Computational Drug Discovery

arget prediction, mode-of-action elucidation, virtual screening, **molecular structure genera**



Quick intro on myself

- MSc in biopharmaceutical sciences (2007)
- PhD in Chem/Bioinformatics (2007 2012)
- Computational chemistry
- Medicinal Chemistry in Leiden
- Personal Grant from Janssen



Quick intro on myself

- MSc in biopharmaceutical sciences (2007)
- PhD in Chem/Bioinformatics (2007 2012)
- Postdoc at European Bioinformatics Institute (2012 2015)
- ChEMBL group
- Marie Curie Fellowship



Quick intro on myself

- MSc in biopharmaceutical sciences (2007)
- PhD in Chem/Bioinformatics (2007 2012)
- Postdoc at European Bioinformatics Institute (2012 2015)
- Assistant Professor in Leiden (2015 ...)
- LACDR & LIACS
- Drug Discovery and Safety
- Data Driven Drug Discovery network (D4N, www.d4n-leiden.nl)
- Center for Computational Life Sciences (www.ccls-leiden.nl, with Dr. Emmerich)

LACDR LIACS

g Discovery and Safety



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Synthesis, Pharmacological Characterization, and Docking Analysis of a Novel Family of Diarylisoxazoles as Highly Selective Cyclooxygenase-1 (COX-1) Inhibitors

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13 Supporting Information

ABSTRACT: 3-(5-Chlorofuran-2-yl)-5-methyl-4-phenylisoxazole (P6), a known selective cyclooxygenase-1 (COX-1) inhibitor, was used to design a new series of 3,4-diarylisoxazoles in order to improve its biochemical COX-1 selectivity and antiplatelet efficacy. Structure-activity relationships were studied using human whole blood assays for COX-1 and COX-2 inhibition in vitro, and results showed that the simultaneous presence of 5-methyl (or -CF3), 4-phenyl, and S-chloro(-bromo or -methyl)furan-2-yl groups on the isoxazole core was essential for their selectivity toward COX-1. 3g, 3s, 3d were potent and selective COX-1 inhibitors that affected platelet aggregation in vitro through the inhibition of COX-I-dependent thromboxane (TX) A2. Moreover, we characterized their kinetics of COX-1 inhibition. 3g, 3s, and 3d were more potent inhibitors of platelet COX-1 and aggregation than P6 (named 6) for their tighter binding to the enzyme. The pharmacological results were supported by docking simulations. The oral administration of 3d to mice translated into preferential inhibition of platelet-derived TXA2 over protective vascular-derived prostacyclin (PGI2).



INTRODUCTION

Cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) catalyze the first step of the biosynthesis of prostanoids from arachidonic acid (AA).1 Different from COX-2 gene that is mainly inducible, COX-I is a housekeeping gene constitutively expressed in almost all mammalian tissues and cells.¹ However, the expression of COX-1 can be regulated in some circumstances, such as during development.² In physiological conditions, COX-1 is highly expressed in the gastrointestinal (GI) tract and platelets,^{3,4} where it is involved in the generation of cytoprotective prostaglandin (PG)E, and platelet proaggregatory thromboxane (TX) A2, respectively.

COX-1 plays a role in several pathological conditions such as thrombosis, atherosclerosis, and tumorigenesis.5-10 Importantly, platelet COX-1 is the target of one of the most efficacious antithrombotic agents used for prevention of vascular occlusive events, i.e., aspirin.

Aspirin irreversibly acetylates Ser529 and Ser516 of COX-1 and COX-2, respectively,12 leading to irreversible enzyme inactivation. Because of pharmacokinetics features (i.e., short half-life of 20 min) and pharmacodynamics features (higher

while they cause only a marginal and transient inhibitory effect on COX-2 and extra-platelet cellular COX-1. Low doses of aspirin cause an almost complete suppression (≥95%) of platelet TXA₂ generation ex vivo, persisting throughout the dosing interval (i.e., 24 h).11.13 This is a fundamental requisite to obtain an antiplatelet effect,14 since even tiny concentrations of TXA, may activate platelets and importantly they synervize with other platelet agonists.15 The antiplatelet effect of aspirin is strictly related to platelet turnover.11,12 Thus, enhanced platelet turnover rate detected in some cardiovascular (CV) conditions, such as diabetes, might decrease the efficacy of the drug to halt almost completely platelet TXA2 generation.16 Moreover, we have recently shown that platelets contain COX-1 mRNA and the enzymatic machinery for protein synthesis; thus, these anucleated megacaryocyte fragments are able to synthesize de novo COX-1 in response to platelet activation in vitro.17 Thus,

potency to inhibit COX-1 than COX-2), low doses of aspirin (75-100 mg once daily) act by affecting platelet COX-1 activity

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and a second sec	
5H), 7.39 (dd, 1H), 7.58–7.80 (m, 4H), 8.82 (s, 2H), 9.04 (s, 2H), Anal. (C ₂₇ H ₂₀ N ₂ O ₂ ·HCI-0.5 H ₂ O) C, H, N, CI.	fragment residues 55–65. ²⁰ Cocrystals were generated by soaking crystals with mother liquer containing 1 mM inhibitor
3-[(4-Methylamino-3-nitro-benzoyl)pyridin-2-yl-amino]-	Data were collected on a MAR Research imaging plate (X-ray
propionic Acid Ethyl Ester (29d). Starting from 4-methy-	Research, Hamburg, Cermany) mounted on a fogatu R020
lamine-3 nitre-benzoic acid and 3 (pyridin-2-ylamino)propienic acid atbol ester, the title compound was preserved following	HKL ²¹ Model building and rafinement were carried out with
the procedure described for the synthesis of intermediate 29a;	MAIN ²² and CNS. ²³ The X-ray structures of compounds 2 are
mp 86-88 °C. H NMR (DMSO-di): & 1.11 (t, 3H), 2.65 (t, 2H),	24 were deposited in the Brookhaven data bank. The accession
2.90 (d, 3H), 3.97 (q, 2H), 4.18 (t, 2H), 6.82 (d, 1H), 7.08 (d,	minibers are IK11 and IK15, respectively. Measurement of Theoretics Jobibition. The elements
110), 7.21 (m, 110, 7.31 (dd, 110), 7.70 (dt, 110), 7.92 (d, 110), 9.27 (s. 110), 9.44 (dd, 110), Appl. (C. H. N.O.), C. H. N.	inhibitory effects (ICa) of the commands were determined
2.02.10. Carbaminideal obenvlamino)mothell. Length	with a commercially available chromogenic assay (Roche
yl-1H-benzoimidazole-5-carbonyl]pyridia-2-yl-amino)-	Mannheim, Germany). Human thrembin (Roche) (0.042 UlanL
propionic Acid Ethyl Ester Hydrochloride (30). Com-	was preincubated for 10 min at 37 °C with 10 different
pound 29d (16.7 g, 44.7 mmol) was hydrogenated (Pd on	compaunds dissalved in DMSO or with DMSO as control. Upon
enarceal, 10A) in 200 mL or methanol at room temperature to afford 3.1(2 amine 4 methodamine herosoftesytelin. 2 st amine).	addition of the preincubation mixture to the chromogenia
propionic acid ethyl ester (10.0 g, 65%) after purification via	substrate, tosyl-glycyl-prelyl-arginine-4-nitranilide acetate, ni
chromatography (silica gol, dichloromethans/methanol 30:1).	traniline is cleaved by thrombin and the increase in absorbance
This intermediate (2.1 g, 6.1 mmol) was added to a solution of	at 400 mill, related to the new intramine, is magaree in a meetrophotometer (Spactrumax, Melecular Devices, Summe
(4-cyano-phenylamino) acetic acid unidazelide (7.3 mmel) in 50 red. of THE and refluend for 24 b. The reaction mixture was	vale, CA). By plotting the absorbance at 405 rm vs the
then concentrated in vacuo, and the residue was disselved in	concentration of the test compound, the concentration that
30 mL of glacial acid and heated under reflux for 1 h. The	induced a 50% thrombin inhibition (IC ₅₀) was calculated. Al
solution was diluted with 100 mL of water and neutralized	values of both determinations are represented.
wen concernated ammonium hydroxone. Extraction with edityl acotate and purification via chromatography (silica sel, dichio,	Measurement of the aPTT. aPTT was measured in a
romethane/methanel 40:1) gave 3-([2-[(4-cyano-phenylamino)-	coagulometer (Biomatic B10, Sarstedt, Cermany) using the
methyl]-1-methyl-1H/benzeimidazole-5-carbenyl]pyridin-2-yl-	PTT reagent of Boehringer, Mannheim, Cormany, according
amine)propionic acid ethyl ester (1.8 g. 61%), which was	coagulant effect of the respective commound. Bloodsamake
Pinner reaction described above vield: 1.4 a 71% LPMS	were collected in sodium citrate solution (final concentration
$(EKA); (M + H)^{+} = 500, (M + H + Na)^{2+} = 261.8, (M + 2H)^{2+}$	0.313%). Each native blood sample (0.1 mL) was pipetted into
= 250.8. ³ H NMR (DMSO-ok): 3 1.12 (t. 3H), 2.68 (t. 2H), 3.77	a test tabe prevaried to 37 °C. The PTT reagent (0.1 mL
(s, 316), 3.97 (q, 214), 4.22 (t, 214), 4.86 (d, 214), 6.88 (m, 314),	chloride solution (0.1 mL), prewarmed to 37 °C, was added in
7.13 (m, 210, 7.30-7.00 (m, 411), 7.05 (t, 210, 8.40 (m, 110), 8.67 (s, 210, 890 (s, 21))	order to activate the congulation cascade, and the time (aPTT
3.(12.1(4.Carbamimidev4.ohenvlamino)methyll.1.meth.	in seconds) was determined that elapsed from the addition o
yl-114-benzoimidazole-5-carbonyl]pyridia-2-yl-amino)-	calcium chioride to the onset of clotting.
propionic Acid (24). Compound 30 (1.0 g. 1.86 mmol) was	Acknowledgment. We thank Ingrid Christ, Jose
added to a solution of solution hydroxide (0.24 g, 6.0 minot) in	Eiband, Herbert Fischbach, Monika Kink-Eiband, Verr
temperature for 2 h. The mixture was then diluted with 60	Koch, Michael Koehler, Angela Schmid, Johanna Schur
mL of water and neutralized with acetic acid. The precipitate	er, and Elisabeth waldhlann for their skullul technica
was isolated and washed with water and other to afford the	assistance.
276-277 °C 1 DMS (TEA): (M + U)* = 472 (M + U + N a)**	Supporting Information Available: X-ray crystallogra
= 247.6, (M + 2H) ²⁺ = 236.7, (M + 2Na) ²⁺ = 258.6. ¹ H NMR	phy of complexes of compounds 2 and 24 with human a throm him data collection and referenced statistics. Combustion
(DMSO-dg + ² HCh: 8 2.87 (t, 2H), 4.01 (s, 3H), 4.17 (t, 2H),	analysis of all new compounds. This material is available free
5.07 (s, 2H), 6.97 (d, 2H), 7.28-7.40 (m, 2H), 7.45 (dd, 1H),	of charge via the Internet at http://pubs.acs.org.
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(31). Compound au (1.0 g, 1.66 mmol) was dissolved in 50 mL of THF and 10 mL of water. Potansium carborate i0.83 a 6.0	Fuster, V. Guide to Anticoaguiant Therapy, Fart 2 Ora
mmol) was added, and the mixture was stirred at room	(2) In Fritman R. L. Kiman, S.: Lindbratt, S.: Baur, M.: Bad.
temperature for 15 min. Then, n heavyl chloroformate (0.21 g.	D.: Torticim, C.: Kasiebo, P.: Close, P. Provertion of thromboon
1.86 ramol) was added and stirring was continued for another	point with use if recombinant hirudin-Results of a double blind, multicanter trial comparing the efficace of desirudia
sodium sulfate, and concentrated in varue. The residue was	(Fevasc) with that of untractionated heparin in patients having
chromatographed (silica gel, dichloromethane/methanel 19:1)	a total hip replacement. J. Bane Jr. Surg. Am. Vol. 1007, 700 Nile TID. D. Britman, B. L. Wile, Israeman, P. Kaskin, P.
to afford the title compound as colorises crystals; mp 128-	Mearet, P.; Rosercher, N.; Beesch, P.; Baar, M.; Elonan, S.
129 °C; 0.61 g, 51%. LRMS (EKA): (M + H) ² = 628, (M + H	Lindbratt, S.; Close, P. A comparison of recombinant hirudia with law protocolog and the bosonic to promote the anti-
+ (Na)** = 320.7, (M + 2H)*** = 314.7, 'H NMR (DMSO-dg); 8 0.86 it 2H) 1.12 it 2H) 1.29 im 6H) 1.58 im 2H) 2.68 it	complications after total hip replayment. N. Bogl. J. Med 1993
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Exploring 'Drug-Like' Chemical Space



Challenges

Target description for side-effect prediction

- Bias in the data (ligand size, explored proteins, etc..)
 - Models will display tendency to regress to the mean for extremes
- Errors in the data (experimental errors and annotation errors)
- Sparseness of the data
 - Typical coverage is 2%...
- Synthesizability
 - Can molecules suggested by the computer be generated?

The data is out there ..

Machine Learning in Drug Discovery

Off-target prediction, mode-of-action elucidation, virtual screening, molecular structure



Storage is cheap*, computing power is cheap*, measurements are easy** .. (and cheap*)..

....scientific data is becoming open / public....

...every research project can profit from data / analytic

rely compared to historic prices Id also be taken with a grain of salt and is relative to history...