Evaluation of a Published in Silico Model and Construction of a Novel Bayesian Model for Predicting Phospholipidosis Inducing Potential

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The identification of phospholipidosis (PPL) during preclinical testing in animals is a recognized problem in the pharmaceutical industry. Depending on the intended indication and dosing regimen, PPL can delay or stop development of a compound in the drug discovery process. Therefore, for programs and projects where a PPL finding would have adverse impact on the success of the project, it would be desirable to be able to rapidly identify and screen out those compounds with the potential to induce PPL as early as possible. Currently, electron microscopy is the gold standard method for identifying phospholipidosis, but it is low-throughput and resource-demanding. Therefore, a low-cost, high-throughput screening strategy is required to overcome these limitations and be applicable in the drug discovery cycle. A recent publication by Ploemen et al. (*Exp. Toxicol. Pathol*. **2004**, *55*, 347−55) describes a method using the computed physicochemical properties pK_a and ClogP as part of a simple calculation to determine a compound's potential to induce PPL. We have evaluated this method using a set of 201 compounds, both public and proprietary, with known in vivo PPL-inducing ability and have found the overall concordance to be 75%. We have proposed simple modifications to the model rules, which improve the model's concordance to 80%. Finally, we describe the development of a Bayesian model using the same compound set and found its overall concordance to be 83%.

INTRODUCTION

Compound-induced phospholipidosis (PPL) is a generalized condition in humans and animals that is characterized by an intracellular accumulation of phospholipids and the concurrent development of concentric lamellar bodies.[43] It can occur in multiple tissue types but is often observed in lymphocytes, lung, and liver and is usually only seen at compound doses that are many multiples of the expected efficacious dose. In addition, the condition is generally considered to be reversible, with disappearance of the phospholipidotic inclusions within days or weeks following the cessation of compound administration.[43]

While the risk associated with compound-induced phospholipidosis is considered manageable, due to the effect being seen only at doses that are many multiples of the intended therapeutic dose (i.e., a wide therapeutic index), its observation can lead to delays in compound development while additional studies are carried out to demonstrate reversibility. Factors relating to the intended indication of the compound such as duration of therapy and target population (e.g., pediatric) can also alter the comfort level a development team has for this finding. Additionally, induction of phospholipidosis in certain tissue types (e.g., central nervous system) may require larger therapeutic indices.

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It has long been recognized that cationic amphiphilic drugs are associated with induction of phospholipidosis. Compounds of this type generally contain a hydrophobic region (typically an aromatic ring or ring system) and a hydrophilic side chain that is positively charged at physiological pH.[17,43] The recent publication by Ploemen et al.[41] of an in silico model based on calculation of the physicochemical properties pK_a and ClogP has raised the possibility of a high-throughput, low-cost screening tool for predicting phospholipidosis-inducing potential. Presented here are the results of work aimed at defining the predictive capacity of the published model as well as our efforts to improve upon the model, including modifications to the existing model rules and a Bayesian model build using a common validation list of compounds.

DATA SET

Literature mining along with research into a proprietary database were used to identify compounds that could be used to test the predictive capacity of the Ploemen in silico model. From the literature, compounds associated with induction of PPL in vivo were identified through keyword searching and subsequently followed up to confirm that phospholipidosis was observed ultrastructurally using electron microscopy. Proprietary in vivo positive compounds were identified in a similar way from internal study reports.

The approach taken to generate a reasonable list of phospholipidosis-negative compounds began with the identification of "drug-like" compounds from the published

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Table 1. Phospholipidosis-Positive Literature Compounds Including Reference for Tissue Observed in and Species

compound	CAS	tissue[a]	species[a]	reference
1-chloro-10,11-dehydroamitriptyline	58084-74-5	Lu, Mac, L, Lym	R,	32
1-chloroamitriptyline	52845-72-4	Lu, Mac, L, N, E, Lym, M	R	20
20,25 diazacholesterol	313-05-3	N, Ad	M	6
6-hydroxydopamine	1199-18-4	Ad	R	54
AC-3579	39038-32-9	L	R	51
amikacin	37517-28-5	K	R	52
amiodarone	1951-25-3	Lu, Mac, N, E, Lym, M, Ad	H, R	14, 37
amitriptyline	50-48-6	Lym	R	31
amodiaquine	86-42-0	E	H	15
AY-9944	366-93-8	Lu, Mac, N, E, Ad	R, Ra, M	22, 23, 42
boxidine	10355-14-3	Lu, Mac	R	18
chlorcyclizine	82-93-9	Mac, L, E, M, Ad, T	R	18
chloroquine	54-05-7	Lu, Mac, L, K, N, E, Lym, B, M, T	R, Mo, H, D, M	9, 12, 18, 46
chlorphentermine	461-78-9	Lu, Mac, L, K, N, E, Lym, B, M, Ad, T	R, M, D, Ra	34
chlorpromazine	50-53-3	E, Lym	R, D	7, 53
citalopram	59729-33-8	Lu, L, K, N, Lym, Ad	R	33
clindamycin	18323-44-9	L	D, R	11
clomipramine	303-49-1	Lu, Mac, L, N, E, Lym	R	32
clozapine	5786-21-0	L, Lym, Ad	R	13
coralgil	69-14-7	Lu, Mac, L, K, E, Lym, B, M	H, R, Ra, Ha, M, D	5, 47
cyclizine	82-92-8	Lu, Mac, L	R	18
dibekacin	34493-98-6	K	R	52
di-isobutamide	68284-69-5	L, K, H, Lu	R, D, Mo	24
emetine	483-18-1	L	R	19
erythromycin	114-07-8	Lu, Mac, L, Lym	D, R	11
ethyl fluclozepate	29177-84-2	Lu, Mac	R	38
fenfluramine	458-24-2	Lu, Mac, L, Lym, B, Ad	R, G, D	35
fluoxetine	54910-89-3	Lu, Mac, Ad	R	1
gentamicin	1403-66-3	K	D, R, H	25, 27, 49
homochlorcyclizine	848-53-3	Lu, Mac, L, Tes	R	18
hydroxyzine	68-88-2	Lu, Mac, L	R	18
IA3	43218-55-9	L, Lym, Ad	R	13
imipramine	50-49-7	Lu, Mac, L, E, Lym, M	R	7
indoramin	26844-12-2	L	R	13
iprindole	5560-72-5	Lu, Mac, L, N, E, Lym, B, M	R	7
ketoconazole	65277-42-1	L	M	40
maprotiline	10262-69-8	Mac, L	R	50
meclizine	569-65-3	Mac, L	R	18
netilmicin	56391-56-1	K	R	52
norchlorcyclizine	303-26-4	Lu, Mac, L	R	18
paraquat	4685-14-7	K	M	10
perhexiline	6621-47-2	Lu, Mac, L, N, E, Lym, B, M, Ad	R, H, M	8, 28
phenacetin	62-44-2	K	R	44
quinacrine	83-89-6	Mac, L, E, M	R	7
R-800	34887-52-0	M	R	30
RMI 10,393	21826-47-1	Lu, Mac, Lym	R	20
SDZ 200-125	102636-45-3	Mac, L, K, Lym, Ad	R	45
SKF-14336-D	5310-55-4	B	R	39
stilbamidine	122-06-5	B	H	21
suramin	129-46-4	Tes	R	48
tamoxifen	10540-29-1	Mac, N, E, Lym, Ad	R	29
tilorone	27591-97-5	L, K, E, B	R, H	26, 55
tobramycin	32986-56-4	K	R, H	4, 52
triparanol	78-41-1	Lu, Mac, L, K, N, E, M, Ad, Tes	R, M, Ha	2, 6, 18
trospectomycin sulfate	97673-66-0	L	R	3
zimelidine	56775-88-3	Mac, L, N, E, Lym, Ad	R, D	36

[a] Species abbreviations: human (H), rat (R), mouse (M), dog (D), rabbit (Ra), hamster (Ha), monkey (Mo). Tissue abbreviations: lung (Lu), macrophage (Mac), liver (L), kidney (K), nerve (N), eye (E), heart (H), lymph (Lym), blood (B), muscle (M), adrenal (Ad), thymus (T), testis (Tes).

Table 2. Phospholipidosis-Negative Compounds from the Literature

compound	CAS	compound	CAS
anticoman	301-15-5	diclofenac	15307-86-5
chlortetracycline	57-62-5	methapyrilene	91-80-5
azaserine	115-02-6	thioacetamide	62-55-5
doxapram	309-29-5	tacrine	321-64-2
doxycycline	564-25-0	acetylsalicylic acid	50-78-2
hypoglycin-A	156-56-9	amineptine	57574-09-1
piroxicam	36322-90-4	demeclocycline	127-33-3
rolitetracycline	751-97-3	zidovudine	30516-87-1
galactosamine	7535-00-4	abacavir	136470-78-5
methadone	76-99-3	ciprofibrate	52214-84-3
desferal	70-51-9	clofibrate	637-07-0
felbamate	25451-15-4	fenofibrate	49562-28-9
stavudine	3056-17-5	gemfibrozil	25812-30-0
flutamide	13311-84-7	sulindac	38194-50-2
zileuton	111406-87-2	methyldopa	555-30-6
carbamazepine	298-46-4	dantrolene	7261-97-4
phenobarbital	50-06-6	diflunisal	22494-42-4
Wy-14,643	50892-23-4	colchicine	64-86-8
cyproterone acetate	427-51-0	17-α-ethynylestradiol	57-63-6
3-methylcholanthrene	56-49-5	ANIT (1-naphthyl isothiocyanate)	551-06-4
rifampin	13292-46-1	etoposide	33419-42-0
bicalutamide	90357-06-5	hydrazine	302-01-2
bupropion	34911-55-2	caffeine	58-08-2
AY-25329	49780-10-1	metformin	657-24-9
methotrexate	59-05-2	physostigmine	57-47-6
acetaminophen	103-90-2	temozolomide	85622-93-1
carbon tetrachloride	56-23-5	donepezil	120011-70-3
chloroform	67-66-3	ceftzidime	72558-82-8
L-ethionine	13073-35-3	famotidine	76824-35-6
hydroxyurea	127-07-1	procaine	59-46-1
valproate	99-66-1		

((pKa- basic)^2 + (ClogP)^2)≥ 90 = predicted positive

provided pKa ≥ 8 and ClogP ≥ 1

If result < 90, or pKa < 8 , or ClogP < 1,

then compound predicted negative

Figure 1. In silico model rules for Ploemen model.

literature with well-established adverse event profiles in multiple species that did not include the observation of phospholipidosis. Proprietary in vivo negative compounds were identified as compounds with no evidence of phospholipidosis throughout the conduct of extensive preclinical toxicology testing. In general, this included evaluation of each compound at multiple doses and time points in at least two species.

This process resulted in a total of 201 compounds, of which 85 were identified as positive inducers of PPL and 116 were classified as negative. A total of 56 of the positive compounds came from literature sources (see Table 1), and 29 were from Pfizer's internal database. Of the negative compounds, 61 were from literature sources (see Table 2), while 55 came from Pfizer's internal database. Information on the PPL-positive literature compounds also includes a representative list of species and tissues where this effect has been observed.

ANALYSIS AND DISCUSSION

Ploemen Model. The in silico model rules as suggested by Ploemen et al. can be found in Figure 1. Following the calculation of the most basic pK_a (ACD/Labs 7.0) and CLogP (BioByte CLogP4.0), these parameters are squared and then summed to produce a score. If this score is equal to or greater than 90, then the compound is predicted to be positive. If it is less than 90, it is predicted negative. The compound is also predicted negative if either the most basic pK_a is less than 8 or the ClogP is less than 1. For dibasic compounds, the first basic pK_a is used.

The primary goal of this work was to derive a better understanding of the predictive ability of the model published by Ploemen et al.[41] Its simplified binary output and readily calculable descriptors, which appear clearly associated with the recognized cationic amphiphilic nature of known phospholipidosis inducing compounds, suggests that this tool could be useful as a rapid screening aid to the medicinal chemist. However, its use at such a critical phase of the drug discovery process requires an in-depth understanding of the model's ability to accurately predict this finding.

From the perspective of compound development risk management, we were interested in optimizing the negative predictive value (NPV) of the model without disproportional negative effect on other model statistics. This followed from our view that, to have value as a structure−activity relationship tool, the output of the in silico model would have to be trustworthy enough to take negative compounds directly into in vivo studies. Observation of phospholipidosis in these models could trigger resource-consuming efforts aimed at both verifying the condition using electron microscopy as well as reversibility of the condition.

If alteration of the model rules to optimize the negative predictive value resulted in a higher false-positive rate, we felt these compounds could be followed up using an in vitro assay. We therefore set out visually examining the data using Spotfire (see Figure 2) in an effort to modify the Ploemen model rules to increase the NPV. Improvements to the model

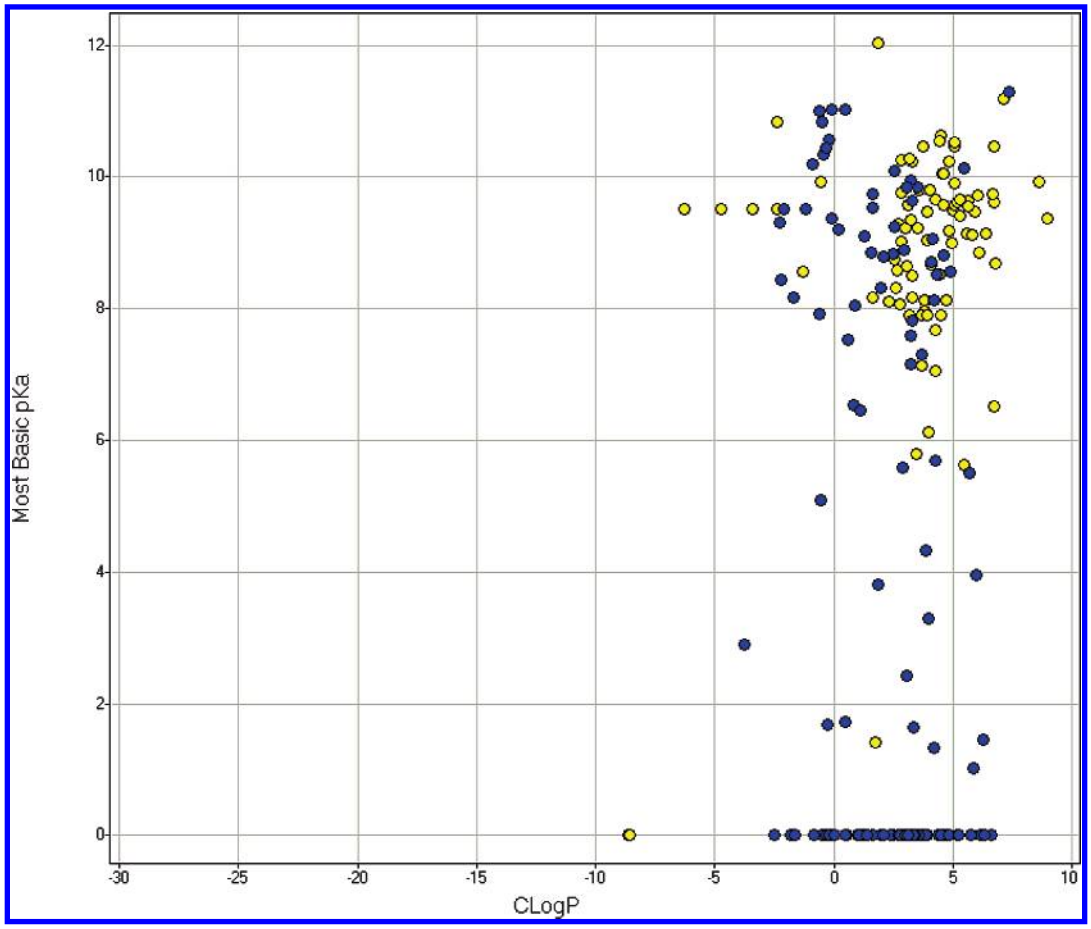

Figure 2. Relationship between most-basic pK_a and ClogP for each compound in the validation list. Yellow circles = phospholipidosis-positive compounds. Blue circles = phospholipidosis-negative compounds.

$((\text{pKa- basic})^2 + (\text{ClogP})^2) \geq 50$ = predicted positive

provided pKa $\geq$ 6 and ClogP $\geq$ 2

If result <50, or pKa < 6 , or ClogP < 2,

then compound predicted negative

Figure 3. Model rules for modified Ploemen model.

parameters were identified by manually adjusting the descriptor values to optimize the separation between positive and negative compounds.

We then used the validation list of compounds described in the data set section to assess the relative performance for each of the models. Table 3 summarizes the results and includes calculations of basic pK_a, ClogP, and the summed square of each for all compounds in the evaluation list. The model performances regarding true and false predictions have been summarized in Table 4.

As regards the published Ploemen model, it appears that this model exhibits high specificity, but relatively low sensitivity. The predictive value of a positive or negative call is about equal (77% vs 74%). Overall, the model was making correct calls for 75% of the compounds.

Modified Ploemen Model. The modified Ploemen model rules can be found in Figure 3. The calculations are the same, but the cutoff values have been altered slightly. If the value of the summed squares is equal to or greater than 50, then the compound is predicted to be positive. If it is less than 50, it is predicted to be negative. The compound is also predicted to be negative if either the most basic pK_a is less than 6 or the ClogP is less than 2. For dibasic compounds, the first basic pK_a is used.

With the modified rules, we managed to increase NPV by 10% with only a 7% loss in specificity, still a respectable 80%, while increasing overall model concordance by 5% (see Table 5). An examination of the false-negative predictions led to the realization that most were antibiotics including many from the aminoglycoside class. Removal of the antibiotic compounds from the validation list increased model performance, in particular the PVN (sensitivity, 85%; specificity, 79%; PVP, 73%; PVN, 89%; concordance, 82%).

Bayesian Model. In parallel with our efforts to modify the Ploemen model, we also explored an alternative model for PPL prediction. Using Pipeline Pilot, version 4.5.1 (Scitegic Inc., www.scitegic.com), a Bayesian model was trained that incorporated the descriptors pK_a, CLogP, the sum of their squared values, amphiphilic moment,[54] the number of basic and acidic centers, and a Scitegic proprietary structural fingerprint, FCFP_4. The FCFP_4 fingerprint allows for the generation of a bit string that encodes local structural and atom class information (i.e., polar, nonpolar, donor, etc.) in a form that is amenable for Bayesian model building. These descriptors were chosen largely on the basis of knowledge of the chemistry and empirical data already established for PPL. In particular, the observation that the presence of a single acid group seemed to ameliorate the risk of PPL led to the inclusion of the number of acidic and basic centers in the model.

Table 3. Property Calculations and Model Predictions for Validation List Compounds[a]

compound	in vivo PPL	pK_a	ClogP	pK_a^2 + CLogP^2	Ploemen	modified Ploemen	Bayesian
chlorphentermine	pos	9.8	2.9	103.4	pos	pos	pos
fenfluramine	pos	10.2	3.3	115.5	pos	pos	pos
amiodarone	pos	9.4	8.9	167.8	pos	pos	pos
coralgil	pos	9.9	8.6	172.5	pos	pos	pos
perhexiline	pos	11.2	7.2	176.4	pos	pos	pos
tilorone	pos	9.7	6.0	130.8	pos	pos	pos
clindamycin	pos	8.7	2.6	83.0	neg	pos	pos
erythromycin	pos	8.2	1.6	69.2	neg	pos	pos
gentamicin	pos	9.5	−2.4	96.4	neg	neg	pos
tobramycin	pos	9.5	−4.7	112.9	neg	neg	pos
dibekacin	pos	9.5	−3.4	102.3	neg	neg	pos
netilmicin	pos	10.8	−2.4	123.2	neg	neg	pos
amikacin	pos	9.5	−6.3	130.3	neg	neg	pos
RMI 10,393	pos	8.9	6.1	115.5	pos	pos	pos
amitriptyline	pos	9.2	4.9	107.8	pos	pos	pos
1-chloroamitriptyline	pos	9.1	5.6	114.5	pos	pos	pos
1-chloro-10, 11-dehydroamitriptyline	pos	9.1	5.8	116.7	pos	pos	pos
citalopram	pos	9.6	3.1	101.4	pos	pos	pos
clomipramine	pos	9.5	5.9	124.5	pos	pos	pos
fluoxetine	pos	10.1	4.6	121.9	pos	pos	pos
imipramine	pos	9.5	5.0	115.4	pos	pos	pos
iprindole	pos	9.6	5.7	125.0	pos	pos	pos
maprotiline	pos	10.6	4.5	133.3	pos	pos	pos
zimelidine	pos	7.9	3.2	72.8	neg	pos	pos
cyclizine	pos	8.0	3.8	77.9	neg	pos	pos
meclizine	pos	6.5	6.7	87.8	neg	pos	pos
tamoxifen	pos	8.7	6.8	122.0	pos	pos	pos
chlorcyclizine	pos	7.9	4.5	82.6	neg	pos	pos
homochlorcyclizine	pos	8.5	4.4	92.2	pos	pos	pos
norchlorcyclizine	pos	9.0	3.9	97.0	pos	pos	pos
chloroquine	pos	10.5	5.1	135.2	pos	pos	pos
quinacrine	pos	10.5	6.7	154.8	pos	pos	pos
AY-9944	pos	9.1	6.4	124.2	pos	pos	pos
boxidine	pos	9.6	5.7	123.2	pos	pos	pos
triparanol	pos	9.6	6.7	137.5	pos	pos	pos
chlorpromazine	pos	9.4	5.3	116.6	pos	pos	pos
IA3	pos	9.7	6.7	139.2	pos	pos	pos
AC-3579	pos	0.0	2.4	5.6	neg	neg	pos
clozapine	pos	7.1	3.7	64.8	neg	pos	pos
hydroxyzine	pos	6.1	4.0	53.4	neg	pos	pos
indoramin	pos	9.0	2.8	89.2	neg	pos	pos
20,25-diazacholesterol	pos	9.6	5.1	117.1	pos	pos	pos
ethyl fluclozepate	pos	0.0	3.2	10.2	neg	neg	neg
6-hydroxydopamine	pos	9.9	−0.5	98.7	neg	neg	neg
paraquat	pos	0.0	−8.7	74.9	neg	neg	neg
phenacetin	pos	1.4	1.8	5.1	neg	neg	neg
R-800	pos	9.3	2.7	93.4	pos	pos	pos
SKF-14336-D	pos	9.6	5.2	119.1	pos	pos	pos
trospectomycin sulfate	pos	8.6	−1.3	74.9	neg	neg	pos
SDZ 200−125	pos	8.1	2.3	71.3	neg	pos	pos
amodiaquine	pos	5.6	5.5	61.5	neg	pos	pos
di-isobutamide	pos	10.5	5.1	136.3	pos	pos	pos
ketoconazole	pos	9.2	3.0	94.3	pos	pos	neg
suramin	pos	0.0	−8.6	73.6	neg	neg	neg
emetine	pos	9.0	4.9	105.5	pos	pos	pos
stilbamidine	pos	12.0	1.8	148.3	pos	pos	pos
PFE 1	pos	8.6	2.6	80.7	neg	pos	pos
PFE 2	pos	8.7	4.1	91.9	pos	pos	pos
PFE 3	pos	8.6	3.1	84.0	neg	pos	pos
PFE 4	pos	9.7	5.3	121.4	pos	pos	pos
PFE 5	pos	8.3	2.6	76.0	neg	pos	pos
PFE 6	pos	8.5	3.3	83.1	neg	pos	pos
PFE 7	pos	7.7	4.3	77.4	neg	pos	pos
PFE 8	pos	9.8	3.6	109.0	pos	pos	pos
PFE 9	pos	9.7	4.2	111.1	pos	pos	pos
PFE 10	pos	10.0	4.6	122.1	pos	pos	pos
PFE 11	pos	9.8	4.0	112.2	pos	pos	pos
PFE 12	pos	10.3	2.8	113.2	pos	pos	pos

Table 3. (Continued)

compound	in vivo PPL	pK_a	ClogP	pK_a^2 + CLogP^2	Ploemen	modified Ploemen	Bayesian
PFE 13	pos	10.3	3.2	115.4	pos	pos	pos
PFE 14	pos	7.1	4.3	67.9	neg	pos	pos
PFE 15	pos	9.5	3.9	105.3	pos	pos	pos
PFE 16	pos	10.5	3.7	123.4	pos	pos	pos
PFE 17	pos	10.6	4.5	131.2	pos	pos	pos
PFE 18	pos	5.8	3.5	45.9	neg	pos	pos
PFE 19	pos	9.3	3.3	97.9	pos	pos	pos
PFE 20	pos	9.6	4.6	112.7	pos	pos	pos
PFE 21	pos	8.1	2.8	72.9	neg	pos	pos
PFE 22	pos	9.2	3.5	97.3	pos	pos	pos
PFE 23	pos	8.2	3.3	77.8	neg	pos	pos
PFE 24	pos	8.1	3.8	80.8	neg	pos	pos
PFE 25	pos	8.1	4.7	88.3	neg	pos	pos
PFE 26	pos	7.9	3.7	76.0	neg	pos	neg
PFE 27	pos	7.9	3.9	77.9	neg	pos	pos
PFE 28	pos	9.9	5.1	123.5	pos	pos	pos
PFE 29	pos	10.2	4.8	127.9	pos	pos	pos
anticoman	neg	0.0	0.5	0.3	neg	neg	neg
chlortetracycline	neg	11.0	−0.1	121.2	neg	neg	neg
azaserine	neg	0.0	−2.5	6.3	neg	neg	neg
doxapram	neg	7.6	3.2	68.0	neg	pos	pos
doxycycline	neg	10.8	−0.5	117.8	neg	neg	neg
hypoglycin-A	neg	9.5	−2.1	94.9	neg	neg	neg
piroxicam	neg	3.8	1.9	18.0	neg	neg	neg
rolitetracycline	neg	11.0	0.5	121.4	neg	neg	neg
galactosamine	neg	8.4	−2.2	75.8	neg	neg	pos
methadone	neg	9.1	4.2	99.3	pos	pos	pos
desferal	neg	10.6	−0.2	111.5	neg	neg	neg
felbamate	neg	0.0	0.5	0.2	neg	neg	neg
stavudine	neg	0.0	−0.5	0.2	neg	neg	neg
flutamide	neg	0.0	3.3	11.1	neg	neg	neg
zileuton	neg	0.0	2.5	6.2	neg	neg	neg
carbamazepine	neg	0.0	2.4	5.7	neg	neg	neg
phenobarbital	neg	0.0	1.4	1.9	neg	neg	neg
Wy-14,643	neg	0.0	4.6	20.9	neg	neg	neg
cyproterone acetate	neg	0.0	3.8	14.2	neg	neg	neg
3-methylcholanthrene	neg	0.0	6.6	43.8	neg	neg	neg
rifampin	neg	7.3	3.7	67.1	neg	pos	neg
bicalutamide	neg	0.0	2.7	7.3	neg	neg	neg
bupropion	neg	7.2	3.2	61.6	neg	pos	neg
AY-25329	neg	8.1	4.2	83.6	neg	pos	pos
methotrexate	neg	5.1	−0.5	26.2	neg	neg	neg
acetaminophen	neg	1.7	0.5	3.2	neg	neg	neg
carbon tetrachloride	neg	0.0	2.9	8.3	neg	neg	neg
chloroform	neg	0.0	2.0	3.8	neg	neg	neg
L-ethionine	neg	9.5	−1.2	92.1	neg	neg	neg
hydroxyurea	neg	0.0	−1.8	3.2	neg	neg	neg
valproate	neg	0.0	2.8	7.6	neg	neg	neg
diclofenac	neg	0.0	4.7	22.3	neg	neg	neg
methapyrilene	neg	8.9	3.0	87.9	neg	pos	pos
thioacetamide	neg	1.7	−0.3	2.9	neg	neg	neg
tacrine	neg	9.6	3.3	103.6	pos	pos	pos
acetylsalicylic acid	neg	0.0	1.0	1.0	neg	neg	neg
amineptine	neg	8.8	2.5	84.1	neg	pos	neg
demeclocycline	neg	11.0	−0.6	121.3	neg	neg	neg
zidovudine	neg	0.0	0.0	0.0	neg	neg	neg
abacavir	neg	6.5	0.8	43.3	neg	neg	neg
ciprofibrate	neg	0.0	3.2	10.1	neg	neg	neg
clofibrate	neg	0.0	3.7	13.5	neg	neg	neg
fenofibrate	neg	0.0	5.2	27.4	neg	neg	neg
gemfibrozil	neg	0.0	3.9	15.5	neg	neg	neg
sulindac	neg	0.0	3.2	10.0	neg	neg	neg
methyldopa	neg	9.3	−2.3	91.6	neg	neg	neg
dantrolene	neg	0.0	1.6	2.7	neg	neg	neg
diflunisal	neg	0.0	4.4	19.3	neg	neg	neg
colchicine	neg	0.0	1.2	1.4	neg	neg	neg
17-α-ethynylestradiol	neg	0.0	3.9	14.9	neg	neg	neg
ANIT (1-naphthyl isothiocyanate)	neg	0.0	4.5	20.1	neg	neg	neg
etoposide	neg	0.0	0.0	0.0	neg	neg	neg
hydrazine	neg	8.2	−1.7	69.6	neg	neg	neg

Table 3. (Continued)

compound	in vivo PPL	pK_a	ClogP	pK_a^2 + CLogP^2	Ploemen	modified Ploemen	Bayesian
caffeine	neg	0.0	0.0	0.0	neg	neg	neg
metformin	neg	0.0	−1.6	2.7	neg	neg	neg
physostigmine	neg	8.3	2.0	73.0	neg	pos	neg
temozolomide	neg	0.0	−0.8	0.7	neg	neg	neg
donepezil	neg	8.8	4.6	98.6	pos	pos	neg
ceftzidime	neg	2.9	−3.8	22.5	neg	neg	neg
famotidine	neg	7.9	−0.6	63.2	neg	neg	neg
procaine	neg	9.2	2.5	91.8	pos	pos	pos
PFE 30	neg	0.0	3.0	9.0	neg	neg	neg
PFE 31	neg	0.0	−0.3	0.1	neg	neg	neg
PFE 32	neg	5.7	4.3	50.6	neg	pos	pos
PFE 33	neg	7.8	3.3	72.2	neg	pos	pos
PFE 34	neg	1.5	6.3	41.2	neg	neg	pos
PFE 35	neg	0.0	4.8	23.2	neg	neg	neg
PFE 36	neg	0.0	1.0	1.1	neg	neg	neg
PFE 37	neg	0.0	6.1	37.7	neg	neg	neg
PFE 38	neg	8.9	1.6	81.0	neg	pos	pos
PFE 39	neg	1.0	5.9	35.5	neg	neg	neg
PFE 40	neg	0.0	5.8	33.3	neg	neg	neg
PFE 41	neg	0.0	0.5	0.2	neg	neg	neg
PFE 42	neg	9.4	−0.1	87.8	neg	neg	pos
PFE 43	neg	9.2	0.2	84.7	neg	neg	neg
PFE 44	neg	4.3	3.8	33.4	neg	neg	neg
PFE 45	neg	8.8	2.1	81.5	neg	pos	pos
PFE 46	neg	0.0	4.9	23.7	neg	neg	neg
PFE 47	neg	0.0	5.2	27.0	neg	neg	neg
PFE 48	neg	0.0	5.2	27.5	neg	neg	neg
PFE 49	neg	0.0	4.9	23.6	neg	neg	neg
PFE 50	neg	9.1	1.3	84.5	neg	pos	neg
PFE 51	neg	5.5	5.7	63.1	neg	pos	neg
PFE 52	neg	7.5	0.6	56.9	neg	neg	neg
PFE 53	neg	4.0	6.0	51.3	neg	neg	pos
PFE 54	neg	6.5	1.1	42.9	neg	pos	neg
PFE 55	neg	0.0	6.3	39.9	neg	neg	neg
PFE 56	neg	5.6	2.9	39.6	neg	pos	pos
PFE 57	neg	0.0	5.2	27.2	neg	neg	pos
PFE 58	neg	3.3	4.0	26.9	neg	neg	neg
PFE 59	neg	1.3	4.2	19.5	neg	neg	neg
PFE 60	neg	2.4	3.1	15.3	neg	neg	neg
PFE 61	neg	1.6	3.4	14.1	neg	neg	neg
PFE 62	neg	0.0	3.6	13.0	neg	neg	neg
PFE 63	neg	0.0	3.4	11.5	neg	neg	neg
PFE 64	neg	0.0	3.4	11.3	neg	neg	neg
PFE 65	neg	0.0	3.1	9.7	neg	neg	neg
PFE 66	neg	0.0	2.1	4.5	neg	neg	neg
PFE 67	neg	0.0	1.4	2.0	neg	neg	neg
PFE 68	neg	0.0	−0.2	0.0	neg	neg	neg
PFE 69	neg	0.0	0.0	0.0	neg	neg	neg
PFE 70	neg	8.5	4.3	91.3	pos	pos	pos
PFE 71	neg	8.6	4.9	97.1	pos	pos	pos
PFE 72	neg	9.8	3.5	109.2	pos	pos	pos
PFE 73	neg	8.7	4.1	92.5	pos	pos	pos
PFE 74	neg	9.9	3.3	109.4	pos	pos	pos
PFE 75	neg	10.1	2.5	108.2	pos	pos	pos
PFE 76	neg	10.3	−0.5	107.1	neg	neg	neg
PFE 77	neg	10.2	−0.9	104.9	neg	neg	neg
PFE 78	neg	9.7	1.7	97.6	pos	pos	pos
PFE 79	neg	9.5	1.7	93.8	pos	pos	pos
PFE 80	neg	9.8	3.1	106.1	pos	pos	pos
PFE 81	neg	10.1	5.5	132.6	pos	pos	pos
PFE 82	neg	11.3	7.4	181.6	pos	pos	pos
PFE 83	neg	8.1	0.9	65.6	neg	neg	neg
PFE 84	neg	10.4	−0.3	109.1	neg	neg	neg

[a] Internal compounds denoted by PFE followed by number.

The model was built on a subset of the overall compound list containing 125 compounds, 84 of which were positive and 41 of which were negative for phospholipidosis. A table containing the Bayesian descriptor values for each of the literature compounds of Tables 1 and 2 is provided as Supporting Information. While they have not been provided, it is reasonable to expect that descriptor values for the proprietary compounds can be produced with the same accuracy and fidelity as the literature compounds.

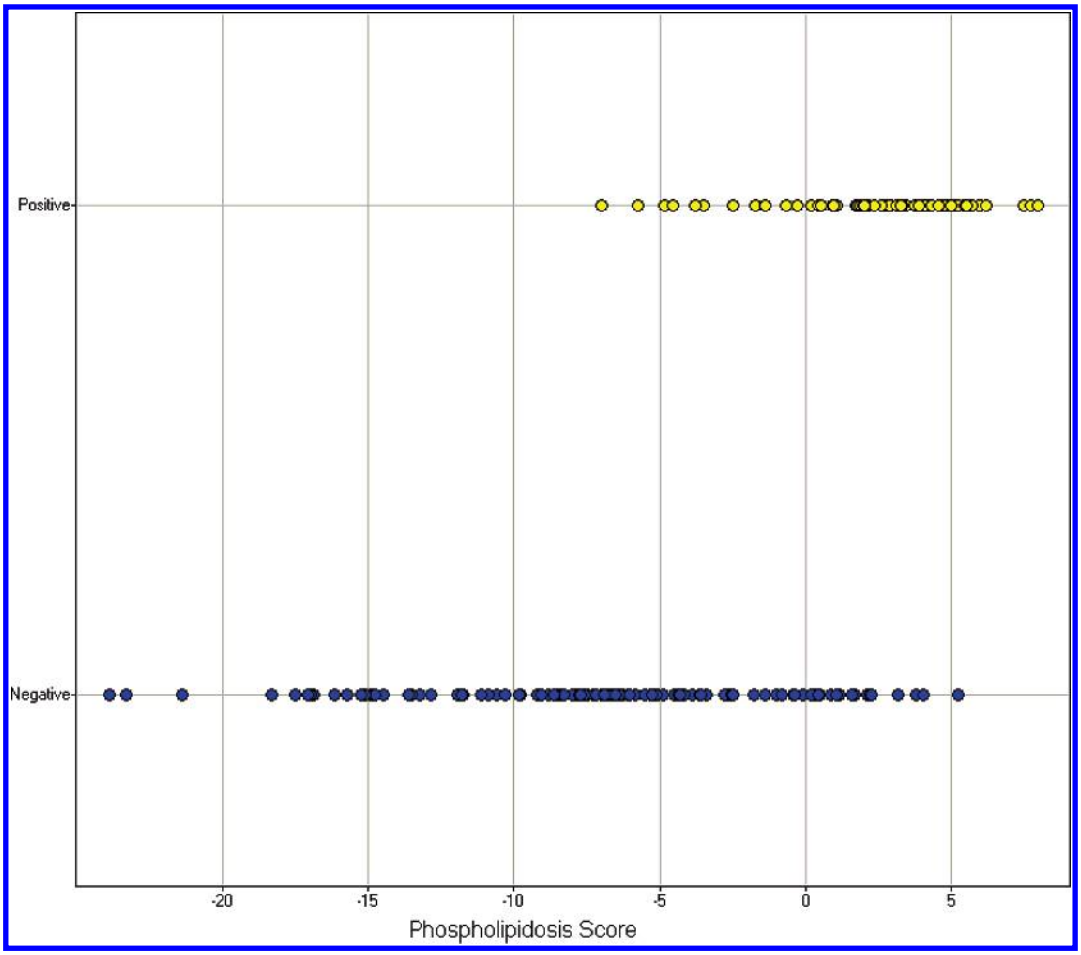

Figure 4. Relationship between phospholipidosis induction potential and Bayesian model phospholipidosis score.

Table 4. Pivot Tables and Predictive Statistics for Ploemen Model

Pivot Table		
	pos	neg
predicted positive	49	15
predicted negative	36	101
Predictive Statistics (%)		
sensitivity	58	
specificity	87	
positive predictive value	77	
negative predictive value	74	
concordance	75	

Table 5. Pivot Tables and Predictive Statistics for Modified Ploemen Model

Pivot Table		
	pos	neg
predicted positive	67	23
predicted negative	18	93
Predictive Statistics (%)		
sensitivity	79	
specificity	80	
positive predictive value	74	
negative predictive value	84	
concordance	80	

A model developed on this small of a data set has inherent liabilities, not the least of which is overfitting. Several steps were taken to mitigate this risk. The 125 compounds were randomly split into two sets, each containing approximately half of the compounds. Each set was used to generate a model, which was then tested against the other. Both of these were quite predictive (ROC curve area > 0.8) with a reasonable separation of positive and negative predictions. This showed that the data had strong predictive capabilities. It is important to note that each of the descriptors was evaluated as to their worthiness for inclusion in the model. This included an evaluation of the relative strength of each descriptor's contribution to the model (as reported by Pipeline Pilot) as well as a visual inspection to ensure that the correlation made sense scientifically (i.e., a high pK_a and high ClogP correlates with strong potential to induce PPL). Molecular descriptors that were tried, but failed this latter test, included polar and nonpolar surface areas; molecular volume; dipole moments; and other measures of charge separation, molecular weight, and number of donors and acceptors.

The final model was trained using all 125 compounds, and the output was carefully scrutinized to ensure that the extracted trends continued to make sense as regards PPL induction potential. Following this, the model was evaluated against the entire 201 compounds in the validation list. The model output includes a phospholipidosis score, a unitless value associated with phospholipidosis-inducing potential (see Figure 4), that ranged from -23.89 (negative end of the range) to $+7.97$ (positive end of the range). In order to produce a binary yes/no output, an optimized cutoff of -2 was chosen to apply to the resulting score. Output scores

Table 6. Pivot Tables and Predictive Statistics for Bayesian Model

Pivot Table		
	pos	neg
predicted positive	78	27
predicted negative	7	89
Predictive Statistics (%)		
sensitivity	92	
specificity	77	
positive predictive value	74	
negative predictive value	93	
concordance	83	

greater than this cutoff were considered positive predictions for phospholipidosis, while scores less than the cutoff were considered negative predictions.

The inclusion of the additional descriptors of amphiphilic moment, number of basic and acidic centers, and FCFP_4 fingerprint has improved the predictive capacity of the model with gains in sensitivity and NPV outpacing losses in specificity (see Table 5). Overall concordance has risen to 83% using this model (Table 6). Importantly, the NPV has been increased even further over the improvements made by the modifications to the Ploemen model to 93%. This level of performance, we feel, will lead to an increased confidence that the compounds selected to advance through the development pipeline will carry minimal risk of inducing phospholididosis in preclinical safety studies.

CONCLUSIONS

In this paper, we have expanded our understanding of the predictive ability of the published Ploemen model using a rigorously selected list of positive and negative compounds for the evaluation. Further, it appears we have improved upon the model's performance by a simple tweaking of the model rules. Finally, we have constructed a Bayesian model using newer methods of computational chemistry which appears to be even more predictive, particularly as regards its ability to identify negative compounds for advancement through the drug discovery and development process.

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Supporting Information Available: Bayesian model descriptor information for the literature compounds in Tables 1 and 2 is available as Supporting Information. This information is available free of charge via the Internet at http://pubs.acs.org.

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