Second basic pKa: An overlooked parameter in predicting phospholipidosis-inducing potential of diamines

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Second basic pKa: An overlooked parameter in predicting phospholipidosis-inducing potential of diamines.

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Abstract

In this paper, we present the phospholipidosis-inducing potential (PLIP) of forty fragment-sized diamines derived from *N*-benzyl-4-(methylamino)piperidine and discuss the relationship between their PLIP and the physicochemical properties. Our results demonstrate that the previously reported methods are not suitable for predicting the PLIP of fragment-sized diamines; the second basic pKa can distinguish PLIP-positive diamines from PLIP-negative diamines more accurately than ClogP or most basic pKa. To the best of our knowledge, this is the first report describing the relationship between PLIP and second basic pKa.

Keywords: phospholipidosis, diamine, second basic pKa

We have identified a novel heterocyclic pyrimidine derivative SUN13837, which exhibits biological activities similar to that of basic fibroblast growth factor (bFGF). This is possible because of the modulation of the signal transduction pathway of the fibroblast growth factor receptor by the pyrimidine derivative.[1] Although SUN13837 exhibited significant pharmacological activities that could aid the treatment of neurodegenerative diseases, *in vitro* examination showed that one of its major metabolites, *N*-benzyl-4-(methylamino)piperidine (BMP, **1**), had high phospholipidosis-inducing potential (PLIP) (Figure 1). Phospholipidosis is a pathological condition in which phospholipids accumulate in tissues, and this is characterized by the formation of lysosomal lamellar bodies. The accumulated phospholipids interfere with cellular functions and may sometimes prove fatal.[2] In our efforts to develop the follow-up compounds of SUN13837, we realized that it is necessary to identify the factors influencing the PLIP of BMP-like diamines, so that alternative diamines with reduced PLIP can be developed.

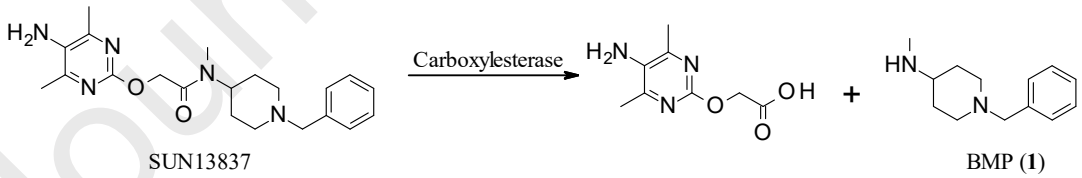


Figure 1. Carboxylesterase-catalyzed hydrolysis of SUN13837.

A number of central nervous system (CNS) drugs, such as SUN13837, that can produce diamines by hydrolysis have been reported (Figure 2). Therefore, we also considered that studying the PLIP of fragment-sized diamines should help assess the risk of phospholipidosis caused by these drugs and guide the selection and development of a drug candidate.

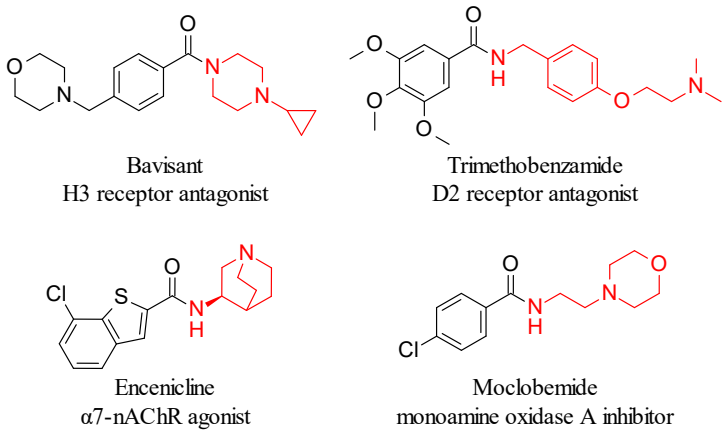


Figure 2. CNS drugs that can produce diamines by hydrolysis

Physicochemical approaches are a useful way of predicting the PLIP of newly designed compounds, and various methods have been reported for this purpose.[3] One of the simplest models was described by Ploemen *et al*., in which they used only two descriptors, ClogP and the most basic pKa, to represent the lipophilicity and basicity, respectively, of a compound.[4] Tomizawa et al. modified Ploemen's model to include the net charge at the lysosomal pH, instead of the most basic pKa, and suggested that diamines are highly likely to exhibit high PLIP.[5] It should, however, be noted that each prediction model was constructed using different datasets, and hence, the application of these models to different datasets may not give the same accuracy as originally reported.

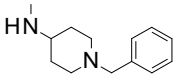
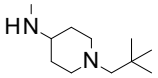
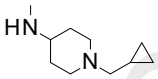
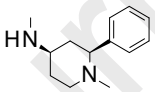
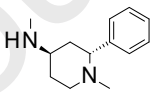
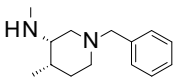
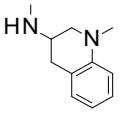
In this study, we evaluated the PLIP of forty fragment-sized diamines derived from **1** and found that there were diamines with low PLIP. Furthermore, a new criterion based on the second basic pKa was introduced, which could distinguish PLIP-positive diamines from PLIP-negative diamines more accurately than ClogP or most basic pKa.

According to the study conducted by Kasahara *et al*,[6] the PLIP can be evaluated using normalized value (NV), which indicates the amount of phosphatidylcholine-conjugated dye taken up per living cell. NV is correlated to the degree of pathological changes and compounds with NV greater than 1.5 are likely to induce cytoplasmic lamellar bodies. With the aim of obtaining diamines with lower PLIP even at higher concentrations, we measured NV at the concentration of 300 μM, which is ten times higher than that in Kasahara's study. Thus, in this study, high-PLIP diamines are defined as those with NV greater than 1.5 at 300 μM.

We began our study by examining the PLIP of diamines **1**–**7**,[7,8] in order to understand the behavior of the PLIP of BMP-like compounds and also to investigate the relationships between their PLIP and physicochemical properties such as ClogP, pKa_1, and pKa_2 (Table 1).[9] Furthermore, we examined the scope of application of the previously reported prediction methods.[10] The NV of **1** was more than 1.5,

indicating that **1** has high PLIP. The NV of the *tert*-butyl derivative **2** was lower than that of **1** but still higher than 1.5. The NV of the cyclopropyl derivative **3** was as high as that of **1**, despite its very low lipophilicity. The NVs of **4** and **5**, which are regioisomers of **1**, were as high as that of **1**. However, the NVs of 3-aminopiperidine **6** and fused-ring piperidine **7** were less than 1.5, despite their high lipophilicity. The PLIP of **3**–**7** observed in the *in vitro* assay could not be accurately predicted by previously reported models, suggesting that these methods are not suitable for predicting the PLIP of BMP-like diamines. Based on the above results, we hypothesized that pKa_2 would be a more accurate predictor of the PLIP, especially for fragment-sized diamines, because the pKa_2 values of **6** and **7** were lower than those of compounds **1–5**. Thus, to verify our hypothesis, we examined other BMP-like diamines which have low pKa_2 values.

Table 1. Normalized values and physicochemical properties of BMP-like diamines.

Cmpd.	Structure	Clog P [c]	pKa_1 [d]	pKa_2 [d]	Normalized value	Prediction by Ploemen's model	Prediction by modified Ploemen's model	Prediction by Tomizawa's model
BMP (**1**)		1.3	10.2	6.3	3.3	positive	negative	positive
2 [a]		1.2	10.3	7.6	2.3	positive	negative	positive
3 [a]		0.3	10.2	7.5	3.2	negative	negative	positive
4 [a,b]		0.7	9.8	6.4	3.0	negative	negative	positive
5 [a,b]		0.7	9.8	6.4	2.7	negative	negative	positive
6		2.8	10.2	5.0	1.4	positive	positive	positive
7 [a,b]		2.0	9.3	2.8	1.1	positive	positive	positive

[a] 2 HCl salt.

[b] Racemate.

[c] Calculated using ChemBioDraw Ultra 12.0.

[d] Calculated using ADMET predictor, version 7.2.0001.

The pKa_2 value of **1** corresponds to the basicity of the piperidyl nitrogen, because the benzyl group acts as an electron-withdrawing group (EWG). Generally, introduction of EWGs in the vicinity of an amine center or relocation of EWGs closer to an amine center reduces its basicity.[11] Thus, we designed and synthesized some diamines with reduced basicity of the piperidyl nitrogen, according to the options shown in Figure 3.

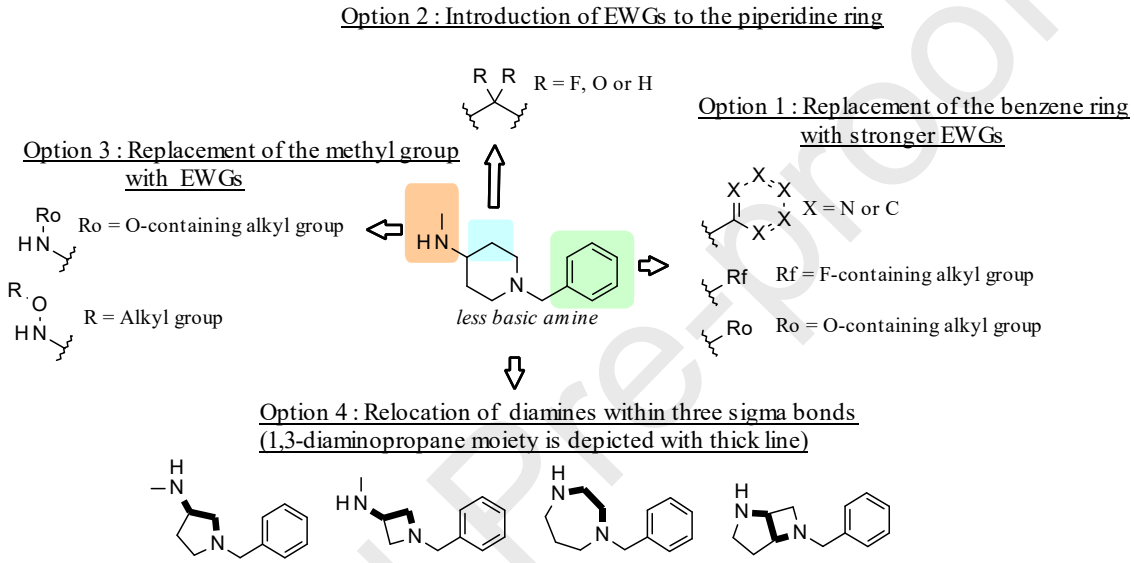
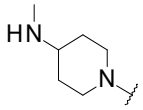


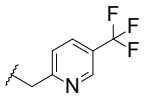
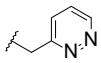
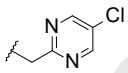
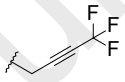
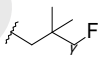
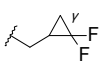
Figure 3. Design of diamines with lower pKa_2 values

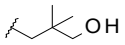
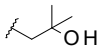
Table 2 shows the NVs and physicochemical properties of the diamines in which EWGs stronger than the phenyl group are introduced (Option 1 in Figure 3). It is worth noting that the pKa_1 values of the newly designed compounds **8**–**24** are almost similar to that of compound **1**, while the pKa_2 values are different from each other. For the heteroaromatic derivatives such as pyridine (**8**, **9**), pyrazine (**10**), pyridazine (**11**), and pyrimidine (**12**, **13**), the pKa_2 values are lower than that of **1**, and the NVs are lower than 1.5. Introduction of alkyne functionality as an EWG (**14**, **15**) also reduced the pKa_2 values, and the NVs were lower than 1.5. For the fluoroalkyl derivatives, the pKa_2 value changes depending on the number of fluorine atoms and the distance between fluorine and the piperidyl nitrogen atom. At the γ-position of the piperidyl nitrogen (**16**, **17**), at least two fluorine atoms were required to make the pKa_2 value lower than that of **1**. The NV was lower than 1.5 only for **17**. At the β-position of the piperidyl nitrogen (**18-20**), only one fluorine atom was enough to make the pKa_2 value lower than that of **1**. The NVs of these diamines were not higher than 1.5. Since the capacity of oxygen to lower the basicity is weaker than that of fluorine, the pKa_2 values of the oxyalkyl derivatives (**21-24)** were higher than that of **1**. The NVs of **21** and **22** were higher than 1.5, despite their very low lipophilicity,

indicating that lowering the lipophilicity alone does not decrease the NV. Contrary to our expectation, the NVs of **23** and **24** were less than 1.5.

Table 2. Normalized values and physicochemical properties of diamines shown in Option 1 (Figure 3).



Compd.	Structure	ClogP [d]	pKa_1 [e]	pKa_2 [e]	Normalized value
1		1.3	10.2	6.3	3.3
8 [a]		0.1	9.9	5.1	1.0
9		0.9	10.0	5.4	1.2
10		-1.1	9.8	5.4	1.1
11		-1.4	9.7	5.3	1.1
12		-1.1	9.8	5.2	0.9
13		-0.4	9.6	5.1	0.8
14 [b]		0.0	10.1	6.1	0.8
15 [b]		0.3	10.1	4.9	1.1
16 [b]		0.6	10.2	6.8	1.7
17 [b,c]		-0.3	10.1	5.9	1.0
18		0.6	10.2	6.2	1.4
19 [b]		0.0	10.1	4.7	1.0

20 [b]		0.2	10.0	3.2	0.9
21		-0.2	10.2	7.2	2.7
22 [b]		-0.5	10.2	7.0	2.5
23		-1.0	9.8	6.4	1.2
24		-0.5	9.9	6.8	1.0

[a] 3 HCl salt.

[b] 2 HCl salt.

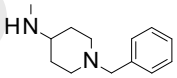
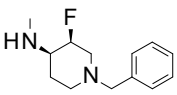
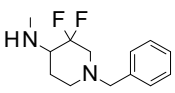
[c] Racemate

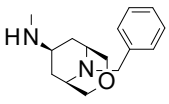
[d] Calculated using ChemBioDraw Ultra 12.0.

[e] Calculated using ADMET predictor, version 7.2.0001.

Table 3 shows the NVs and physicochemical properties of the diamines in which EWGs are introduced on the piperidine ring (Option 2 in Figure 3). Introduction of one fluorine atom reduced the pKa_2 value by 1.4, and the NV of **25** was below 1.5. Difluorination of piperidine ring further reduced the pKa_2 value, and the NV of **26** was less than 1.5. In cyclic ether **27**, the basicity of the piperidyl nitrogen is reduced due to the two σ-bonds connected to the nitrogen atom, while the basicity of the methylamino group at 4-position is mostly unaffected. Therefore, the pKa_2 value of **27** could be rendered lower than that of **1**, with the pKa_1 value remaining unchanged. The pKa_2 value of **27** was similar to that of mono fluorinated compound **25**, and the NV was lower than 1.5.

Table 3. Normalized values and physicochemical properties of diamines shown in Option 2 (Figure 3).

Compd.	Structure	ClogP [d]	pKa_1 [e]	pKa_2 [e]	Normalized value
1		1.3	10.2	6.3	3.3
25 [a,b,c]		1.6	8.6	4.9	1.2
26 [b,c]		2.5	7.4	3.8	1.1

27 [b]		0.3	9.7	4.6	0.9

[a] A mixture of cis/trans isomers (cis/trans = 4/1)

[b] Racemate

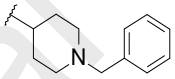
[c] HCl salt

[d] Calculated using ChemBioDraw Ultra 12.0.

[e] Calculated using ADMET predictor, version 7.2.0001.

Table 4 shows the NVs and physicochemical properties of the diamines exemplified in Option 3 in Figure 3, in which methyl group is replaced with an EWG to increase the inductive effect of the amino group at the 4-position of the piperidine ring. *In silico* calculations suggest that the protonation mode of these diamines are different from that of **1** (Figure 4).[12] The nitrogen atoms of **1** are protonated in a stepwise manner, because the difference in the basicities of the two nitrogen atoms are relatively large. On the contrary, those in **28** or **29** will be protonated almost simultaneously, because their basicities are similar to each other.[13] In **30**, protonation will occur in a stepwise manner, though the order will be reverse of that in **1**. The decrease in both pKa_2 values and NVs, regardless of the protonation behavior, suggests the universality of the pKa_2-based hypothesis.

Table 4. Normalized values and physicochemical properties of diamines shown in Option 3 (Figure 3).



Compd.	Structure	ClogP [b]	pKa_1 [c]	pKa_2 [c]	Normalized value
1		1.3	10.2	6.3	3.3
28		1.6	8.6	5.8	1.0
29		2.2	8.6	5.9	1.5
30 [a]		1.5	8.1	2.9	0.8

[a] 2 HCl salt.

[b] Calculated using ChemBioDraw Ultra 12.0.

[c] Calculated using ADMET predictor, version 7.2.0001.

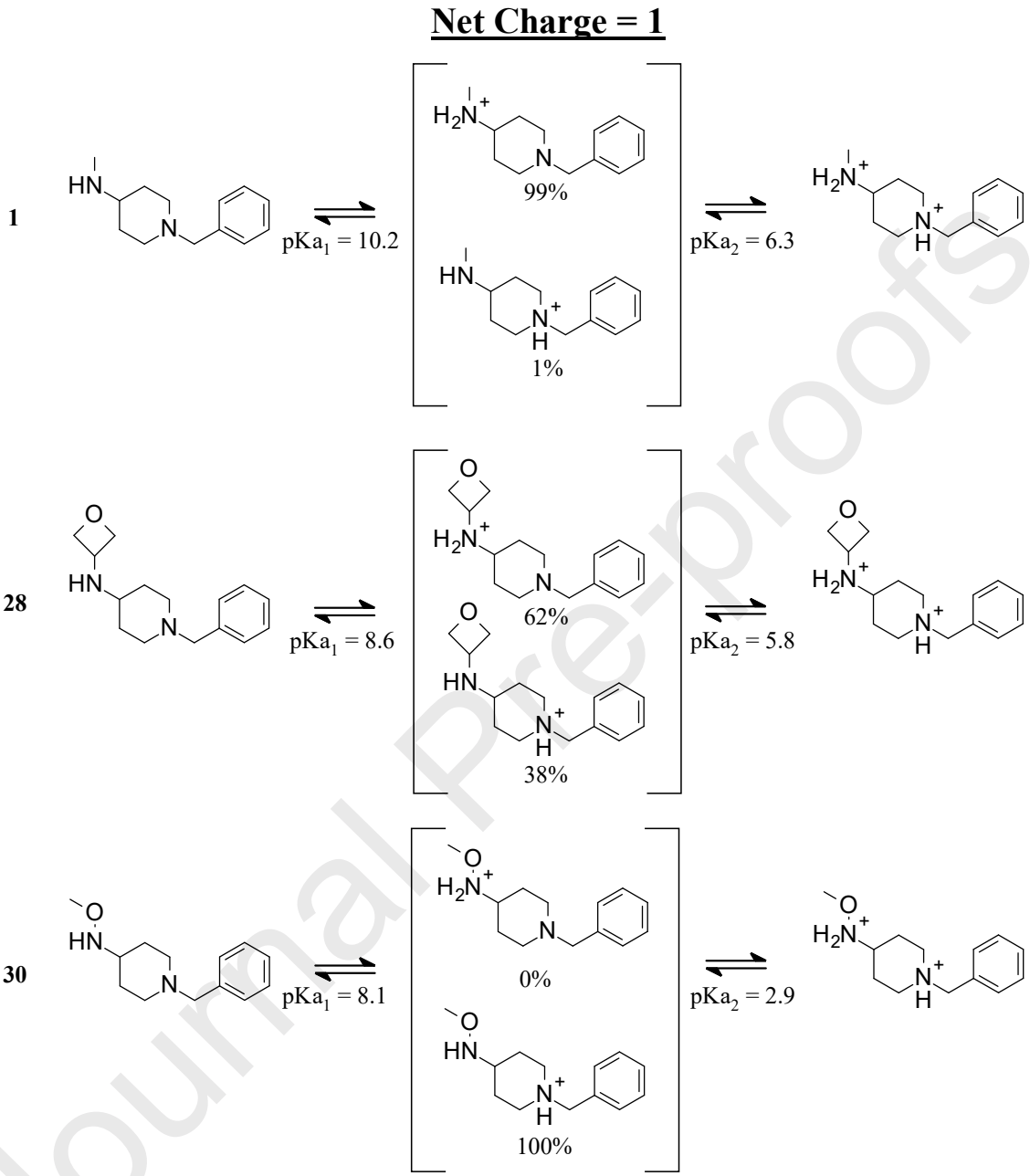
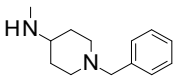
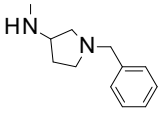
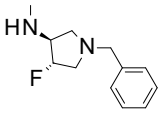
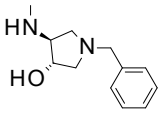
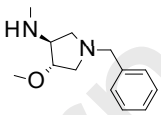
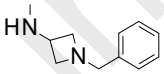
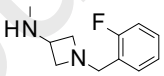
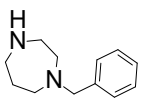
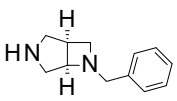


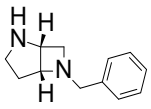
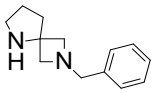
Figure 4. Protonation behaviors of diamines **1**, **28**, and **30**

Table 5 shows the NVs and physicochemical properties of the diamines in which the intramolecular nitrogen atoms are closer to each other (Option 4 in Figure 3). Replacement of piperidine (**1**) with pyrrolidine (**31**) reduced both the pKa_2 value and NV, while no significant reduction in ClogP and pKa_1 were observed. This supports our pKa_2-based hypothesis. Introduction of a fluorine atom or hydroxyl group on the pyrrolidine ring (**32, 33**) further reduced the pKa_2 values, and their NVs decreased below 1.5. Methylation of the hydroxyl group in **33** increases its lipophilicity by 0.9, but no

changes were observed in the pKa_2 value or NV. For the azetidine derivative **35**, both pKa_2 and the NV were lower than that of **1**. Introduction of a fluorine atom on the benzene ring (**36**) reduced both pKa_2 and the NV, although there were no significant changes in ClogP and pKa_1 as compared with **35**. These observations also support our pKa_2-based hypothesis. Furthermore, the pKa_2 values of ring-expansion analogs such as homopiperazine derivative **37**, [3,2,0]diazabicycloheptane derivatives **38** and **39**, and spiro compound **40** were also lower than that of **1**, and the NVs of these diamines were less than 1.5.

Table 5. Normalized values and physicochemical properties of diamines shown in Option 4 (Figure 3).

Compd.	Structure	ClogP [c]	pKa_1 [d]	pKa_2 [d]	Normalized value
1		1.3	10.2	6.3	3.3
31 [a,b]		1.7	10.0	5.2	1.6
32 [b]		2.2	8.7	3.8	1.0
33 [a,b]		1.4	9.4	4.6	0.9
34 [a,b]		2.3	9.4	4.4	0.8
35		2.1	9.5	4.5	1.5
36 [a]		2.3	9.3	3.9	1.0
37		1.9	9.9	5.3	1.3
38 [b]		1.8	9.6	5.1	1.3

39		1.6	9.5	4.1	1.1
40 [a]		2.2	9.7	4.2	1.4

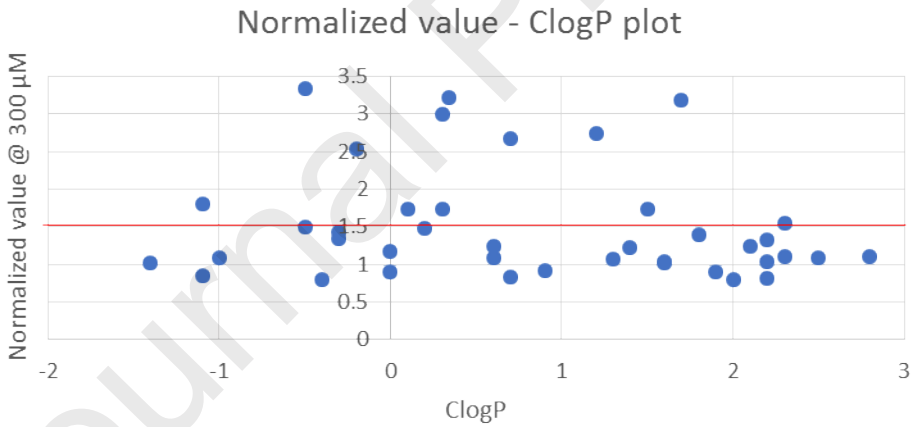
[a] 2 HCl salt.

[b] Racemate.

[c] Calculated using ChemBioDraw Ultra 12.0.

[d] Calculated using ADMET predictor, version 7.2.0001.

To further verify our hypothesis, we analyzed the relationship between the NV and the physicochemical properties for the forty diamines examined in this study (Figure 5). No definite correlation between NV and ClogP or pKa_1 was observed. However, it was clear that the NVs were greater than 1.5 when the pKa_2 values exceed 6.2 with the exception of **23** and **24**. Since both these compounds have an oxetane moiety, we speculate that the basicity-lowering effect of oxetane moiety may be underestimated in the *in silico* calculations performed in this study.



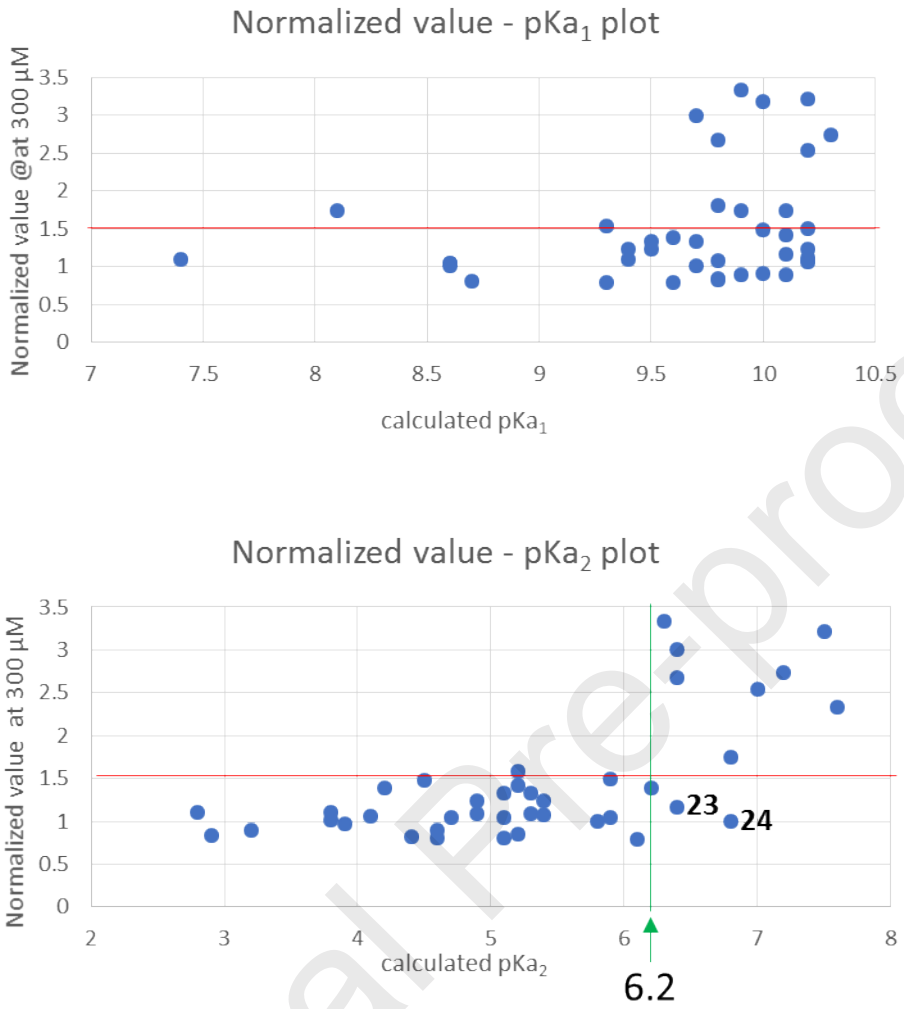
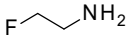
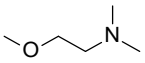
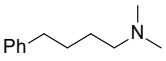
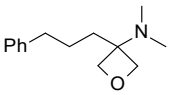


Figure 5. Relationships between NV and ClogP, pKa_1 or pKa_2

In order to verify the above speculation and confirm the accuracy of the *in silico* predicted pKa values, we compared the calculated and experimentally obtained basic pKa values of the representative aliphatic amines (Table 6).[14] For the amines with and without fluorine or oxygen atom, the ΔΔpKa values were 0.4 and 0.3, respectively, indicating that the *in silico* calculations can predict the basicity-lowering effects of these atoms almost accurately. On the other hand, the ΔΔpKa value for oxetanyl group was as high as 1.9, indicating that the basicity-lowering effect of an oxetanyl group was grossly underestimated in the *in silico* calculations performed in this study. Assuming that the pKa_2 values of **23** and **24** are reduced by 1.9, the corrected pKa_2 values would be 4.5 and 4.9, respectively; thus, these compounds will no longer be an exception to our hypothesis.

Table 6. Difference in pKa-lowering effect as obtained from the calculated and experimental values.

Compd.	Structure	$pKa_{calculated}$ *a*	$\Delta pKa_{calculated}$	$pKa_{experimental}$ *b*	$\Delta pKa_{experimental}$	$\Delta\Delta pKa$ *c*
42		10.4		10.7		
			-1.3		-1.7	0.4
43		9.1		9.0		
44		9.8		10.2		
			-0.9		-1.2	0.3
45		8.9		9.0		
46		9.6		9.9		
			-0.8		-2.7	1.9
47		8.8		7.2		

[a] calculated using ADMET predictor, version 7.2.0001.

[b] experimentally measured pKa values reported in ref. 13

[c] $\Delta\Delta pKa$ is defined as $\Delta pKa_{calculated} - \Delta pKa_{experimental}$. It gives the difference in basicity-lowering effects as obtained from the calculated and the experimental values.

Phospholipidosis is caused by lysosomal accumulation of a compound.[2] For a compound to be accumulated into lysosomes, it first need to enter the lysosomes, which is typically driven by the lipophilicity of a compound. Once incorporated into the lysosome, the compound is protonated under lysosomal pH and cannot leave the lysosome, because cationic species are unlikely to passage across biological membrane. Therefore, the more basic a compound is, the more accumulated in lysosomes. Indeed, almost all prediction models are based on lipophilicity and basicity of a compound. That is why ClogP and pKa_1 are considered to be the key physicochemical attributes of phopholipidosis inducers. However, our results showed that there are little correlation between NV and ClogP or pKa_1. Although we have no convincing explanation, we speculate that the differences in the dataset would be one of the reasons, That is, we focus on the fragment-sized diamines, while the previous studies have investigated the marketed drugs and their derivatives. Some monocationic amines have been reported to passively traverse the lysosome membranes if they are small and not very hydrophilic.[15] In such a situation where monocations could escape from lysosomes, lysosomal accumulation of diamines will depend on their tendency toward the formation of dicationic state, since dications are much less permeant than monocations. This further predicts that pKa_2 is the dominant predictor for the PLIP of the fragment-sized diamines.

In conclusion, we have revealed that the previously reported methods to predict the PLIP are not suitable for fragment-sized diamines. Then, we have proposed a new descriptor, pKa_2, which can distinguish PLIP-positive diamines from PLIP-negative diamines more accurately than ClogP or pKa_1. To the best of our knowledge, this is the first report focusing the relationship between pKa_2 and the PLIP. The pKa_2-based prediction might be adopted only when examining small diamines; however, their toxicological behavior is worth investigating in the drug discovery process. Synthetic methods for conformationally restricted small diamines have been frequently reported recently,[16] and some of these diamines are commercially available.[17] Under these circumstances, it is expected that compounds that release a small diamine as a metabolite are frequently synthesized during drug discovery programs,[18] and our study can contribute in evaluating the toxicological potentials of these compounds.

[1] (a) Benjamin, L.; James, L.; Hubert C.; Dennis M. *Clinical Neurology and Neuroscience*, **2018**, *2*, 1. (b) Sakai, H., Inoue, H., Toba, T., Murata, K., Narii, N., Shimmyo, Y., Igawa, Y., Matsumoto, T., Takemoto, N. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 2332.

[2] Hanumegowda U. M.; Regueiro-Ren A. *Top. Med. Chem.* **2015**, *9*, 261.

[3] See ref. 2 and references therein.

[4] According to Ploemen's model, PLIP is predicted to be positive if $(\text{most basic pKa})^2 + (\text{ClogP})^2 \geq 90$ and most basic pKa ≥ 8 and ClogP ≥ 1. It is predicted to be negative if even one of these requirements is not satisfied. Ploemen, J. P.; Kelder, J.; Hafmans, T.; van de Sandt, H.; van Burgsteden, J. A.; Salemink, P.J.; van Esch, E. *Exp. Toxicol. Pathol*. **2004**, *55*, 347.

[5] Compounds with NC equal to 1.0 are more likely to cause phospholipidosis as ClogP increases. For those with NC above 1.0, phospholipidosis is induced regardless of ClogP. Tomizawa, K.; Sugano, K.; Yamada, H.; Horii, I. *J. Toxicol. Sci.* **2006**, *31*, 315.

[6] Kasahara, T.; Tomita, K.; Murano, H.; Harada, T.; Tsubakimoto, K.; Ogihara, T.; Ohnishi, S.; Kakinuma, C; *Toxicol Sci.* **2006**, *90*, 133.

[7] The stoichiometric ratio of amine and HCl has not been experimentally determined. We however consider that all basic nitrogen atoms are protonated under strong acidic conditions because previous studies have confirmed by elemental analysis that diamines form dihydrochloride when treated with hydrochloric acid[(a)] or saturated solution of hydrogen chloride in ethanol.[(b),(c)]

(a) Reiter, J.;Trinka, P.; Bartha, F.L.; Pongo,L.;Volk,B.; Simig, G. *Org. Process Res. Dev.* **2012**, *16*, 1279.

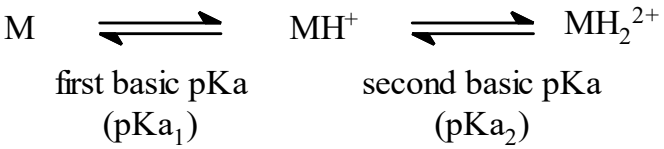
(b) Veselov, I.S.; Trushkov, I.V.; Zefirov, N.S.; Grishina, G.V.; *Russ. J. Org. Chem*. **2009**, *45*, 1050.

(c) Ganina, O.G.; Veselov, I.S.; Grishina, G.V.; Fedorov, A.Y.; Beletskaya, I.P. *Russ. Chem. Bull.,Int. Ed.* **2006**, *55*, 1642.

[8] Culture media used in the PLIP assay contains inorganic salts such as sodium bicarbonate or disodium phosphate, which compose acid-base buffering systems to keep the pH constant. The media

also contains Phenol Red as a pH indicator. We have confirmed that the color of the media didn't change significantly between when free base was used and when HCl salt was used, indicating the difference between free base and HCl salt does not affect physiological pH of evaluated conditions. Detailed assay conditions are described in the supplementary material.

[9] Diamines have two basic pKa. The first and second basic pKa of diamines are expressed as pKa_1 and pKa_2, respectively (see the scheme below). Unless otherwise noted, pKa values were calculated using ADMET predictor, Simulation.plus.inc. Version 7.2.0001.



[10] In addition to Ploemen's model (see ref. 4) and Tomizawa's model (see ref. 5), modified Ploemen's model[(a)] was used.

(a) Pelletier D.J.; Gehlhaar, D.; Tilloy-Ellul, A.; Johnson, T.O.; Greene, N. *J. Chem. Inf. Model.* **2007**, *47*, 1196.

[11] Morgenthaler, M.; Schweizer, E.; Hoffmann-Röder, A.; Benini, F.; Martin, RE.; Jaeschke, G.; Wagner, B.; Fischer, H.; Bendels, S.; Zimmerli, D.; Schneider, J.; Diederich, F.; Kansy, M.; Müller K. *ChemMedChem.* **2007**, *2*, 1100.

[12] The protonation behaviors are simulated by ADMET predictor, version 7.2.0001. All pKa_1 and pKa_2 values listed in Table 1 to 5 have been specified as shown in Figure 4.

[13] Even though the basicities of two nitrogen atoms are similar to each other, pKa_1 and pKa_2 values are not necessarily close to each other. This is because pKa_1 and pKa_2 values do not represent the basicity of a particular nitrogen atom but the pH at which the whole positive charge of the compound is 0.5 or 1.5, respectively.

[14] Experimentally measured pKa values, cited from the tabulation at http://www.zirchrom.com/organic.htm and references therein. [Accessed: 1-Sep-2019]

[15] (a) Andrew, C. L.; Klemm, A.R.; Lloyd, J. B.; *Biochim Biophys Acta.* **1997**, *1330*, 71. (b) Ohkuma, S.; Poole, B.; *J Cell Biol.* **1981**, *90*, 656.

[16] Grygorenko, O. O.; Radchenko, D.S.; Volochnyuk D. M.; Tolmachev A. A.; Komarov, I. V. *Chem Rev.* **2011,** *111*, 5506.

[17] http://www.enamine.net/download/catalogs/Enamine_BicyclicAmines_2014.pdf [Accessed: 1-Sep-2019]

[18] Roughley, S. D.; Jordan, *A. M. J Med Chem.* **2011**, *54*, 3451.

Declaration of interests

☐ 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.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

This research was conducted with a research fund from Asubio pharma, which the authors belonged to.