhuber contributed equally.

✉ Cosima Rhein
cosima.rhein@uk-erlangen.de

[1] Department of Psychiatry and Psychotherapy, University Hospital Erlangen, Friedrich-Alexander Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

[2] Pharmaceutical Chemistry, Friedrich-Alexander Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

[3] Department of Molecular Biology, University of Duisburg-Essen, Essen, Germany

[4] Present Address: Optical Imaging Centre Erlangen (OICE), Friedrich-Alexander Universität Erlangen-Nürnberg (FAU), Erlangen, Germany

Published online: 06 September 2018

strong ASM inhibition should be achieved by a FIASMA to be beneficial in this context (Kornhuber et al. 2014), without inducing side effects.

The FIASMAs imipramine, desipramine, and fluoxetine are among the most prominent antidepressant drugs. For desipramine, experimental data showed that it accumulates at the lysosomal membrane and causes displacement of ASM from the membrane (Kölzer et al. 2004). ASM is released into the lysosomal lumen and subjected to degradation by lysosomal mechanisms via proteases (Hurwitz et al. 1994). This effect on ASM activity can be measured and quantified by an enzyme activity assay (Gulbins and Kolesnick 2000) in cell culture systems (Kornhuber et al. 2011), clinical studies on cultured human blood cells (Kornhuber et al. 2005) or murine tissues (Gulbins et al. 2013).

However, the application of FIASMAs also results in the side effect of phospholipidosis (PLD), which is the accumulation of phospholipids (Anderson and Borlak 2006), and is probably caused by the degradation of lysosomal phospholipases (Halliwell 1997). This effect occurs in lysosomal storage disorders, but is also induced by different drugs, and then called drug-induced phospholipidosis (Lüllmann et al. 1978). Affected organs develop inflammatory reactions and histopathological changes (Anderson and Borlak 2006). Phospholipid content in cells can be monitored using specific staining (Muehlbacher et al. 2012).

Antidepressant drug development thus aims to generate potent ASM inhibitors with a low potential of inducing PLD. Relevant for this approach are those factors that influence lysosomal targeting of compounds. Cationic amphiphilic drugs (CADs) are known for their optimal lysosomotropic characteristics. FIASMAs are a subgroup of CADs and also feature high lysosomal accumulation as predicted by a chemo-theoretic approach (Trapp et al. 2008).

CADs enter the lysosomal cell compartment by a mechanism known as lysosomotropism or lysosomal trapping (de Duve et al. 1974; Trapp et al. 2008). The efficiency of this mechanism is highly dependent on the chemical properties of the respective compound (Kornhuber et al. 2011; Trapp et al. 2008). The log *P* value (logarithmic partition coefficient) of a chemical compound is a measure of its lipophilicity, and the p*K*a value (logarithmic acid dissociation constant) describes its ability to become protonated in a pH-dependent manner. These two characteristics are the main discriminators of lysosomal trapping (Trapp et al. 2008). Lysosomotropic compounds are rather lipophilic (log *P* between 2 and 9) with a p*K*a value > 4; thus, these compounds are protonated at a low pH level.

Therefore, CADs were supposed to influence the lysosomal metabolism by unspecific, functional mechanisms (Hurwitz et al. 1994; Kölzer et al. 2004; Kornhuber et al. 2010) that affect lysosomal hydrolases like ASM and phospholipases in general (Kodavanti and Mehendale 1990), but not in a specific or differential manner. But experimental data showed that the inhibitory potential of FIASMAs on ASM activity is broad (Kornhuber et al. 2011), and that only a few of the lysosomal enzymes were affected by FIASMA application (Kornhuber et al. 2010), whereas other enzymes retained their function. These data led to the hypothesis that CADs can indeed exert specific effects on selected lysosomal enzymes. For an overview of the different lysosomal parameters, see Fig. 1. It could be experimentally demonstrated that the FIASMAs imipramine, desipramine, and fluoxetine exert differential patterns of influence on ASM activity (Kornhuber et al. 2011) and phospholipid accumulation (Muehlbacher et al. 2012), that point to specific lysosomal action of CADs with need for optimized parameters in the context of MDD.

Therefore, we aimed to generate modified derivatives of imipramine, desipramine, and fluoxetine that should increase ASM activity inhibition and reduce PLD induction compared with the original drugs. The systematic modification of imipramine, desipramine, and fluoxetine resulted in the generation of a library of 45 new CADs. All substances were synthesized and characterized. In addition, the effects of the compounds on ASM activity and PLD induction were assessed in cell culture systems. The results demonstrate that minor chemical modifications changed the properties regarding ASM activity and PLD induction for some of the compounds significantly. This approach will help to define specific actions exerted by CADs and could lead to improved antidepressants with a reduced spectrum of side effects.

Experimental section

Synthesis of imipramine derivatives

Test compounds **RH272B, RH272D, RKZ01, RKZ06, RKZ07** and **HT04** were obtained by reductive alkylation of desipramine (free base) using 5 equivalents of propanal, pentanal, hexanal, cyclohexanol, acetone or 6-hydroxyhexanal, respectively. $Na(OAc)_3BH$ (3 eq.) served as the reducing agent, and CH_2Cl_2 served as a solvent. After stirring the mixture at room temperature for 16 h, the solution was treated with brine and extracted with CH_2Cl_2. After evaporation, the crude product was purified by flash chromatography.

For the synthesis of the derivative **HT23** 5-(4-bromobutyl)-10,11-dihydro-5H-dibenz[*b,f*]azepine (Schmidt et al. 2008) was treated with 1-hexylamine (5 eq.), Na_2CO_3 (1 eq.) and NaI (0.2 eq.) and stirred at reflux temperature in acetonitrile for 16 h. The cooled mixture was treated with brine and extracted with CH_2Cl_2. After evaporation, the crude product was purified by flash chromatography.

The amide derivatives **LP05** and **HT13** were synthesized by acylation of desipramine (free base) with 6-bromo

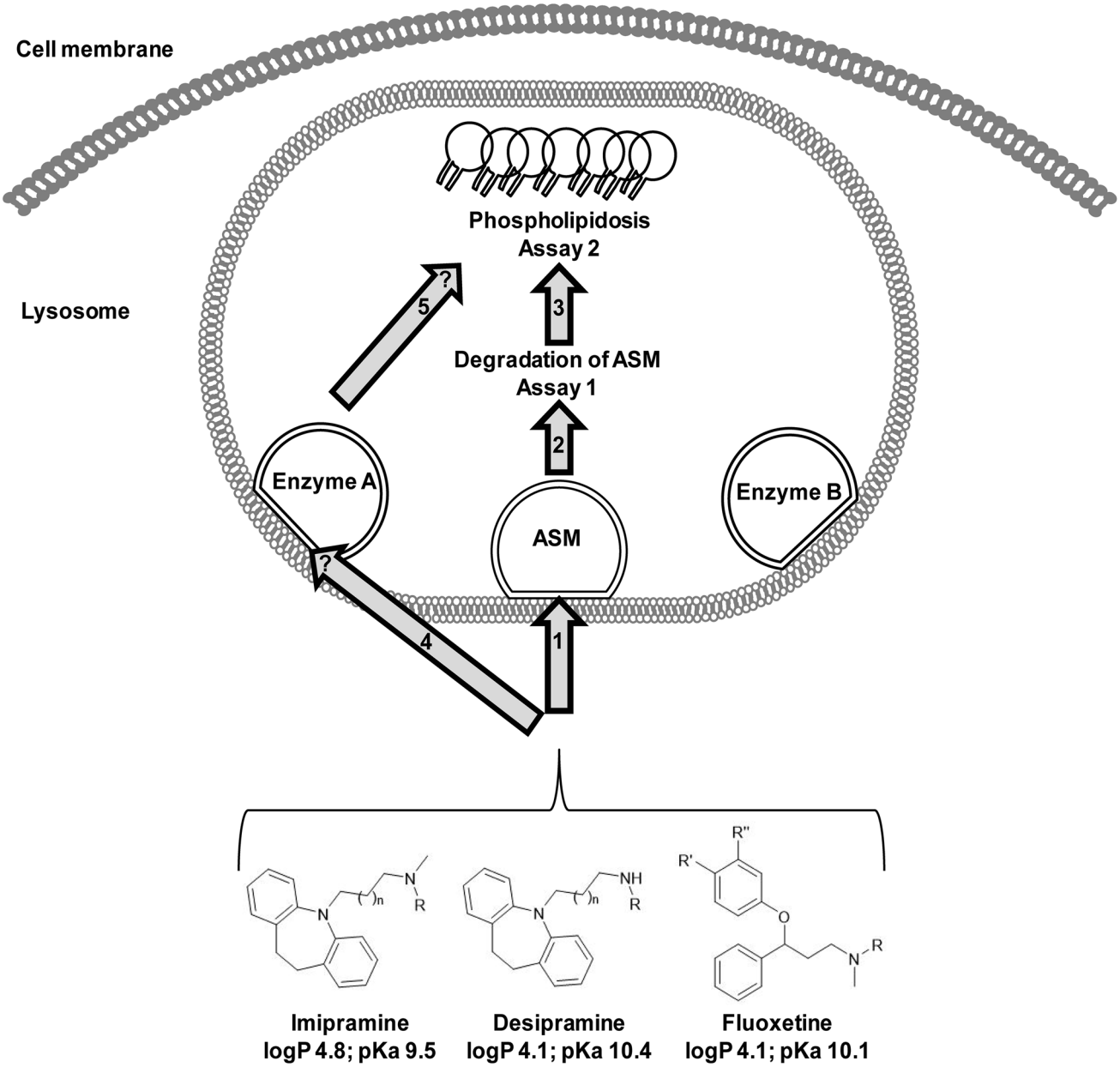


Fig. 1 Overview of relevant lysosomal parameters. The FIASMAs imipramine, desipramine, and fluoxetine show values for lipophilicity (log *P*) and protonation (p*K*a) that allow efficient trapping in the lysosome. The inner surface charge of the lysosomal membrane is negative (Gallala and Sandhoff 2011; Kolter and Sandhoff 2010) due to the high abundance of bis-(monoacylglycero)-phosphate (Kobayashi et al. 1998; Wilkening et al. 1998). ASM is positively charged at lysosomal pH and binds to lysosomal membrane structures (Kölzer et al. 2004). The drugs enter the lysosome, get protonated, accumulate in the lysosomal membrane and cause the displacement of ASM from the membrane (Arrow 1). ASM is released into the lysosomal lumen and subjected to degradation by lysosomal mechanisms via proteases (Arrow 2) (Hurwitz et al. 1994). This indirect inhibition of ASM activity can be measured by an enzyme activity assay ("Assay 1"). The degradation of ASM by FIASMAs results in the accumulation of phospholipids ("Phospholipidosis"; arrow 3) that can be measured using LipidTOX ("Assay 2"). FIASMAs are supposed to influence lysosomal enzymes other than ASM, e.g., phospholipases ("Enzyme A"; arrow 4), that leads to further phospholipid accumulation (Arrow 5). In contrast, other lysosomal enzymes might be unaffected by FIASMAs ("Enzyme B"). Basic motifs from © motifolio.com

hexanoic acid chloride (1.5 eq) in the presence of triethylamine (2 eq.) in CH_2Cl_2 at room temperature for 2 h. The resulting bromo hexanoylamide was purified by flash chromatography and reacted in DMF at 50 °C for 20 h with dimethylamine or piperidine (4 eq.), respectively. The products were purified by flash chromatography.

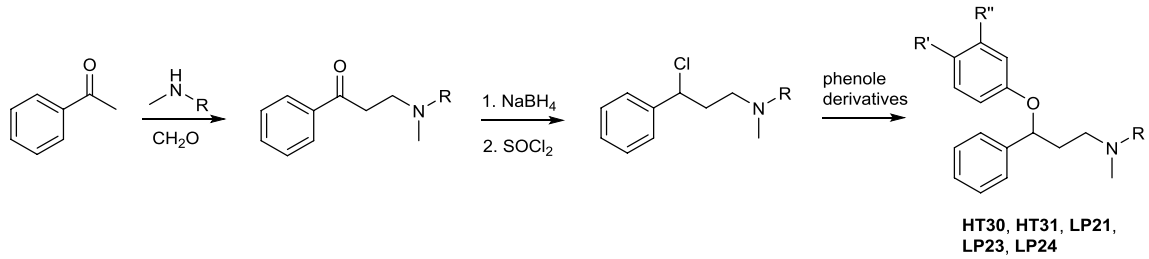
The piperazine derivative **RKZ11** was derived from 5-(3-chloropropyl)-10,11-dihydro-5H-dibenzo[*b,f*]azepine

(Dollinger et al. 2006) by reaction with *N*-methyl piperazine following the procedure described for the synthesis of **HT23**.

Synthesis of desipramine derivatives

Test compounds **RH281A, HT21** and **HT25** were obtained by nucleophilic substitution of 5-(4-bromobutyl)-10,11-dihydro-5H-dibenz[*b,f*]azepine (Dollinger et al. 2006) or 5-(4-bromobutyl)-10,11-dihydro-5H-dibenz[*b,f*]azepine (Schmidt et al. 2008), respectively, with n-propylamine, methylamine or n-hexylamine following the procedure described for the synthesis of **HT23**.

Synthesis of fluoxetine derivatives



HT30, **HT31**, **LP21**, **LP23**, **LP24**

The fluoxetine derivatives **HT30, HT31, LP21, LP23** and **LP24** were synthesized according to the depicted scheme. Mannich reaction of acetophenone with the hydrochloride salts of dimethylamine or *N*-methylhexylamine in ethanol at reflux temperature (2.5 h) yielded the respective β-aminoketones. Reduction of the ketone functionality with $NaBH_4$ in MeOH/H_2O (5:1) at room temperature (16 h) followed by treatment with thionylchloride in CH_2Cl_2 for 4 h at reflux temperature resulted in the respective benzyl chlorides. Finally, reaction with 3- or 4-trifluoromethyl phenol, 4-hydroxy benzaldehyde or 4-hydroxy benzonitrile, respectively, in DMF at 80 °C in the presence of K_2CO_3 (3 h) yielded the desired fluoxetine derivatives. After treatment with aqueous NaOH, extraction with CH_2Cl_2 and evaporation of the solvent, the crude products were purified by flash chromatography.

All compounds were dissolved in water or DMSO at a concentration of 10 mM and stored at − 20 °C. For an overview of chemical structures of test compounds, see Fig. 2.

Cell culture

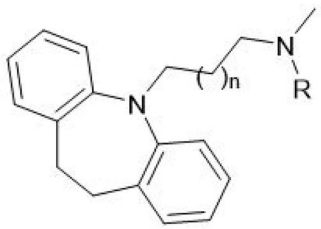
Human brain neuroglioma H4 cells were purchased from Promochem (Wesel, Germany). The cells were cultivated in Dulbecco's modified Eagle's medium (Biochrom, Berlin, Germany) supplemented with 10% (v/v) fetal bovine serum (FBS) and 4 mM glutamine (Biochrom, Berlin, Germany). The cell line was maintained at 37 °C in a humidified atmosphere containing 8.5% CO_2 and was routinely split at a ratio of 1:6. The cells were regularly tested for mycoplasma contamination by an ELISA-based assay (Lonza, Basel, Switzerland), and the tests were always negative.

Acid sphingomyelinase activity assay

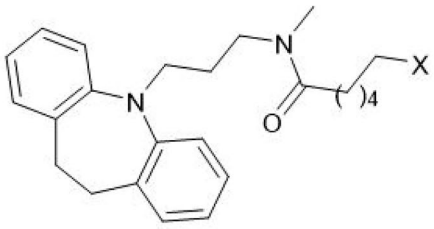
The activity of ASM was determined in whole cell lysates, as previously described (Gulbins and Kolesnick 2000). After the substance was added to the growth medium at a final concentration of 10 µM, cells were kept at 37 °C in a humidified atmosphere at 8.5% CO_2 for 30 min. Results are given as residual ASM activity (%) normalized to control cells treated with the solvent alone and represent mean values of three independent experiments, each with a standard deviation of approx. 16%. A residual ASM activity ≤ 50.0% in H4-cells was rated as positive.

Phospholipidosis assay

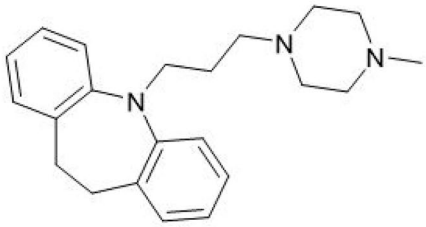
H4 cells were seeded in 96-well white dishes (Nunc, Langenselbold, Germany) at a density of 4×10^3 cells per well. After 48 h, the medium was replaced with fresh medium that included the test substances and HCS LipidTOX Green phospholipidosis detection reagent (Invitrogen, San Diego, US) at their respective final concentrations. Each test substance was diluted from the stock solution with medium and was applied at 2.5 µM for an additional 24 h. All tests were performed in quadruplicate. During the test period, the cells were cultured at 37 °C in a humidified atmosphere containing 8.5% CO_2. To quantify the lysosomal phospholipid content, the cells were washed with phosphate-buffered saline (PBS), counterstained with DAPI (Carl Roth GmbH, Karlsruhe, Germany) and fixed with 10% neutral buffered formalin (AppliChem GmbH, Darmstadt, Germany). Fluorescent signals were quantified with a fluorescence reader (Perkin Elmer, Waltham, US) at an excitation wavelength of 485 nm and an emission wavelength of



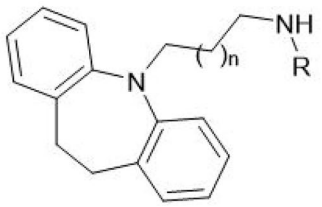
imipramine: n= 1; R= methyl
RH272B: n= 1; R= propyl
RKZ01: n= 1; R= pentyl
RH272D: n= 1; R= hexyl
RKZ06 : n= 1; R= cyclohexyl
RKZ07: n= 1; R= isopropyl
HT04: n= 1; R= -$(CH_2)_6OH$
HT23: n= 2; R= hexyl



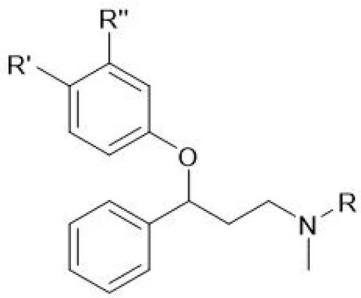
LP05: X= -$N(CH_3)_2$
HT13: X= piperidin-1-yl



RKZ-11



desipramine: n= 1; R= methyl
RH281A: n= 1; R= propyl
HT21: n= 2; R= methyl
HT25: n= 2; R= hexyl



fluoxetine: R= H; R'= CF_3; R"= H
HT30: R= methyl; R'= CF_3; R"= H
HT31: R= methyl; R'= H; R"= CF_3
LP21: R= hexyl; R'= CF_3; R"= H
LP23: R= hexyl; R'= CHO; R"= H
LP24: R= hexyl; R'= CN; R"= H

Fig. 2 Chemical structures of imipramine, desipramine and fluoxetine derivatives

535 nm for the lysosomal dye (LipidTOX Green) and an excitation wavelength of 355 nm and an emission wavelength of 470 nm for the nuclear dye (DAPI). The results were corrected by subtraction of the background and are reported as percent fluorescence of the corresponding control averaged over four experiments. A chemical-induced reduction in cell number greater than 25% (DAPI fluorescence < 75% compared with untreated control) was rated as a toxic effect and prompted the exclusion of the respective compound from further analysis. Maximal doubling of the cellular phospholipid content (LipidTox fluorescence ≤ 200.0% compared with control) was rated as an absent PLD side effect and thus a non-toxic result (Muehlbacher et al. 2012).

Calculation of p*K*a and log *P* values

The p*K*a and log *P* values for the substances were calculated using ACD/Log *D* Suite (program version 10.02, Toronto, Canada) as previously described (Kornhuber et al. 2008). Briefly, the p*K*a prediction algorithms of the ACD program are based on a fragmental method using generic substructures and Hammett type equations to cover the most popular ionizable functional groups using either a database or the estimation of electronic substituent constants. The log *P* prediction algorithms of the ACD program are based on a fragmental method with correction factors. Further details of the prediction algorithms are presented on the ACD webpage (http://www.whocc.no/atcddd).

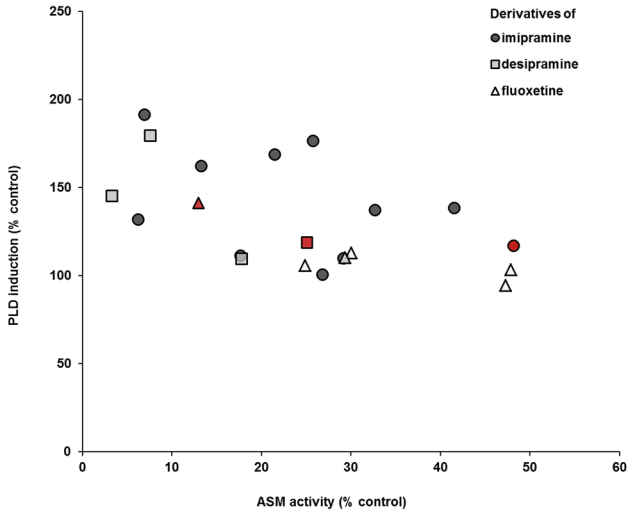


Fig. 3 Derivatives of imipramine, desipramine, and fluoxetine with beneficial effects regarding the residual ASM activity and PLD induction ($n = 18$). H4 cells were treated with 10 µM of antidepressant drug derivatives for 30 min, harvested and subjected to ASM activity assays. In parallel, cells were treated with 2.5 µM lipidTOX for 24 h, stained with DAPI and analyzed for PLD induction. The original drug values are indicated in red. Only compounds that exhibit ASM inhibition ≥ 50% and PLD induction ≤ 200% are presented. Both parameters are significantly negatively correlated ($r = -0.5$; $p < 0.05$)

Statistical analysis

SPSS software (Statistical Package for the Social Sciences, Chicago, IL, for Windows, Version 21.0) was used for statistical analysis. Correlations between ASM activity and PLD induction were calculated using Pearson's correlation coefficients. Correlations were considered significant with p values < 0.05 (two-tailed).

Results

A subset of derivatives exhibits beneficial potential regarding residual ASM activity and PLD induction

A reduction in ASM activity of at least 50%, thus resulting in the classification of the substance as a FIASMA (Kornhuber et al. 2008), and a non-toxic PLD induction with less than 200% induction compared with control (Muehlbacher et al. 2012) comprise the beneficial range for substances in our study. Of the 45 compounds generated, 18 compounds exhibited effects in a beneficial range after application in our cell culture model (Fig. 3). Of note, a significant negative correlation ($r = -0.5$, $p < 0.05$) between ASM inhibition and PLD induction was observed. This finding indicates that increased functional inhibition of ASM activity correlates with increased PLD induction.

Table 1 Derivatives with reduced residual ASM activity or/and decreased PLD induction levels compared with the original drugs, and corresponding log *P*, p*K*a and molar mass values

Derivative of	Compound	Residual ASM activity (% control ± SD)	PLD induction (% control ± SD)	log *P*	p*K*a	Molar mass (g/mol)
–	Imipramine	48.1 ± 10.6	115.0 ± 5.5	4.8	9.5	280.4
Imipramine	HT04	26.8 ± 6.1	101.3 ± 8.3	5.5	9.5	366.5
	RH272B	33.8 ± 8.2	110.5 ± 8.1	5.9	9.6	308.5
	RH272D	28.4 ± 12.6	112.1 ± 3.9	7.5	9.7	350.5
	RKZ-11	41.6 ± 10.9	143.5 ± 3.6	3.8	8.2	335.5
	LP05	6.2 ± 2.0	132.8 ± 7.4	4.6	9.7	407.6
	RKZ-7	32.7 ± 5.4	142.1 ± 7.5	5.7	9.7	308.5
	HT13	6.9 ± 0.6	197.7 ± 16.0	5.8	9.9	447.7
	RKZ-6	25.8 ± 8.1	182.6 ± 6.7	6.6	5.4	342.5
	RKZ-1	30.3 ± 12.6	174.6 ± 14.9	6.9	9.6	336.5
	HT23	13.0 ± 3.2	163.0 ± 3.6	7.5	9.9	364.6
–	Desipramine	25.1 ± 10.9	123.6 ± 7.3	4.1	10.4	266.4
Desipramine	RH281A	17.8 ± 4.9	110.4 ± 2.1	5.2	10.6	294.4
	HT21	3.3 ± 0.5	146.0 ± 2.9	4.4	10.6	280.4
	HT25	7.5 ± 1.6	180.3 ± 8.6	7.0	11.0	350.5
–	Fluoxetine	13.0 ± 2.8	141.3 ± 15.7	4.1	10.1	309.3
Fluoxetine	HT31	30.0 ± 4.4	113.5 ± 9.3	4.8	9.3	323.4
	HT30	24.9 ± 4.1	106.5 ± 2.4	5.0	9.2	323.4
	LP23	47.3 ± 3.6	110.9 ± 3.3	6.0	9.5	353.5
	LP24	29.4 ± 2.9	104.2 ± 4.4	6.1	9.5	350.5
	LP21	47.9 ± 6.5	103.0 ± 1.9	7.7	9.5	393.5

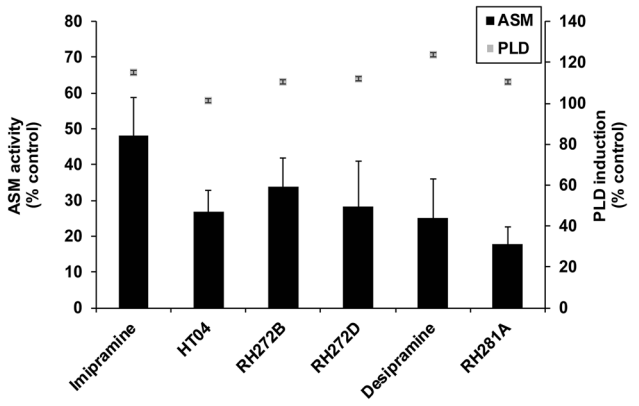


Fig. 4 Derivatives with increased beneficial effects compared with their original drugs. H4 cells were treated with 10 µM of the respective substance for 30 min, harvested and subjected to ASM activity assays. In parallel, cells were treated with 2.5 µM lipidTOX for 24 h, stained with DAPI and analyzed for PLD induction. Only those derivatives that exhibit both reduced residual ASM activity levels and reduced PLD induction compared with their original drug are presented. The results are presented as the residual ASM activity (%) normalized to control cells representing the mean values of three independent experiments with the respective standard deviations or PLD induction (%) normalized to control cells representing the mean values of quadruplicate measurements with the respective standard deviations

Imipramine derivatives with increased beneficial potential compared with imipramine

Compared with the original drug imipramine, 10 compounds exhibited reduced residual ASM activity, a reduced risk of PLD induction, or both. All 10 compounds exhibited enhanced ASM inhibition compared with imipramine itself. Three compounds resulted in less PLD induction compared with imipramine (Table 1). The compounds HT04, RH272B and RH272D exhibited increased beneficial effects for both parameters (Fig. 4). RH272B and RH272D share a tricyclic structure with imipramine, but have an elongated residual chain (propyl and hexyl, respectively) that results in a similar p*K*a value but in an increased log *P* value compared with imipramine (Fig. 2; Table 1). Compound HT04 seems to be a promising compound given that its application resulted in an approximately 20% reduced PLD level and it inhibited ASM activity approximately twice effectively compared with its original drug imipramine. A $(CH_2)_6OH$ residue is added to the tricyclic structure, resulting in a similar p*K*a value but in an increased log *P* value compared with imipramine (Fig. 2; Table 1).

Desipramine derivatives with increased beneficial potential compared with desipramine

Of the desipramine derivatives, three compounds inhibited ASM activity more efficiently compared with the original drug (Table 1). Compound RH281A exhibited increased beneficial effects for both parameters: an approximately 10% reduced PLD risk compared with desipramine, and ASM activity inhibition was increased by approximately 10% compared with the original drug (Fig. 4). This compound features a propyl residue that results in a similar p*K*a value but in an increased log *P* value compared with desipramine (Fig. 2; Table 1).

Fluoxetine derivatives with increased beneficial potential compared with fluoxetine

None of the fluoxetine derivatives exhibited enhanced ASM activity inhibition compared with fluoxetine. Five compounds exhibited approximately 20–40% reduced PLD induction compared with fluoxetine. However, the efficacy of PLD induction was achieved at the expense of residual ASM activity levels that increased disproportionally by 3- to 4-fold compared with fluoxetine treatment (Table 1).

Discussion

In this study, we demonstrate that small changes in compound structure can markedly change the properties of the indicated drugs. Compared with their original drugs, derivatives exhibited varied potential to inhibit ASM activity and PLD induction. As expected, according to earlier studies on FIASMAs and PLD (Muehlbacher et al. 2012), strong functional inhibition of ASM correlated with high levels of PLD induction. Drug-induced PLD is a side effect of CADs, especially with regard to the common practice of polypharmacy in the aged population (Glock et al. 2016; Kukreja et al. 2013). Several lysosomal mechanisms that cause PLD are currently discussed, including direct or indirect inhibition of different phospholipases, induction of non-degradable complexes of lipids and drugs, increased lipid synthesis, and inhibition of enzyme transport processes in the lysosome (Nonoyama and Fukuda 2008). Interestingly, it was predicted in silico that different CADs induce PLD via distinct underlying lysosomal mechanisms (Lowe et al. 2010). This prediction is consistent with our observations given that some of the derivatives did not induce high levels of PLD despite their significant effect on ASM activity. These chemical compounds indicate the presence of a PLD-inducing mechanism independent of ASM biology. Thus, our data demonstrate that CADs indeed seem to exert effects on specific lysosomal enzymes. Regarding the commonly used antidepressant fluoxetine, none of the derivatives yielded significantly better results for ASM inhibition and PLD induction given that both cellular mechanisms vary among the derivatives. In contrast,

derivatives of the tricyclic antidepressant drugs imipramine and desipramine exhibited beneficial results. Three imipramine derivatives, HT04, RH272B and RH272D, and one desipramine derivative, RH281A, exhibit improved values compared with the original drugs. The imipramine derivative HT04 seems to be a promising compound as it reduces PLD by 20% and inhibits ASM twice as efficiently as the original drug imipramine. This substance could therefore be an interesting molecule with therapeutic potential. Given that both ASM inhibition and PLD induction require proper lysosomal accumulation of the CAD, modifications that increase the lysosomotropic potential of the respective compound could account for the different effects. Of note, similar lysosomal accumulation was demonstrated for all derivatives by a chemo-informatic prediction algorithm in silico and thus does not explain those effects. Interestingly, beneficial derivatives could be identified only for tricyclic antidepressants. Fluoxetine seems to be the most efficient structure in our context and does not benefit from modification. When assessing the properties of the beneficial tricyclic compounds HT04, RH272B, RH272D and RH281A, it is obvious that these compounds exhibit similar p*K*a values compared with their original drugs, and the modifications do not seem to influence their effects. In contrast, the log *P* value was increased for all compounds compared with their original drugs. HT04 features the longest residue. However, the increase in log *P* value is limited to the beneficial range due to the OH group. Thus, the lipophilic property is a main factor in our context. Additional explanations for the beneficial potential of these compounds remain elusive.

Limitations of the study are that the new compounds were not tested for other side effects and additional properties. More studies are needed to specify the mechanisms of CAD action in the lysosome. Our CAD library serves as a valuable tool for in vitro studies that seek to vary ASM inhibition and PLD induction independently.

Acknowledgements We thank Michaela Henkel and Alice Konrad for excellent technical assistance. We are grateful to Lydia Pettermann and Heike Thomas for generating the chemical compounds. We thank Christiane Mühle for helpful comments on the manuscript and preparation of Fig. 3. This work was supported by funding from the Forschungsstiftung Medizin at the University Hospital Erlangen, the German Federal Ministry of Education and Research (BMBF, 01 EE1401C, to J. K.), and DFG grants KO 947/13-1 and GU 335/29-1 (to J.K. and E.G.).

Author contributions PT and JK conceived and designed the experiments; PT and SL designed and performed the experiments and wrote parts of the paper; CR, JK and EG analyzed the data; SL, PG and JK contributed reagents/study materials/analysis tools; CR and PT wrote the paper. All authors read the paper and provided intellectual input.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Albouz S, Le Saux F, Wenger D, Hauw JJ, Baumann N (1986) Modifications of sphingomyelin and phosphatidylcholine metabolism by tricyclic antidepressants and phenothiazines. Life Sci 38:357–363

Anderson N, Borlak J (2006) Drug-induced phospholipidosis. Febs Lett 580:5533–5540

de Duve C, de Barsy T, Poole B, Trouet A, Tulkens P, Van Hoof F (1974) Commentary. Lysosomotrop Agents Biochem Pharmacol 23:2495–2531

Dollinger S, Löber S, Klingenstein R, Korth C, Gmeiner P (2006) A chimeric ligand approach leading to potent antiprion active acridine derivatives: design, synthesis, and biological investigations. J Med Chem 49:6591–6595. https://doi.org/10.1021/jm060773j

Gallala HD, Sandhoff K (2011) Biological function of the cellular lipid BMP-BMP as a key activator for cholesterol sorting and membrane digestion. Neurochem Res 36:1594–1600. https://doi.org/10.1007/s11064-010-0337-6

Glock M, Muehlbacher M, Hurtig H, Tripal P, Kornhuber J (2016) Drug-induced phospholipidosis caused by combinations of common drugs in vitro. Toxicol In Vitro 35:139–148. https://doi.org/10.1016/j.tiv.2016.05.010

Gulbins E, Grassme H (2002) Ceramide and cell death receptor clustering. Biochim Et Biophys Acta 1585:139–145

Gulbins E, Kolesnick R (2000) Measurement of sphingomyelinase activity. Methods Enzymol 322:382–388

Gulbins E, Kolesnick R (2002) Acid sphingomyelinase-derived ceramide signaling in apoptosis. Subcell Biochem 36:229–244

Gulbins E et al (2013) Acid sphingomyelinase-ceramide system mediates effects of antidepressant drugs. Nat Med 19:934–938. https://doi.org/10.1038/nm.3214

Halliwell WH (1997) Cationic amphiphilic drug-induced phospholipidosis. Toxicol Pathol 25:53–60

Hurwitz R, Ferlinz K, Sandhoff K (1994) The tricyclic antidepressant desipramine causes proteolytic degradation of lysosomal sphingomyelinase in human fibroblasts. Biol Chem Hoppe Seyler 375:447–450

Kobayashi T, Stang E, Fang KS, de Moerloose P, Parton RG, Gruenberg J (1998) A lipid associated with the antiphospholipid syndrome regulates endosome structure and function. Nature 392:193–197. https://doi.org/10.1038/32440

Kodavanti UP, Mehendale HM (1990) Cationic amphiphilic drugs and phospholipid storage disorder. Pharmacol Rev 42:327–354

Kolter T, Sandhoff K (2010) Lysosomal degradation of membrane lipids. FEBS Lett 584:1700–1712

Kölzer M, Werth N, Sandhoff K (2004) Interactions of acid sphingomyelinase and lipid bilayers in the presence of the tricyclic antidepressant desipramine. FEBS Lett 559:96–98

Kornhuber J, Medlin A, Bleich S, Jendrossek V, Henkel AW, Wiltfang J, Gulbins E (2005) High activity of acid sphingomyelinase in major depression. J Neural Transm 112:1583–1590

Kornhuber J, Tripal P, Reichel M, Terfloth L, Bleich S, Wiltfang J, Gulbins E (2008) Identification of new functional inhibitors of acid sphingomyelinase using a structure-property-activity relation model. J Med Chem 51:219–237. https://doi.org/10.1021/jm070524a

Kornhuber J et al (2010) Functional Inhibitors of acid sphingomyelinase (FIASMAs): a novel pharmacological group of drugs with broad clinical applications. Cell Physiol Biochem 26:9–20. https://doi.org/10.1159/000315101

Kornhuber J et al (2011) Identification of novel functional inhibitors of acid sphingomyelinase. PloS One 6:e23852. https://doi.org/10.1371/journal.pone.0023852

Kornhuber J, Müller CP, Becker KA, Reichel M, Gulbins E (2014) The ceramide system as a novel antidepressant target. Trends Pharmacol Sci 35:293–304. https://doi.org/10.1016/j.tips.2014.04.003

Kukreja S, Kalra G, Shah N, Shrivastava A (2013) Polypharmacy in psychiatry: a review. Mens Sana Monogr 11:82–99. https://doi.org/10.4103/0973-1229.104497

Lowe R, Glen RC, Mitchell JBO (2010) Predicting phospholipidosis using machine learning. Mol Pharm 7:1708–1714

Lüllmann H, Lüllmann-Rauch R, Wassermann O (1978) Lipidosis induced by amphiphilic cationic drugs. Biochem Pharmacol 27:1103–1108

Muehlbacher M, Tripal P, Roas F, Kornhuber J (2012) Identification of drugs inducing phospholipidosis by novel in vitro data. ChemMedChem. https://doi.org/10.1002/cmdc.201200306

Nonoyama T, Fukuda R (2008) Drug-induced phospholipidosis—pathological aspects and its prediction. J Toxicol Pathol 21:9–24

Rhein C et al (2017) Alternative splicing of SMPD1 coding for acid sphingomyelinase in major depression. J Affect Disord 209:10–15. https://doi.org/10.1016/j.jad.2016.09.019

Schmidt M, Teitge M, Castillo ME, Brandt T, Dobner B, Langner A (2008) Synthesis and biochemical characterization of new phenothiazines and related drugs as MDR reversal agents. Arch Pharm 341:624–638. https://doi.org/10.1002/ardp.200800115

Trapp S, Rosania GR, Horobin RW, Kornhuber J (2008) Quantitative modeling of selective lysosomal targeting for drug design. EBJ 37:1317–1328. https://doi.org/10.1007/s00249-008-0338-4

Wilkening G, Linke T, Sandhoff K (1998) Lysosomal degradation on vesicular membrane surfaces. Enhanced glucosylceramide degradation by lysosomal anionic lipids and activators. J Biol Chem 273:30271–30278