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An aminomethylpyrimidine DPP-IV inhibitor with improved properties

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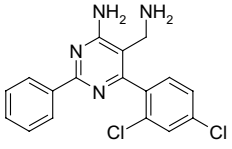
Abstract—A recently identified DPP-IV inhibitor (**1**) was found to induce phospholipidosis and to inhibit CYP3A4. A small series of less lipophilic and less amphiphilic analogues was synthesized in an effort to overcome these issues. One compound from this series was equipotent to **1**, did not induce phospholipidosis and showed a reduced CYP3A4 inhibition.

Glucagon-like peptide 1 (GLP-1) has become a prominent target for the treatment of type 2 diabetes.[1] GLP-1 is secreted by the gastrointestinal tract in response to food intake, stimulates insulin secretion, and inhibits hepatic glucose production.[2] Continuous intravenous infusion of GLP-1 nearly normalizes blood glucose levels in type 2 diabetic patients.[3] However, subcutaneous bolus injections of GLP-1 have proven ineffective[4] because GLP-1 is rapidly metabolized by dipeptidyl peptidase IV (DPP-IV).[5,6] Inhibition of DPP-IV is an indirect way to increase the levels of circulating GLP-1,[7] and DPP-IV inhibitors have been shown to improve glucose excursion in patients with type 2 diabetes.[8]

In a search for novel DPP-IV inhibitors, we identified aminomethylpyrimidine **1** (Table 1) as a lead structure.[9] Compound **1** has many favorable properties, such as high solubility,[10] high membrane permeability,[11] and high metabolic stability in human liver microsome preparations.[12] However, **1** was also found to be an inhibitor of cytochrome P450 3A4 (CYP3A4)[13] and was found to induce phospholipidosis in cultured fibroblasts at all test concentrations (2.5–20 μM) in a concentration-dependent manner.[14] Inhibition of CYP3A4 is a leading cause of drug–drug interactions,[15] and phospholipidosis might have adverse physiological consequences.[16] For these reasons, we felt that these latter issues had to be addressed to improve the quality of our lead.

Table 1. Properties of **1**



1

IC_{50}(DPP-IV)	=	10nM
$logD_{7.4}$	=	3.0
IC_{50} (CYP 3A4)	=	5.4μM

• phospholipidosis induction

Compound **1** is rather lipophilic[17] and has a pK_a of 7.8.[18] As a consequence, **1** should be a cationic, amphiphilic compound under physiological conditions.[19] Lipophilicity is a recognition criterion for CYP3A4,[20] and amphiphilicity is predictive for the phospholipidosis potential of cationic compounds.[21] Therefore, we anticipated that less lipophilic and less amphiphilic analogues of **1** should be less likely to show CYP3A4 inhibition and phospholipidosis induction. In an attempt to obtain such improved analogues, we replaced the lipophilic 2-phenyl group of **1** by small motifs like Me (**2**, Table 2), MeO (**3**), and NH_2 (**4a**). However, these compounds had a reduced inhibitory activity at DPP-IV.[22]

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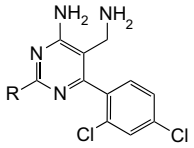
Table 2. Exploration of small substituents R

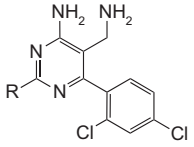


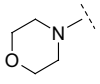
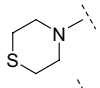
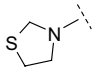
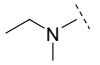
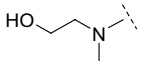
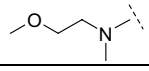
	R =	IC_{50} (DPP-IV) (nM)	log $D_{7.4}$
2	Me	250	1.4
3	OMe	79	1.6
4a	NH_2	270	0.4

To identify similar compounds with restored activity and low CYP3A4/phospholipidosis potential, we prepared a number of N-alkylated analogues of **4a** that were predicted by computational tools to be less lipophilic (KOW_ClogP)[23] and less amphiphilic (CAFCA)[24] than compound **1** (**4b**–**h**, Table 3). More specifically, we aimed for compounds with a calculated free energy of amphiphilicity ($\Delta\Delta G_{AM}$) greater than −6 kJ/mol, because it was found previously that such compounds have a low probability of being positive in the fibroblast phospholipidosis assay, whereas $\Delta\Delta G_{AM}$ values below −6 kJ/mol were found to indicate compounds with a high risk of phospholipidosis.[21]

The in silico results and the measured inhibitory activities of **4b**–**h** are documented in Table 3.

Table 3. Exploration of amine substituents R



	R =	KOW_ClogP	$\Delta\Delta G_{AM}$[a] (kJ/mol)	IC_{50} (DPP-IV) (nM)
1	Ph	2.6	−6.6	10
4b		1.4	−5.3	140
4c		2.3	−5.7	0.2
4d		1.8	−5.6	11
4e		2.2	−5.7	24
4f		2.3	−5.8	55
4g		0.9	−6.0	0.5
4h		1.6	−5.6	9

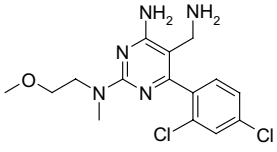
[a] Calculated free energy of amphiphilicity as a measure of phospholipidosis potential.

In **4b**, the 2-Ph substituent of **1** is replaced by a morpholino substituent. Compound **4b** is twofold more active than **4a**, but is still much less active than **1**. The thiomorpholino compound **4c** is structurally closely related to **4b**, but is in contrast to **4b** an outstandingly active DPP-IV inhibitor. The calculated lipophilicity of **4c** is, however, rather high. Formal removal of one methylene unit led to the smaller thiazolidine analogue **4d**, which is less lipophilic but also less active than **4c**. Compound **4e** and its acyclic analogue **4f** have an even further decreased activity. The introduction of a hydroxyl function gave a highly active DPP-IV inhibitor, **4g**, with a greatly reduced lipophilicity, but a borderline $\Delta\Delta G_{AM}$ value. The high absolute $\Delta\Delta G_{AM}$ value for **4g** is an exception to the otherwise generally observed correlation between $\Delta\Delta G_{AM}$ and KOW_ClogP within the series of **4**, and is due to the conformer selection procedure in CAFCA.[24] The corresponding methyl ether, **4h**, had the most appealing balance between activity, lipophilicity, and amphiphilicity, and was further characterized. Compound **4h** is comparable to **1** with respect to solubility, membrane permeability, microsomal stability, and inhibitory activity at DPP-IV. Compound **4h** has a much reduced measured lipophilicity (as compared to **1**), which correlates well with the predicted value. Gratifyingly, **4h** shows a sufficiently reduced CYP3A4 inhibition, and was found not to induce phospholipidosis in cultured fibroblasts up to the highest test concentration (20 μM) (Table 4).

Aminomethylpyrimidines **1**–**4** were obtained according to Scheme 1: benzylamidine (**5**, R = Ph), O-methylisourea (**5**, R = OMe), or urea (**5**, R = NH_2) were reacted with 2,4-dichlorobenzylidenemalononitrile (**6**) under basic conditions with a subsequent oxidative step to give the corresponding 5-cyanopyrimidines **7**.[25] Reduction of the nitrile functionality furnished **1**–**4a**. S-Methylisothiourea (**5**, R = SMe) and **6** were converted to pyrimidine **7**-SMe. Oxidation provided sulfone **7**-SO_2Me. Nucleophilic displacements with a variety of amines R_2NH gave **7b**–**h**,[26] which were reduced to aminomethylpyrimidines **4b**–**h**. For a detailed procedure for the preparation of **4h**, see Ref. 27.

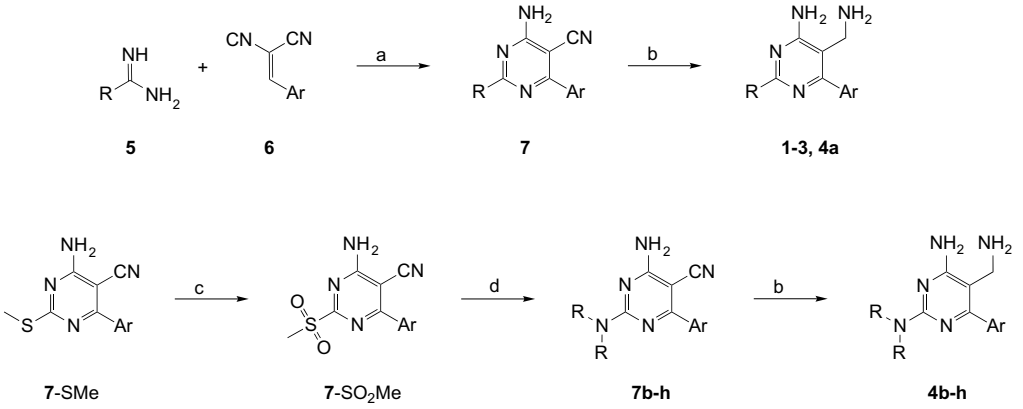
In summary, we addressed the phospholipidosis liability and the CYP3A4 inhibition in a series of aminomethylpyrimidine DPP-IV inhibitors. We speculated that compounds with lowered lipophilicity and amphiphilic-

Table 4. Properties of **4h**



4h

IC_{50} (DPP-IV) = 9nM
$logD_{7.4}$ = 1.6
IC_{50} (CYP 3A4) = 30μM
• no phospholipidosis



Scheme 1. Ar = 2,4-dichlorophenyl. Reagents and conditions: (a) K_2CO_3, MeOH, 50 °C, 3 h, then, after evaporation of solvent, $KMnO_4$, acetone, rt, 3 h (e.g., **7**-Ph 52%; **7**-SMe 88%); (b) $LiAlH_4$, THF, 40 °C, 3 h (e.g., **1** 40%; **4h** 18%); (c) MCPBA, CH_2Cl_2, 15 min 0 °C → rt (quant.); (d) R_2NH, dioxane, 2 h, rt (e.g., **7h** 84%).

ity might be superior in terms of CYP3A4 inhibition and phospholipidosis potential. Predictive computational tools guided us in the synthesis of a series of such compounds. From this series we identified a low nanomolar DPP-IV inhibitor, **4h**, that did not induce phospholipidosis and showed a reduced CYP3A4 inhibition. Additionally, sub-nanomolar inhibitors of DPP-IV (**4c**,**g**, Table 3) were found in the course of our work.

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22. DPP-IV inhibitors were measured for their ability to inhibit DPP-IV mediated cleavage of Ala-Pro-7-amido-4-trifluoromethylcoumarin in a fluorogenic assay. All compounds were measured in triplicate at 5–7 concentrations in the range of 100 μM to 100 pM. IC_{50} values were calculated with a nonlinear best fit regression model. All assays were calibrated with NVP-DPP728 as internal standard inhibitor. NVP-DPP728 under the conditions of the assay showed an IC_{50} of 15 ± 4 nM (M ± SD, $n = 12$) at 50 μM substrate concentration and a K_i of 11 ± 3 nM determined at substrate concentration range of 10–600 μM. IC_{50} values of unknown compounds were accepted when the IC_{50} (x) measured for NVP-DPP728 in the assay was $11 < x < 19$ nM.
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27. Preparation of **4h**: (a) **7**-SMe: S-Methylisothiourea sulfate (**5**-SMe, 4.49 g, 16.1 mmol) 2,4-dichlorobenzylidenemalononitrile (**6**, 6.00 g, 27.0 mmol), and K_2CO_3 (6.50 g, 47.0 mmol) were suspended in MeOH (50 mL) and heated to reflux for 90 min. The solvent was then evaporated and the residue was distributed in EtOAc/H_2O. The organic layer was separated and dried (Na_2SO_4). After evaporation of the solvent, the residue was taken up in acetone and treated with $KMnO_4$ at rt overnight. After filtration (dicalite) and evaporation, **7**-SMe (7.40 g, 88%) was isolated by column chromatography (silica gel, AcOEt/hexane = 1:2). ^{1}H NMR (DMSO-d_6): δ 2.46 (3H, s), 7.57–7.62 (2H, m), 7.83 (1H, s), 7.80–8.30 (2H, br s); (b) **7**-SO_2Me: At 0 °C, MCPBA (865 mg, 5.01 mmol) was added to a suspension of **7**-SMe (1.30 g, 4.18 mmol) in CH_2Cl_2 (40 mL). The mixture was stirred for 1 h at rt, during which time the product partly crystallized. CH_2Cl_2 was added, the mixture was washed with satd $NaHCO_3$ and H_2O, and dried (Na_2SO_4). The product, **7**-SO_2Me (1.49 g, 100%), was used without further purification.^{1}H NMR ($CDCl_3$): δ 1.55 (2H, br s), 3.33 (3H, s), 7.45 (2H, s), 7.59 (1H, s); (c) **7h**: A mixture of N-2-(methoxyethyl)methylamine (62 mg, 0.699 mmol) was added to **7**-SO_2Me (200 mg, 0.583 mmol) in dioxane (4 mL). The mixture was stirred for 2 h at rt during which time it turned into a clear brown solution. The solvent was evaporated and the residue was taken up in EtOAc. The mixture was washed (1 N HCl, brine) and dried (Na_2SO_4). After evaporation, **7h** (173 mg, 84%) was obtained by column chromatography (silica gel, AcOEt/hexane = 1:2). ^{1}H NMR (DMSO): δ 3.13 (3H, s), 3.21 (3H, s), 3.48–3.50 (2H, m), 3.71–3.76 (2H, m), 7.35 (2H, br s), 7.49–7.51 (1H, m), 7.55–7.57 (1H, m), 7.79 (1H, s); (d) **4h**: At a temperature of 0 °C, a solution of **7h** (19 mg, 0.054 mmol) in THF (2 mL) was added to a suspension of $LiAlH_4$ (20 mg, 0.53 mmol) in THF (1 mL). The mixture was stirred for 2 h at rt and then quenched with H_2O (0.5 mL). After filtration and evaporation of the solvent, **4h** (3.4 mg, 18%) was isolated from the reaction mixture by preparative HPLC [Pro 18 column, solvent gradient 5–95% CH_3CN in 0.1% TFA(aq) over 6.0 min, λ = 230 nm, flow rate 40 mL/min]. ^{1}H NMR ($CDCl_3$): δ 1.61 (2H, br s), 3.01 (3H, s), 3.23 (3H, s), 3.31 (2H, s), 3.45–3.48 (2H, m), 3.61–3.65 (2H, m), 6.70 (2H, br s), 7.38–7.40 (1H, m), 7.46–7.48 (1H, m), 7.67 (1H, s).