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Optimization of Halopemide for Phospholipase D2 inhibition

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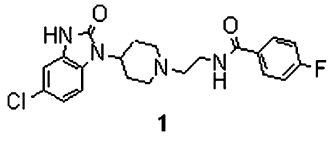
Abstract—Halopemide, which was identified by HTS to inhibit phospholipase D2 (PLD2), provided the basis for an exploratory effort to identify potent inhibitors of PLD2 for use as inflammatory mediators. Parallel synthesis and purification were utilized to rapidly identify orally available amide analogs derived from indole 2-carboxylic acids with superior potency versus PLD2.

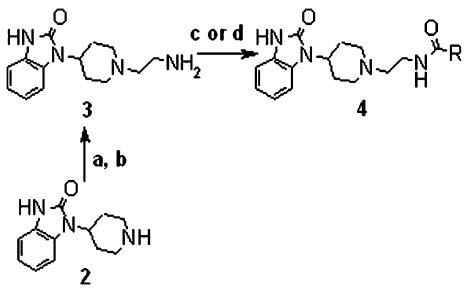
Phospholipase D (PLD) produces phosphatic acid (PA) and choline by hydrolysis of phosphatidylcholine (PC), representing the first committed step to lipid signaling pathway mediated by PA and its downstream messengers, such as diacyl glycerol (DAG). Consistent with its consequent implication in inflammation, PLD can be activated by a variety of inflammatory mediators, including tumor necrosis factor alpha (TNFα), interleukin-1 (IL-1), and lipopolysaccarides (LPS). The molecules described in this publication were targeted for arthritis and inflammatory indications, although PLD is implicated in additional disease processes.[1] To date, few small molecule PLD inhibitors have been disclosed.[2] Halopemide **1**,[3] originally reported by Janssen for neuroscience indications, was identified by high throughput screening to target the PLD2 enzyme, one of the two PLD isoforms.



1

Deletion of the chloride from **1** gave compound **4a** and equivalent PLD2 potency. Therefore, a family of analogs generated to explore SAR of the amide portion began with conversion of commercial piperidine **2** to the intermediate amine **3** (Scheme 1). The desired amides **4** were synthesized in parallel by treatment with an acid chloride or activated ester generated from the corresponding carboxylic acid and a suitable coupling agent, such as TPTU or EDCI, in the presence of an amine base, such as *N*-methylmorpholine (NMM). Following parallel synthesis, the resulting amides were purified in parallel by HPLC.[4] Alternatively, the amides **4** were conveniently accessed in larger quantities by the EDCI coupling above in dioxane and water[5] or by treatment of **3** with a pentafluorophenyl ester in THF, followed by filtration of the amide product and purification by trituration.

Enzyme inhibition data for selected examples appear in Table 1. Conservative modification of the phenyl substi-



Scheme 1. Synthesis of amide analogs. Reagents: (a) *N*-BOC 2-bromoethylamine; (b) HCl, CH_2Cl_2; (c) RCO_2H, TPTU, NMM, DMF or RCO_2H, NMM, EDCI, dioxane, water; (d) $RCO_2C_6F_5$, NMM, THF.

Keywords: Halopemide; Phospholipase D; Inhibitor.
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Table 1. PLD2 inhibition for compounds **1**, **2**, and **5a**–**5k**

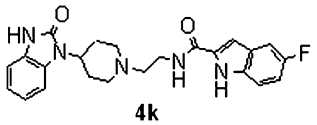
Compound	R	PLD2 IC_{50} (μM)
1	*p*-FPh	1.500
4a	*p*-FPh	1.410
4b	Ph	1.500
4c	*p*-FPhCH_2	0% inh at 1μM
4d	*p*-FPh$(CH_2)_2$	0% inh at 1μM
4e	*i*-Pr	0% inh at 1μM
4f	*i*-Bu	0% inh at 1μM
4g	Benzyloxycarbonyl	0% at inh at 1μM
4h	3-Thienyl	1.000
4i	3-Quinolinyl	0.600
4j	2-Indolyl	0.020
4k	5-Fluoro-2-indolyl	0.020
4l	*N*-Me 2-indolyl	0% at inh at 1μM
4m	2-Benzthienyl	2.000

Table 2. Rat pharmacokinetic parameters measured for **4k** dosed 5 mg/kg p.o. or 1 mg/kg iv in 20% PEG/80% CMC/0.025% Tween 80

$T_{1/2}$ (h)	Cl (L h^{-1} kg^{-1})	C_{max} (μM)	AUC (μM h L^{-1})	%*F*
5.57	2.18	0.363	1.03	18.5

tuent (such as the desfluoro **4b** or the 3-thienyl **4h**), replacement of the phenyl ring with alkyl or arylated alkyl substituents (**4c**–**4f**), and introduction of a carbamate **4g** produced compounds with equivalent or weaker PLD2 potency. Introduction of a 2-indolyl moiety (compounds **4j** and **4k**) produced a significant improvement in potency. Fused bicyclic aromatic compounds lacking the hydrogen bond donor, **4l** and **4m**, showed attenuated activity.

High melting indole-containing compounds **4j** and **4k** displayed limited solubility in a variety of common organic solvents and moderate water solubility under thermodynamic conditions (0.075 mM at pH 6.8). Nevertheless, a set of pharmacokinetic parameters for compound **4k** (shown below in Table 2) show that, despite and high clearance of greater than 2 L h^{-1} kg^{-1}, the compound showed a half-life of greater than 5 h, a C_{max} of greater than 10-fold the IC_{50} versus PLD2, and moderate bioavailability of 18%.



4k

In conclusion, replacement of the *p*-fluorophenyl moiety of halopemide **1** and **4a** with a 2-indolyl moiety (present in analogs, **4j** and **4k**) improved the potency versus PLD2 75-fold that was unmatched in a set of fused bicyclic heteroaromatic compounds. The oral availability and potency of indole **4k** provided a suitable starting point for further optimization of potency and oral availability as well as a useful tool for study in models of arthritis and inflammation.

Acknowledgments

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5. Preparation of **4k**: To amine **3** (0.48 g, 1.85 mmol), NMM (0.22 g, 2.22 mmol), and 5-fluoroindole-2-carboxylic acid (0.56 g, 3.14 mmol) in 6 mL of dioxane/water (1:1) was added EDCI (0.71 g, 3.69 mmol). After 18 h, H_2O (30 mL) was added and the reaction mixture was filtered. The desired product was purified by trituration with hot EtOAc, flash chromatography eluting with 7% MeOH/CH_2Cl_2, and then trituration with CH_2Cl_2. The purified product was converted to the corresponding hydrochloride salt using MeOH and aqueous HCl before being concentrated to a solid under reduced pressure and dried in vacuo for 18 h at 60 °C to give a solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.55–1.71 (m, 2H), 2.06–2.24 (m, 2H), 2.24–2.44 (m, 2H), 2.52–2.63 (m, 2H), 2.96–3.15 (m, 2H), 3.38–3.53 (m, 2H), 4.08–4.26 (m, 1H), 6.86–7.23 (m, 6H), 7.34–7.48 (m, 2H), 8.42–8.62 (m, 1H), 10.84 (s, 1H), 11.71 (s, 1H).