Design, Synthesis, and *In Vitro* Testing of Novel COX-2/COX-1 Inhibitors

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PURPOSE

To design, synthesize, and test new lead molecules that inhibit both COX-1 and COX-2 forms of the cyclooxygenase (COX) enzyme, with higher affinity for COX-2 than for COX-1.

METHODOLOGY

We used the ADMET Design Suite™ (ADMET Predictor™, MedChem Studio™, and MedChem Designer™) and GastroPlus™ software programs [1] with public-domain data [2-19].

Starting with ~550 COX-1 and COX-2 inhibitors, we generated structure-activity relationships (SAR) and identified activity cliffs using MedChem Studio.

Using the ADMET Modeler™ portion of ADMET Predictor, we trained artificial neural network ensemble (ANNE) classification and regression models to predict inhibition and selectivity.

Novel scaffolds were then generated using *de novo* design tools in MedChem Studio. Nine virtual libraries based on these scaffolds were created. *In silico* screening was performed based on predicted activities and ADMET Risk™ scores. GastroPlus™ simulations were run to determine likely dosing regimens that would achieve target plasma concentrations.

Eight compounds were selected to be synthesized and tested for COX-1 and COX-2 inhibition, and for selected physicochemical and biopharmaceutical properties. Only four were successfully synthesized.

Synthesis was performed by the Southern Research Institute (Birmingham, AL) and testing was performed by Eurofins CEREP (Seattle, WA) and Absorption Systems, Ltd. (Exton, PA).

RESULTS

All four lead molecules designed with the ADMET Design Suite™ inhibited both COX-1 and COX-2. One molecule showed about 6-fold greater affinity for COX-2 than COX-1. In addition, measured physical properties were in reasonable agreement with the predicted values used to filter out undesirable candidate compounds.

DISCUSSION

In this case, the challenge was to design high-quality lead molecules that would bind to *two* targets, with a goal of higher binding affinity for COX-2 than for COX-1. This ratio was indicated to be desirable to maintain a proper balance of prostaglandins for vascular health [20].

Activity Models: Figure 1 shows several "activity cliffs" within the public domain data set. Figure 2 shows the classification model performance for COX-2 pIC50 using 3 neurons and 47 descriptors. Figure 3 shows the performance of the ANNE regression model we obtained for COX-2 pIC50 (-log IC50). This model employed 6 neurons and 26 descriptors.

Figure 4 shows the regression model performance for COX-1 pIC50 using 5 neurons and 18 descriptors. Figure 5 shows the performance of a selectivity model using 7 neurons and 25 descriptors.

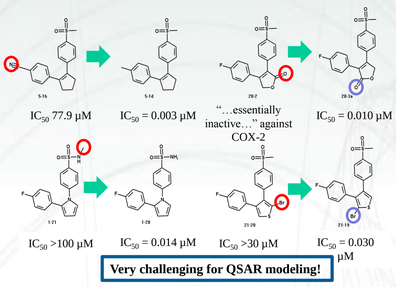
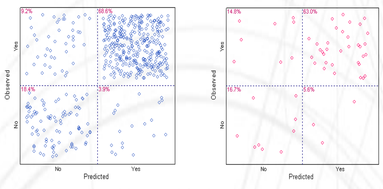


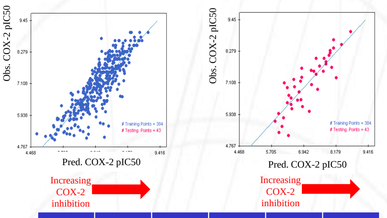
Fig 1. A few COX-2 Activity cliffs



(COX-2 inhibitor was defined as COX-2 IC_{50} < 10 µM)

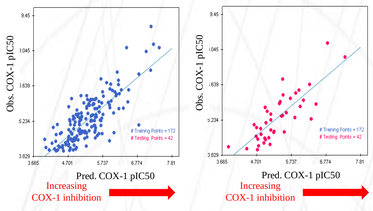
Set	No. of Cmpds.	Spec	Sens	Conc
Train/Ver	490	0.83	0.88	0.87
Test	54	0.75	0.81	0.80

Fig 2. COX-2 ANNE classification model built on IC50 data for 544 molecules



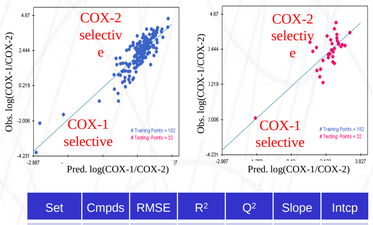
Set	No. Cmpds	RMSE	R^2	Slope	Intcp
Train/Ver	384	0.493	0.746	1.070	-0.474
Test	43	0.485	0.741	1.090	-0.614

Fig 3. COX-2 ANNE regression model built on IC50 data for 427 molecules



Set	Cmpds	RMSE	R^2	Q^2	Slope	Intcp
Train/Ver	172	0.571	0.652	0.682	1.140	-0.755
Test	42	0.561	0.662	0.651	1.150	-0.829

Fig 4. COX-1 ANNE regression model built on IC50 data for 214 molecules



Set	Cmpds	RMSE	R^2	Q^2	Slope	Intcp
Train/Ver	182	0.543	0.792	0.687	1.200	-0.440
Test	32	0.746	0.567	0.564	1.080	-0.176

Fig 5. COX-1 IC_{50}/COX-2 IC_{50} Selectivity

Molecule Design: Scaffold-hopping techniques in MedChem Studio were used to generate novel templates. Ideas containing an sp3 hybridized atom connecting an aromatic sidechain were eliminated because they would incorrectly position the substituents. Nine virtual libraries, each containing 240 new molecules (2160 total) were constructed in MedChem Studio. The sidechains of the libraries were obtained from known COX-2 inhibitors with IC50 < 100 nM. This helped to ensure synthetic feasibility of our candidate molecules.

Screening: Almost 2,000 molecules were removed based on poor predicted activities for COX-2 and COX-1. The remaining molecules were screened using their ADMET Risk scores, which account for a range of predicted physicochemical, biopharmaceutical, and toxicity properties. ADMET Risk is a proprietary scoring system within ADMET Predictor.

GastroPlus™ simulations were then run to assess likely dosing regimens to achieve target plasma concentrations. Finally, eight molecules were selected for synthesis quotes. Four molecules were successfully synthesized from three different scaffolds.

Table 1 lists some of the filtering criteria for eliminating molecules. Table 2 shows the activity predictions and measured activities for the four new molecules. Table 3 shows predicted and measured solubilities and logP values.

Table 1. Filtering Criteria for New Molecules

Filter Criteria	Removed	Remaining
None		2,160
Remove OOS* COX-2 classification model	52	2,108
Remove COX-2 classification=No	700	1,408
Remove OOS COX-1 regression model	87	1,321
Remove OOS COX-2 regression model	140	1,181
Remove COX-1 predicted < 1 µM	248	933
Remove COX-1/COX-2 > 100	691	242

*OOS: out of scope of model

Table 2. Predicted and experimental activities

	Predicted			Experimental		
Name	COX-1 IC_{50} [µM]	COX-2* IC_{50} [µM]	Selectivity Model	COX-1 IC_{50} [µM]	COX-2 IC_{50} [µM]	Selectivity
SLP0012	2.32	0.012	30	23.00	11.00	2.1
SLP0016	1.763	0.017	18	0.51	2.10	0.2
SLP0016_A	0.617	0.054	86	0.14	0.26	0.5
SLP0020	5.601	0.026	34	0.77	0.13	5.9

Table 3. Predicted and experimental solubility and logP for the four new molecules

Name	Predicted Solubility [µM]	Experimental Solubility [µM]	Predicted logP	Experimental logP
SLP0012	5.1	5.8	2.9	2.2
SLP0016	6.6	2.0	2.5	2.0
SLP0016_A	7.1	23.6	2.6	2.1
SLP0020	20.9	9.0	3.5	3.6

CONCLUSIONS

The ADMET Design Suite is a powerful molecule design system for generating high-quality lead molecules meeting both activity and ADMET property requirements.

When sufficient experimental activity data exist, the design and screening of new molecules can often be accomplished using only 2D structures in a fraction of the time and cost typically required in the pharmaceutical industry.

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