Accepted Manuscript

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PII: S0960-894X(17)31053-3
DOI: https://doi.org/10.1016/j.bmcl.2017.10.054
Reference: BMCL 25383

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 25 July 2017
Revised Date: 18 October 2017
Accepted Date: 22 October 2017

Please cite this article as: Forster, A.B., Abeywickrema, P., Bunda, J., Cox, C.D., Cabalu, T.D., Egbertson, M., Fay, J., Getty, K., Hall, D., Kornienko, M., Lu, J., Parthasarathy, G., Reid, J., Sharma, S., Shipe, W.D., Smith, S.M., Soisson, S., Stachel, S.J., Su, H-P., Wang, D., Berger, R., The Identification of a Novel Lead Class for Phosphodiesterase 2 Inhibition by Fragment-Based Drug Design, *Bioorganic & Medicinal Chemistry Letters* (2017), doi: https://doi.org/10.1016/j.bmcl.2017.10.054

The Identification of a Novel Lead Class for Phosphodiesterase 2 Inhibition by Fragment-Based Drug Design

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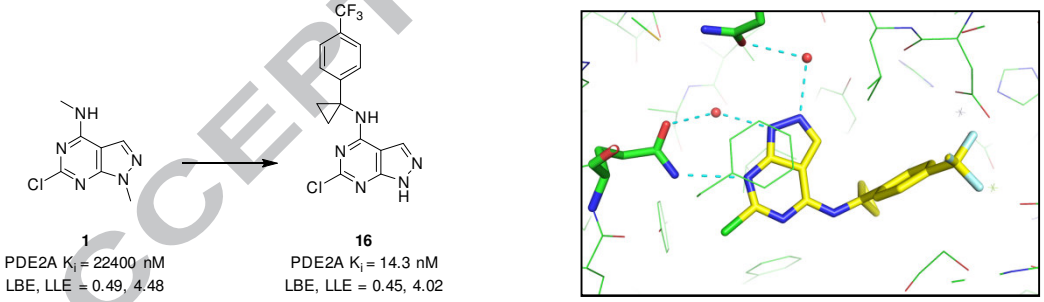
KEY WORDS: PDE2, Phosphodiesterase 2, fragment-based screening, structure-based drug design

ABSTRACT: We have identified a novel PDE2 inhibitor series using fragment-based screening. Pyrazolopyrimidine fragment **1**, while possessing weak potency (K_i = 22.4 μM), exhibited good binding efficiencies (LBE = 0.49, LLE = 4.48) to serve as a start for structure-based drug design. With the assistance of molecular modeling and X-ray crystallography, this fragment was developed into a series of potent PDE2 inhibitors with good physicochemical properties. Compound **16**, a PDE2 selective inhibitor, was identified that exhibited favorable rat pharmacokinetic properties.



Alzheimer's Disease (AD) is a progressive, neurodegenerative disorder affecting an estimated 5.2 million people nationwide and is the sixth leading cause of death in the US.[1,2] Symptoms of AD include decline in cognitive functions such as memory, speech and learning, and eventually lead to the patient's inability to perform routine daily tasks. Current approved therapies provide only temporary symptomatic treatment of AD. No agents are available that cure, prevent or slow disease progression.[1] It is estimated that by 2025 approximately 13.8 million people will be afflicted with Alzheimer's Disease, indicating there is a severe unmet medical need.[1,2]

The cyclic nucleotides, cAMP and cGMP, are secondary messengers which mediate the release of neurotransmitters implicated in the regulation of normal cognitive function.[3-6] Signaling within these neurotransmitter pathways, which heighten synaptic plasticity, learning, and memory is impaired in patients with Alzheimer's Disease.[1,4,7,8] The phosphodiesterases (PDEs) are a superfamily of phosphohydrolases that catalyze the hydrolysis of the 3'-cyclic phosphate bonds of cAMP and cGMP, which is the primary mechanism for their inactivation.[3,4,7] To date, 11 different PDE gene families have been identified which differ broadly in cellular function, location and affinity for cAMP and cGMP.[3-5] The PDE2 isoform is a dual substrate that hydrolyzes both cAMP (K_m = 30 μM) and cGMP (K_m = 10 μM),[3,4,9] and is highly expressed in the hippocampus and frontal cortex,[4] regions of the brain which are associated with cognition, learning and memory.[6-8] It has been demonstrated that pharmacological inhibition of PDE2 enhances cGMP and cAMP signaling within the neurotransmitter pathways involved in cognition.[4,6] In addition, preclinical studies have shown that PDE2 inhibition improves cognition in a variety of rodent behavioral models relevant to AD.[4,6-8,10] Due to the low homology that exists between the catalytic domain of the different PDE isoforms, ranging from 20 – 50%,[4] there is a strong possibility for the identification of a PDE2 selective inhibitor. For this reason PDE2 has been targeted as a treatment for AD-related cognitive deficits.

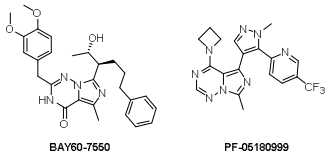


Figure 1. BAY60-7550[7,11] and PF-05180999.[7,12]

PDE2 inhibitors such as BAY60-7550 and PF-05180999 have previously been disclosed in the literature (Fig. 1).[6-8,11,12] In an effort to identify novel chemical matter differentiated from the current landscape, a fragment-based screening (FBS) campaign was initiated in parallel with a traditional high throughput screen (HTS). The concept of fragment-based screening is to identify small molecules that, while displaying weak binding, exhibit this through high binding efficiencies (LBE and LLE).[13] These small fragments also possess more drug-like physicochemical properties than larger and more potent HTS leads.[5,14-16] Such attributes provide an improved starting point for the lead optimization process and increase the probability that these properties could be incorporated into more potent molecules if carefully maintained through the drug discovery process.[15-18]

Biochemical (High Concentration Screening [HCS]) and biophysical (Surface Plasmon Resonance [SPR]) methods were used to screen our proprietary fragment library[19] for PDE2 inhibitors.[14-16] A total of 54 structurally distinct hits with PDE2 K_i and K_d < 200 μM were identified and importantly were found to exhibit competitive binding with known PDE2 inhibitors by SPR. The pyrazolopyrimidine fragment **1** was selected for SAR development based on favorable physicochemical properties and high binding efficiencies (Table 1).

In order to efficiently progress a fragment lead, it is crucial that crystallographic data be available to optimize the compound using structure-based drug design. Initially, a representative inhibitor-bound structure was solved for BAY60-7550 (Fig. 2).[7,10] Analysis of internal and published[7,10] structures observed key ligand interactions and pockets of the PDE2 active site. The heterocyclic core of BAY60-7550 is anchored via π-stacking with F862 and F830 residues (**A** and **B**)[7,10,20] and the pyrimidone functionality interacts through hydrogen bonds with Q859 and Q812, the latter of which is

unique to PDE2 (**C**), while Q859 is conserved among PDEs.[10,20] Interestingly, the propylphenyl group is accommodated through a novel hydrophobic induced binding pocket (**D**) located under L770[7,10] BAY60-7550 also interacts with the surface hydrophobic patch (**E**).[10] It has been previously demonstrated that PDE2 inhibitory potency can be enhanced through all of these interactions, however selective inhibitors have relied on interactions with PDE2 specific Q812 and also through occupying the hydrophobic induced binding pocket.[10,20]

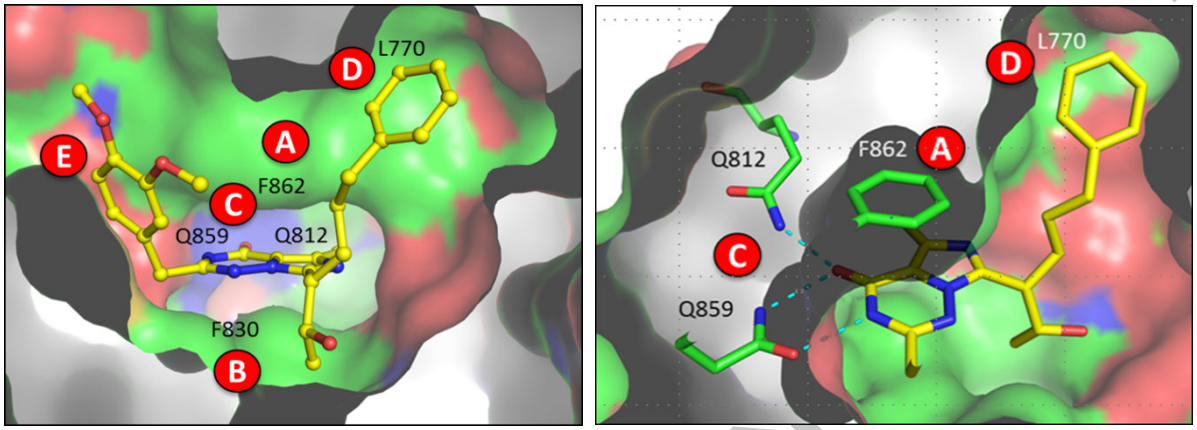
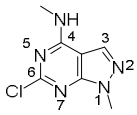


Figure 2. Two views of crystal structure with reference compound BAY60-7550, exemplifying key interactions with the active site. (4HTX PDB)



1

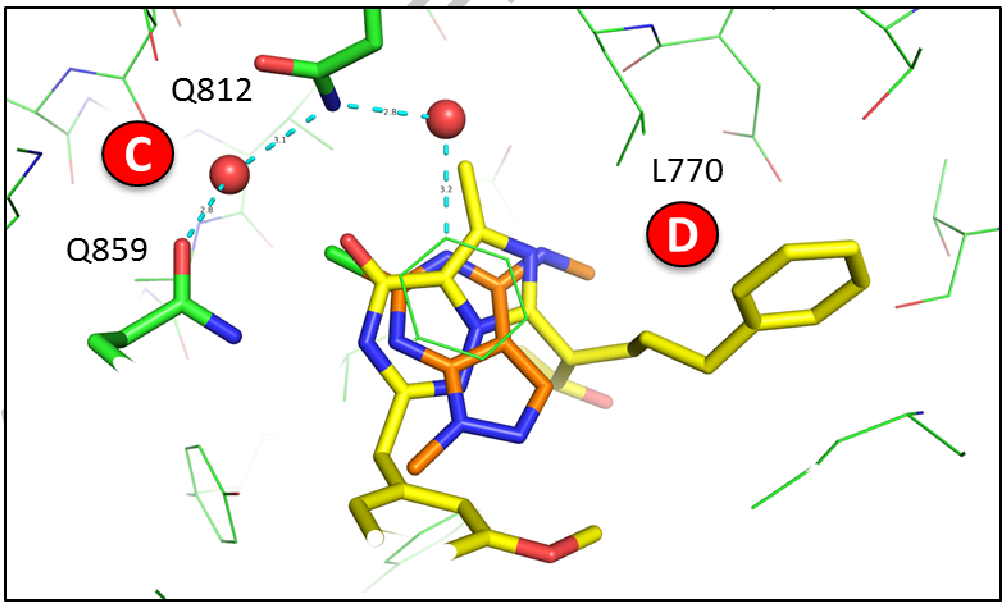
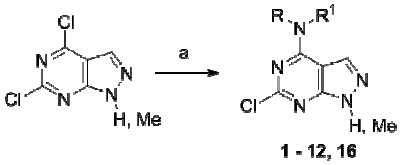


Figure 3. Crystal structure overlay of fragment **1** (orange) with BAY60-6550 (yellow). (6B96 PDB, 4HTX PDB)

Similar to BAY60-6550, the ligand bound PDE2 crystal structure of **1** shows the pyrazolopyrimidine ring anchored in the cAMP binding pocket through a π-stacking interaction with F862 and F830. The structure also showed compound **1**

has a water-mediated hydrogen bond to residue Q812 but does not directly or indirectly interact with Q859 (**C**) (Fig. 3). Examination of the X-ray overlay of **1** and BAY60-6550 also indicated a potential for extending from the aminomethyl moiety into the unoccupied hydrophobic pocket (**D**). To investigate this possibility, a *para*-chloro benzyl group was added (**2**). This modification resulted in a 3-fold increase in potency in support of this hypothesis (Table 1). Based on this result, further SAR development of the fragment hit **1** was explored. An efficient route was devised for rapid analogues of the 4-position to investigate binding from the commercially available 4,6-dichloropyrazolopyrimidines (Scheme 1) Primary and secondary amines were introduced via S_NAR reaction to afford compounds **1** – **12** and **16.** Modifications to the Cl substitution pattern of the R^1 group identified the *para* substitution (**2**) as optimal. Further SAR investigations demonstrated that the *p*-trifluoromethyl substituent (**5**), afforded an additional 3-fold improvement in potency over **2** with comparable LBE, LLE and solubility. Incorporation of the 3-pyridyl moiety (**6**), by analogy to PF-05180999, was less tolerated and led to a 7-fold decrease in potency compared to **5** with concomitantly reduced LBE. To explore the depth of the induced binding pocket, homologation of the linker to a more flexible phenethyl derivative (**7**) resulted in a decrease in potency and significant reduction in LLE.



Scheme 1. (a) RR^1NH, TEA, THF, 50°C

Molecular modeling suggested the trajectory of the aromatic ring to the PDE2 hydrophobic induced binding pocket (**D**) could be improved through structural modifications to the benzylic methylene. Incorporation of the gem-dimethyl substitution as in compound **8** resulted in a 10-fold boost in potency over compound **2**. Further optimization identified the (*R*)-stereoisomer of the mono-methyl analog **9** as the preferred enantiomer, affording a 30-fold improvement in PDE2 inhibitory activity over **2**, while the (*S*)-stereoisomer was equipotent to **2**. With this boost in potency, compound **9** shows enhanced LBE and LLE values despite an increasing AlogP observed with the incorporation of the CF_3 group.

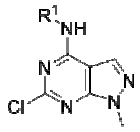
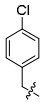
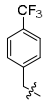
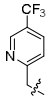
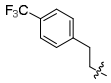
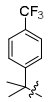
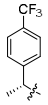


Table 1. Benzylic Optimization

Compound	R^1	PDE2 K_i (μM)	LBE/LLE	Solubility pH6.5 (μM)	AlogP98
1	Me	22.4	0.49/4.48	206	1.16
2		6.90	0.35/1.75	145	3.41
3		7.81	0.35/1.70	78	3.41

4		11.7	0.34/1.52	44	3.41
5		2.34	0.34/1.94	164	3.69
6		15.0	0.29/2.07	176	2.75
7		10.1	0.28/0.99	150	4.01
8		0.642	0.34/1.92	183	4.27
9		0.237	0.38/2.56	159	4.07

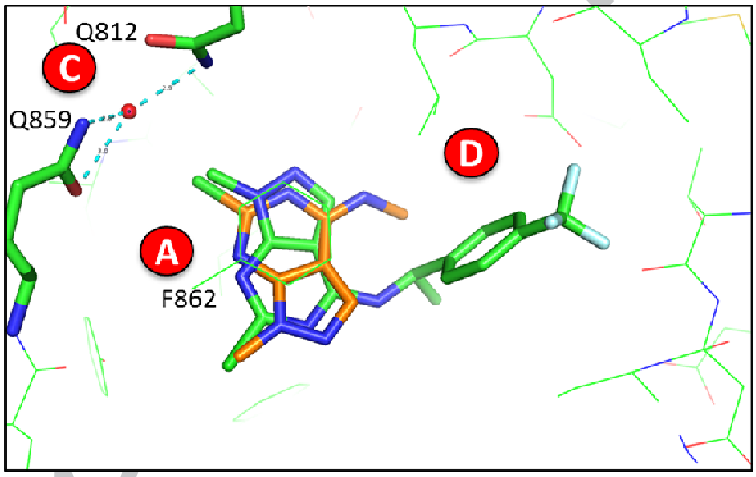


Figure 4. Crystal structure overlay of inhibitor **9** (green) with fragment **1** (orange)**.** The heterocycle of inhibitor **9** has flipped in the hydrophobic clamp relative to **1** and does not interact with either Q859 or Q812. (6B96 PDB, 6B97 PDB)

Having succeeded in identifying a sub-micromolar inhibitor, the co-crystal structure was solved in the PDE2 active site to confirm the design hypothesis. Quite surprisingly, **9** was found to bind in a 180° orientation relative to fragment hit **1** (Fig. 4). These types of observations are common in fragment design, supported by reports that compounds derived from fragments often do not maintain the original binding mode,[14] underscoring the importance of X-ray crystallography for structural confirmation and compound design in fragment advancement. In the new structure orientation, while the π-stacking interaction with F862 and F830 are maintained and the hydrophobic pocket (**D**) is occupied, there is a lack of any

interaction between **9** and Q859 and Q812, (**C**) yielding a non-selective inhibitor (Fig. 4, Table 4). While it is speculated that the interactions in the hydrophobic induced binding pocket contribute to PDE2 inhibitory potency,[10] it is a combination of occupying this pocket and interactions with the glutamine residues that are necessary for PDE isoform selectivity. Therefore, it was hypothesized that the selectivity profile for **9** could be improved by reestablishing these key hydrogen bonds along with further optimization of binding in the hydrophobic pocket.

New molecular modeling predictions based on the ring flip suggested that optimization of the trajectory might be achieved through incorporation of either a cyclic amine (**10** and **11**)[21] or a benzylic cyclopropane (**12**) (Table 2). Cyclized derivatives **10** and **11**, while potent, possess suboptimal LLE's due to the significant increases in their AlogP values. The cyclopropane derivative **12** was found to be superior to **9**, affording a 50-fold improvement in potency while maintaining the favorable LBE, LLE and solubility of fragment hit **1**. Based on crystal structures of **9** and **12**, conformational analysis using Freeform[22] suggested that the bioactive conformation of **12** is more stable than that of **9** relative to their global minimum. The difference is largely due to the preferred torsion angle between cyclopropyl and distal phenyl ring in **12** and supports the observed potency enhancement seen with this analog. The PDE isoform selectivity profile of **12** was also now significantly improved over **9,** likely an effect of the optimized interactions in the hydrophobic induced binding pocket (Table 4).

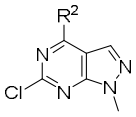
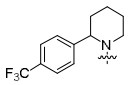
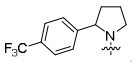
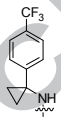
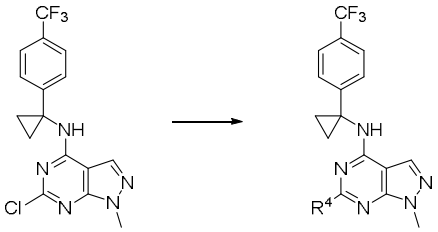


Table 2. Hydrophobic Pocket Optimization

Compound	R^2	PDE2 K_i (μM)	LBE/LLE	Solubility (μM) pH6.5	AlogP98
10		0.152	0.35/1.83	138	4.99
11		0.0972	0.37/2.48	139	4.53
12		0.00430	0.46/4.33	156	4.03

While we were able to realize large increases in potency, these were driven mainly by lipophilic interactions as noted by low LLEs, hence increases in compound AlogP were observed (Table 1, 2). Because high AlogP values have been linked to compound attrition in the clinic,[16-18] we sought to modulate the lipophilic interactions through increasing compound polarity. Two areas amenable for structural modifications not yet explored were the 6-chloro and 1-methyl substituents. Modifications to the 6-position of the pyrazolopyrimidines were derived from compound **12** and are described in Scheme 2. Replacement of the 6-chloro substituent with a hydroxyl (**13**) or a cyclopropane (**14**) modestly reduced the AlogP which led to an overall decrease in LLE compared to **12**. Their decrease in potencies however, led to reduced PDE-selectivity profiles (Table 4). Conversely, the 1,6-dimethyl (**15**) and the 6-chloro-1-des-methyl (**16**) analogs

displayed reduced AlogP values, which afforded improved LLE's and their enhanced potencies contributed to their respectable PDE off-target selectivity profiles (Table 4).



12 **13 - 15**

Scheme 2. (**13**, R^4 = OH) 1M NaOH, Dioxane, 150 °C (**14**, R^4 = cyclopropyl) $CyPrB(OH)_2$, K_3PO_4, $Pd(dppf)Cl_2$, 95:5 Toluene:H_2O, 100 °C (**15**, R^4 = Me) KBF_3Me, Cs_2CO_3, $Pd(dppf)Cl_2$, 10:1 THF:H_2O, 160 °C

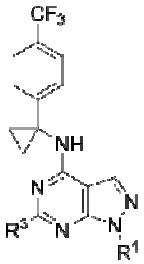


Table 3. Physical Property Optimization

Compound	R^3	R^4	PDE2 K_i (μM)	LBE/LLE	Solubility (μM) pH6.5	AlogP98
13[23]	OH	Me	0.195	0.37/3.25	175	3.46
14		Me	0.0196	0.36/3.25	125	3.89
15	Me	Me	0.00549	0.45/5.12	110[24]	3.14
16	Cl	H	0.0143	0.45/4.02	105	3.82

Table 4. PDE Isoform Selectivity

Compound	PDE Selectivity Ratios[i]
9	1 3 4 5 6 7 8 9 10 11
12	1 3 4 5 6 7 8 9 10 11
13	1 3 4 5 6 7 8 9 10 11
14	1 3 4 5 6 7 8 9 10 11

15	
16	

i. Selectivity ratio = (PDE*x* K_i)/ (PDE2 K_i) Red = 0 – 100-fold selective for PDE2, Orange = 101 – 500-fold selective for PDE2, Green > 500-fold selective for PDE2

Due to their promising activity, selectivity profiles and physical properties, lead molecules **15** and **16** were evaluated for their pharmacokinetic properties (Table 5). While compound **15** displayed sub-optimal PK, compound **16**, lacking both metabolically labile methyl groups, showed a robust profile exemplified by high bioavailability and low *in vivo* clearance. Consistent with the properties of the pyrazolopyrimidine class, compounds **15** and **16** demonstrated good cell permeability and were not substrates for Pgp efflux, and are therefore anticipated to be highly brain penetrant.

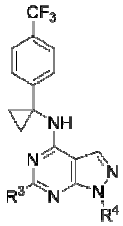


Table 5. Rat Pharmokinetics Data

Compound	R^3	R^4	PDE2 K_i (nM)	Solubility (µM) pH6.5	Cl (mL/min/kg)	AUC_N µM*h*kg/mg	V_d (L/kg)	$t_{½}$ (h)	%F	Rat PPB%	Rat Pgp/Papp $(10^{-6}m/s)^{ii}$
15	Me	Me	5.49	110[24]	44.35	0.24	3.78	1.34	16.9	92.6	0.82/26.7
16	Cl	H	14.3	105	8.44	6.59	4.64	6.33	146	97.4	1.63/26.5

ii. Rat LLC-MDR1a cells

Based on the promising overall properties of compound **16**, a crystal structure of the inhibitor was solved (Fig. 5). As designed, the benzylic amine reaches into the hydrophobic induced binding pocket (**D**) and the heterocycle core π-stacks between F862 (**A**) and F830 (**B**). Compound **16** differentiates itself from other inhibitors through the extensive water network formed with the 1- and 7-nitrogens of the inhibitor with both Q812 and Q859 (**C**). Further investigations into improving upon the selectivity profile while balancing the interactions in the hydrophobic pocket of the series will be the topic of future publications.

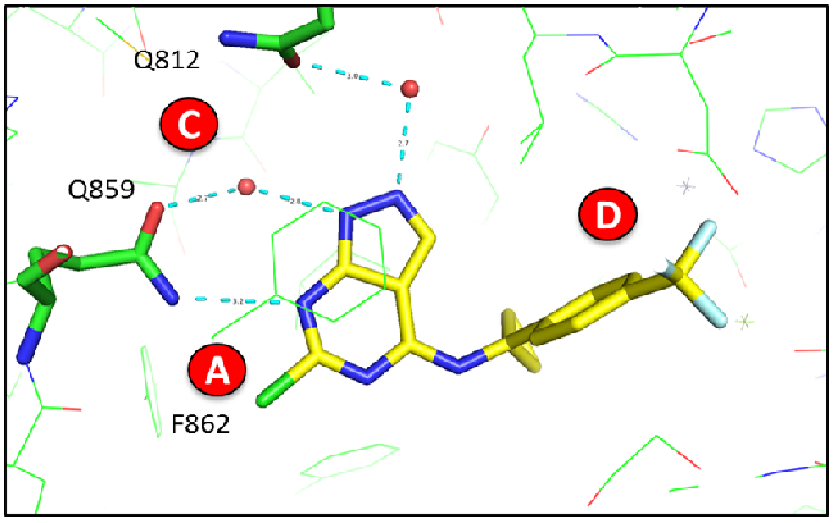


Figure 5. Crystal structure of optimized inhibitor **16.** (6B98 PDB)

In conclusion, with the aid of molecular modeling and X-ray crystallography, fragment **1** was rapidly developed into a viable starting point for a lead optimization chemistry effort. Potency and selectivity were achieved through a small number of analogs (25 singles) by accessing three key interactions with the PDE2 enzyme (**A/B**, **C, D**). It has been demonstrated that X-ray confirmation of structures is important for fragment optimization. In advancing fragment **1**, a large improvement in potency was achieved (>5000-fold, compound **12**), while maintaining favorable LLE, LBE, solubility and AlogP values, as shown with compounds **12**, **15** and **16**. In addition, compound **16** has shown good oral pharmacokinetics in rat, further validating the series as a good starting point for lead optimization efforts. Subsequent efforts to further optimize this structural class will be the subject of future publications.

Acknowlegements: This work was funded by Merck & Co., Inc., Kenilworth, NJ USA. The authors thank the West Point NMR group for structure elucidation support and Jessica Frie for helpful reviews and insights.

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24. A predicted solubility value was used as solubility at this pH was unable to be measured. Predictive solubility values for this series trend accurately with observed data