

Compound screening strategies for polyglutamine diseases

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Overview

- I Brief introduction to PolyQ diseases
- II Compound screen for SCA2 (HTS introduced)
- III Considerations on HTS Design
- IV Review of HTS studies for polyQ diseases(Putative drugs for HD resulting from HTS)
- V Informatics (software links)
- VI Accessing the MLPCN

Polyglutamine Diseases

	MIM	Gene	Normal repeat	Expanded		
Disease	number	product	length	repeat length	Main clinical features	
HD	143100	Huntingtin	6–34	36-121	Chorea, dystonia, cognitive deficits, psychiatric problems	
SCA1	164400	Ataxin1	6–44 ^b	39-82	Ataxia, slurred speech, spasticity, cognitive impairments	
SCA2	183090	Ataxin2	15–24	32-200	Ataxia, polyneuropathy, decreased reflexes, infantile variant with retinopathy	
SCA3	109150	Ataxin3	13-36	6184	Ataxia, parkinsonism, spasticity	
SCA6	183086	CACNA1 _A	4–19	10-33	Ataxia, dysarthria, nystagmus, tremors	
SCA7	164500	Ataxin7	4-35	37-306	Ataxia, blindness, cardiac failure in infantile form	
SCA17	607136	TBP	25-42	47-63	Ataxia, cognitive decline, seizures, and psychiatric problems	
SBMA	313200	Androgen receptor	9–36	38-62	Motor weakness, swallowing, gynecomastia, decreased fertility	
DRPLA	125370	Atrophin	7–34	49-88	Ataxia, seizures, choreoathetosis, dementia	

Table 2 Polyglutamine disordersa caused by a gain-of-function mechanism

From Orr & Zogby, Annu. Rev. Neurosci. 2007. 30:575–621

Huntington's Disease

Molecular features

67 exons 3142 aa protein (aka HTT) 345 kDa

Exon 1:

Encodes 17 aa followed by CAG repeat (Most HTS on HTT used this fragment)



Clinical features

Chorea, Anxiety, sleep disorder, depression, weight loss, social isolation, hypokinesia, rigidity.

Prevalence

5-10 per 100,000 Unevenly distributed 0.1 per 100,000 in Japan Founder effects

Parkinson's Disease & Movement Disorders, 5th Edition, Ch 18, Axelandra Durr

Spinocerebellar Ataxia Type 2 (SCA2)

- SCA2 is a polyglutamine disorder caused by *ATXN2* mutation.
- Gait ataxia, frontal executive dysfunction, slow saccades, and parkinsonism.
- Age of onset is characterized by anticipation where CAG22-23 is normal and CAG>32 causes disease.
- Characterized by Purkinje cell death.
- Gain of normal function (Duvick et al., Neuron 2010).

Ataxin 2 regulates mRNA, Ca²⁺ movement and endocytosis

RNA Binding Proteins

A2BP1/Fox 1	Shibata et al., HMG 9:1303-13; 2000
PABP1	Nonhoff et al., PNAS 98:4409-13; 2001
DDX6	Nonhoff et al., MBC 18:1385-96; 2007
TDP-43	Elden et al., Nature 466:1069-75; 2010

Endocytosis and EGFR Function

Endophilins Nonis et al., Cell Signal 20:1725-39; 2009

Calcium Movement

IP3R Liu et al., J Neurosci 29:9148-62;2009

Hypothesis

Reduction of ataxin-2 expression or mRNA stability provides a therapeutic avenue for SCA2.

- SCA2 phenotype is worse in patients homozygous for the disease allele (Ragothaman and Muthane, 2008).
- SCA2 phenotype is worse in homozygous vs heterozygous *ATXN2* transgenic mice (Huynh et al., 2000).
- *ATXN2* knockout mice are obese (Kiehl et al., 2006; Huynh et al., 2009).
- Reversibility of SCA1&3 transgenic mouse phenotype (Zu et al., 2004; Boy et al., 2009).
- shRNA injection in brains of ATXN1 mouse improved phenotype (Xia et al., 2004).

Pilot HTS to identify compounds inhibiting ATXN2

Assay	ATXN2 promoter-[firefly luciferase]-ATXN2 3'UTR		
Readout	Luminescence		
Library	60,000 compounds tested at 10 uM		
Format	384 well plate		
Detector	Luminometer		
Orthagonal	ATXN2 promoter-[Renilla luciferase]-ATXN2 3'UTR		
Counterscreen	CMV-[firefly luciferase]		
Pre-screening	Evaluation of the signal		
	Promoter Analysis		
Post-screening	Mouse Models		



Conducting a screen



One 384-well plate



Result

Total number of 384 well plates = 197 Total number of compounds = 65,122 Total hits(3SD down)=363 Hit rate=363/65,122 x 100 = 0.5%

Many compounds appearing as hits in our pilot HTS were luciferase inhibitors



With the completion of a pilot study we now will enter the MLPCN to conduct a larger quantitative high throughput screen (qHTS) of 300,000 compounds tested over 7 doses at the NCGC.

As discussed in the next two slides, data from the pilot screen led to identification of luciferase inhibitors useful for positive controls and evaluation of the assay dynamic range... Chembridge compound 5553825, a potent luciferase inhibitor was used with our assay as a positive control to calculate Z'-Factor (see next slide)



Z'-Factor Calculation

By assessing the Z'-Factor you gain an understanding on how suitable your assay is for compound screening.

This is one requirement of the NCGC and MLPCN



Measure of dynamic range Z'-Factor = $1-[(3(\sigma_{exp}+\sigma_{cont}))/|\mu_{exp}-\mu_{cont}|]$ Z'-Factor = 0.8

Z' <0, no assay window Z' >0.5, very good window Z' = 1 is upper limit

Zhang, J-H. et al. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* 4:67-73, 1999

ATXN2 promoter analysis to develop a set of *ATXN2*-luc plasmids with deletions throughout the expression control region.

Establishes tools for evaluating elements in the expression control region required for compound function.

pGL2-ATXN2-Luc ATXN2-Luciferase Expression Construct



ATXN2 promoter deletions and corresponding luciferase assays showed promoter activity was reduced with the region 41-324 was deleted. While this is not surprising since it included the start codon, we also found a region upstream of the start codon important for expression shown on the next slide.



A 65 bp region in the *ATXN2* promoter is critical for expression. Smaller deletion of this region is shown on the next slide...



Identification of a 14 bp promoter region just upstream of the ATXN2 start codon that is critical for expression





Transgenic mouse verifying the ATXN2 promoter fragment expresses in cerebellum

ATXN2-luc expression in mice







Imaging by IVIS (in vivo imaging system)



We verified that the very same promoter fragment used for HTS directs expression of *ATXN2-luc* in cerebellum, the pathogenic tissue in SCA2

Cerebellum localization



Overlay Transparent Overlay

Considerations on HTS Design

Mechanism of disease for the polyQ diseases

Gain of function (enhanced or new) Interacting proteins Calcium movement Transcription and Translation

Gain of toxicity Expanded polyglutamine Aggregates RNA toxicity (Li & Bonini, Trends Neurosci 33:292-8, 2010)

Loss of function

SCA1: Gain of normal function (Duvick et al., Neuron 2010).

Screen Components

Design screen

We chose a cell-based assay to find compounds inhibiting expression of *ATXN2-luc*. This was based on several deciding factors including biology of the disease and knowledge on *ATXN2* function

Prescreening tests

Effects of DMSO on readout Screening window (Z'-factor, S/B) (requires a positive control compound)

Primary Screen

Screen for compounds inhibiting ATXN2-Firefly luc expression

Orthogonal Screen

Test positive hit compounds for their ability to inhibit ATXN2-Renilla luc expression

Counterscreen

Test compounds for their inability to inhibit CMV-Firefly luc expression Test compounds for their ability to inhibit expression of endogenous mutant *ATXN2* Western blot or qPCR

Post-screen experimentation

Testing in a mouse model

Pharmacokinetics

Toxicity testing

SAR Chemistry (Structure Activity Relationship)

HTS

Definition

> 10,000 assays per day = HTS

> 100,000 assays per day = uHTS

qHTS = dosewise uHTS



Molecular cancer therapeutics: strategies for drug discovery and development By George C. Prendergast. 2004 John Wiley & Sons

Format of the screen

Format Relative Cost

Well-plate assay

Well by well (cell based) Fly by fly Fish by fish Mouse by mouse

Plate assays

shRNA screen

Low

Assay Target

Targeting Expression

When the disease-related function of the disease protein is unknown.

Objective: get rid of it...

Target total expression

 Screen for compounds reducing luciferase expression driven by the disease gene's promoter

Target expression of the disease allele

 Screen for reduction of the disease protein by TR-FRET or AlphaLISA

Targeting Function

Targeting function Abnormal function Aggregation (must know aggregation is bad) Known function Enzymatic activity (follow the post-translational modification...see below) Transcription factor binding Protein-protein interaction Calcium movement

Targeting post-translational modification

Proteolysis

Phosphorylation

Glycosylation

Ubiquitination Time Resolved FRET Robers et al., Anal Biochem. 372(2):189-97, 2008 SUMOylation AlphaLISA Eglen et al., Curr Chem Genomics 1:2-10, 2008 Time Resolved FRET Carlsen et al., Assay Drug Dev Technol. 7(4):348-55, 2009



Readouts

Expression Assay	Proximity Assay	Calcium Sensor	High Content
Luciferase GFP qPCR ELISA	FRET HTRF AlphaLISA	Fura-2 Cameleon	Confocal Imaging Expression Mislocalization Colocalization Morphology Aggregation Apoptosis Proliferation
	Proliferation		
	MTT DAPI		


Coelenterates



Renilla (Sea Pansy)



Periphylla (Jellyfish) Extremely bright, used at pH 1-11, can be boiled



Firefly





Proximity Assays

○ FRET

 \circ HTRF

○ AlphaLISA



Wavelength (nm)



2800 compounds screened at 4 ug/ml



Co-overexpressed in COS7



Y-27632 was previously characterized as an inhibitor of Rho activated kinase p160ROCK.

Y-27632 was able to reduce neurodegeneration of Drosophila expressing HTT with Q93

Pollitt, et al., Neuron 40:685–694, 2003

Time Resolved FRET using Rare Earth Elements (lanthanides)



http://www.htrf.com/technology/

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746475



Degorce et al., Current Chemical Genomics 3:22-32, 2009.

HTRF (homogeneous time resolved fluorography) allows collection of a uniform FRET signal after the decay of other background signals.



LanthaScreen by Invitrogen Time Resolved FRET using Terbium



Robers et al., Anal Biochem. 372(2):189-97, 2008

AlphaLISA by PerkinElmer Inc.

AlphaLISA proximity assay

Excited phthalocyanine → oxygen singlet 200 nm range "at least" (limited compared to FRET) Excited europium acceptors luminate at 615 nm



Poster presented at the 2010 Society for Neuroscience Annual Meeting in San Diego This presents an assay to specifically detect mutated HTT suitable for HTS.



Assay Principal, from the SFN poster shown in the previous slide.



In presence of mutated Htt, the two beads are brought into close proximity through specific antibody recognition of the target. Upon excitation of the sample at 680 nM, the Donor beads will produce singlet oxygen that will reach the Acceptor beads and be converted to a light emission at 615 nM.

The antibody shown in magenta detects in the 17 aa upstream region of HTT. The author of the poster said the antibody shown in yellow can be substituted by 1C2 detecting polyglutamines. The assay can be customized for most polyQ disease proteins.

HTS for polyglutamine diseases

There are 18 published drug discovery studies on polyglutamine diseases that utilized some form of high throughput screening

All are on HD except one

	HTS Objective							
	Reduce HTT Aggregation	Reduce HTT Cytotoxicity	Reduce HTT Expression	Enhance HTT Autophagy	Enhance HTT Aggregation			
Compounds	6	4*	2	1	1			
RNAi	2			1				
Peptides	1							

* One on AR

Compounds inhibiting aggregate formation

(Pos. control in many studies on aggregation)



Janek K, et al. Water-soluble beta-sheet models which self-assemble into fibrillar structures. Biochemistry. 1999 Jun 29;38(26):8246-52.

Congo Red

Showed that Congo Red and some other small molecules inhibited the self-assembly of proteins forming β-sheets; hypothesized Congo Red might prevent HTT aggregation.

Heiser et al. (Max-Planck) Inhibition of huntingtin fibrillogenesis by specific antibodies and small molecules: implications for Huntington's disease therapy. PNAS 97(12):6739-44; 2000.

 Showed that Congo Red could inhibit HTT aggregation using the assay in the following slide. In that study they sought other compounds due to poor drug likeliness for Congo Red (IC50 ~3 μM)



Benzothiazoles



Heiser et al., 2002.

Light-emitting component of luciferin found in fireflies.

Used in production of rubber and paper, wood preservation, leather processing. Found as pollutants in municipal waste water.

Screening Assay:

Validation Assay:

Wells with reduced protein amount were considered to contain toxic compounds.

Animal Study:

None yet, except that Riluzole could increase survival of HD mice in another study but appeared as a false positive in the screening assay due to cellular toxicity.

Conclusions:

These studies show promise for Riluzole for polyglutamine diseases since it is an FDA approved drug used for ALS.

Animal studies on the less toxic effective benzothiazoles are proposed (there were 8 in the study).



Trehalose (mycose) Tanaka et al., 2004.

First isolated from beetles, found also in plants and fungi Protects against desiccation A sugar with ~half the calories of table sugar

Used as a protective additive in foods and pharmaceutical formulations

Screening Assay:

Myoglobin-Q35 isolated in large quantity then distributed in 96 well-plates in buffer with compounds. Aggregates forming at 37°C monitored by OD550.

Screened >200 compounds ("LTS")

Validation Assay:

Trehalose increased viability of Neuro2a cells expressing 150Q-EGFP.

Animal Study:

R6/2 mice given 2% trehalose in drinking water:

Trehalose increased body wt., reduced striatal atrophy, reduced ub positive aggregates, improved rotorod performance, clasping and survival (Ns = 5 to 11)

Conclude trehalose may make the mut-HTT resistant to proteolysis and translocation

Clasping Test





Fosfosal Like aspirin but w/ a phosphate in place of acetyl group Levonordefrin Vasoconstrictor and metabolite of methyldopa Nadolol Beta blocker used for hypertension

Screening Assay:

AR-Q65-CFP/YFP → Transfect HEK293 Add 10 uM compound Read CFP-YFP FRET

Validation Assay:

Inhibition of aggregate formation in HTTQ92 inducible PC12 cells

Animal Study:

Reduction of neurodegeneration in a Dorsophila model

(expressing HTTQ93 with reduced rhabdomere numbers)

Conclusions:

Three FDA approved compounds potentially useful in clinical trials for HD Problems:

Effective doses were 5-50 μ M, 0.5 μ M for Nadolol. Medicinal chemistry may be needed.

Pollitt et al., 2003 2800 compounds Y-27632 Desai et al., 2006 4000 compounds Fosfosal Levonordefrin Nadolol **FGFR** inhibitor Caspase inhibitor

Quinazoline Anti-cancer and malaria agent.



None reported

Conclusions:

Four quinazolines with 5b being the best. With these they will conduct mechanistic chemistry to find the target of action.

C2-8

Zhang et al., 2005 David Housman & Aleksey Kazantsev, MIT

Screening Assay:

Yeast expressing Htt-103Q–EGFP were examined for aggregates. Only those with increasing OD600 (increased growth) were examined.

16,000 compounds (Chembridge) studied, 9 were selected.

Validation Assay:

4 compounds inhibited Htt-103Q–EGFP aggregation in PC12 and COS1 cells.

4 compounds inhibited HTT-Q150 aggregation in hippocampal slice cultures from R6/2 mice after 4 weeks.

Animal Study:

Drosophila HD model expressing HTT-Q93 were fed food containing

C2-8 and rhabdomere phenotype was assessed.

Conclusions:

Lead compound requiring medicinal chemistry









C2-8 R6/2 mouse study

Chopra et al. A small-molecule therapeutic lead for Huntington's disease: Preclinical pharmacology and efficacy of C2-8 in the R6/2 transgenic mouse. PNAS 104(42): 16685-89; 2007.

David Housman & Aleksey Kazantsev, MIT

Mice treated with C2-8 (50 mg/kg) for 5 days had 25 μ M C2-8 accumulated in cerebral cortex (gradient chromatography)

C2-8 reduced neuronal atrophy C2-8 treated mice had smaller nuclear aggregates Improved performance in wire hang and rotarod tests



Striatal cell body volume





One compound <u>supporting</u> aggregate formation

B2

Bodner et al., 2006 David Housman & Aleksey Kazantsev, MIT

Screening Assay:

Ecdysone inducible Htt-103Q–EGFP 14A2.6 cells (Drosophila) Screened 37,000 compounds (10 uM) Selected those for which total GFP fluorescence increased 5 compounds showed increases, B2 the highest

Validation Assay:

Proteasome function reporter system... B2 prevents huntingtinmediated proteasome dysfunction and reduces α -synucleinmediated toxicity.

Conclusions:

B2 lowers pathological consequences of HTT and a-synuclein while increasing inclusion formation, perhaps by lowering levels of toxic oligomers

Follow-up Study:

Palazzolo et al. B2 attenuates polyQ AR toxicity in cell and fly models of SBMA. Journal of Neuroscience Research 88:2207–2216 (2010)





Compounds inhibiting cytotoxicity (Neuroprotective) Nipecotic acid, Propafenone, Acivicin, Isoproteronol, and Mycophenolic acid

Wang et al., 2005 Christopher Ross, Johns Hopkins

Screening Assay: 1040 NIH compounds PC12 cells expressing HTT-Q148

Validation Assay:

LDH release

Immunofluorescent evaluation for aggregations, showing reduced aggregation.

Conclusions:

5 FDA approved compounds requiring pharmacokinetics

Cannabinoids

Aiken et al., 2004 Erik Schweitzer, UCLA

Screening Assay:

1040 NIH compounds PC12 cells expressing HTT-Q104-EGFP Cell abundance indicated by GFP expression Validation Assay: Scotter et al., 2010 New Zealand CB1 as a target for HD CB1 is the cannabinoid receptor This study also showed cannabinoids reducing neurotoxicity consistent with Aiken et al., 2004.

LDH release GFP evaluation for aggregations, showing reduced aggregation.

Conclusions:

4 cannabinoids with 50-100% protection (and some other compounds)

Enhancement of Autophagy

Minoxidil, Clonidine, Rapamycin, Rilmenidine

and L-type Ca²⁺ channel agonists (verapamil, loperamide, nimodipine, nitrendipine, amiodarone)

Williams et al., 2008 and Rose et al., 2010 (Rilmenidine) David Rubinsztein, Cambridge

Screening Assay:

253 FDA approved compounds (low throughput) Screened for clearance of α -synuclein in PC12

Validation Assay:

Clearance of mutant HTT fusion protein (EGFP-HDQ74) from PC12 cells Lack of toxicity Reduction of aggregate formation

Animal study:

Drosophila HD model, HDQ120 expressed in eye, photoreceptor degeneration monitored Zebrafish HD model, EGFP-HDQ71 expressed in rods of the eye, aggregation monitored

Conclusions:

Identified mTOR independent pathways suggesting elevated intracellular Ca²⁺ enhances autophagy.

Note that the primary readout is merely a tool for identifying compounds that may be useful for treatment of HD. Many of these compounds have functional overlap.

Some compounds that were identified for their ability to reduce neurotoxicity or enhance autophagy were also shown to inhibit aggregate formation. So while it is unclear whether aggregate formation contributes to pathogenesis, it remains a valid readout for HD and possibly other polyQ diseases.

Informatics (links to free software and tools for informatics)

HTS Corrector

http://www.info2.uqam.ca/~makarenv/HTS/home.php

Makarenkov V. *et al.*, HTS-Corrector: software for the statistical analysis and correction of experimental high-throughput screening data. Bioinformatics 22:1408-1409, 2006.



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Description

Resources // Free Software and Code for Public Use

Utility

>> Resources

PubChem Molecular Libraries Initiative HTS Assay Dev Interest Group NCGC Pubchem Data Guideline NCGC Assay Data in Pubchem Free Software and Code for Public Use

Response	NCGC CurveFit: Public, stand alone open source version of NCGC's curve fitting software. This application automatically fits and classifies thousands of dose response curves. Features include sorting of compounds by quality of their assay response, chemical structure viewing, and advanced filtering of results. Filtering includes ability to search by chemical structure similarity or by curve class or by potency cutoffs. Click here to find out more.
(tît)	PubChem Fingerprint for JChem: Implementation of PubChem Group's fragment-based FP using ChemAxon's JChem library, provided for public use for integration in JChem based software development projects. There are slight differences between PubChem's fingerprint and our implementation due to JChem's aromaticity perception and SSSR determination.
lif	Chemical Structure Processing: A Java class for generating canonical structures from an HTS perspective, so that common chemical entities can be identified from compound collections. We generally use the FDA's definition for chemical entity (see this fda link and also this wikipedia link) with the exception that esters and other covalent modifications are treated as different molecules because most HTS assays are not metabolically competent). The smiles are canonical – identical chemical entities will have exactly the same smiles string. The class uses JChem package and tautomer plug-in. A list of common salts is provided. Molecules are salt-stripped, and the largest remaining component is taken as the chemical entity (sorry, no mixtures). A canonical tautomer form is generated for the chemical entity (not the lowest energy, but a canonical tautomer for comparison). Molecules with unusual atoms (organometalics, dative bonds are particularly difficult to standardize) and simple molecules (generally non HTS molecules) are not salt-stripped but a canonical smiles is still generated. This successfully handles the vast majority of HTS compounds. The output is of the format: Asmiles Name Bsmiles CSmiles. Asmiles is the smiles version of the original structure, for display purposes. Bsmiles is the canonical, stereo smiles. Csmiles is the non-stereo, canonical smiles.
(tit)	Atom Pair Descriptors: Implementation of atom pair descriptors using JChem library, provided for public use for integration in JChem based software development projects.
	JP Clustering: Implementation of basic Jarvis-Patrick clustering. Combined with the descriptors above, you can create your own stand alone clustering tool or incorporate into exisiting development projects.

Free Software and Code for Public Use

http://pubchem.ncbi.nlm.nih.gov/

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For PubChem help: Yanli Wang ywang@ncbi.nlm.nih.gov

AID = Assay ID, CID = Compound ID, SID = Substance ID

Compound Searching and SMILES (Simple Molecular Input Line Entry Specification) With this PubChem tool, you can build the structure of any chemical and search for that molecule in the database, identifying any assays that it appeared in. The SMILES format is useful for searching PubChem as well as vendor sites that might sell the compound.

	hem Structure Search	PubMed Entrez
Search By: Name/ Text Identity/ Similarity Substructure/ Superstructure Image: Constructure CID, SMILES, InChI Structure Image: Constructure CID, SMILES, InChI Structure Image: Constructure Image: Constructure Image: Constructure Image: Constructure Image: Constructure Image: Constructure Image: Constructure Image: Constructure Image: Constructure	Molecular Formula Saved Search File PubChem Sketcher V2	SMILES notation C1=CC=CC2=C1C(=C[N]2)CO $f \in f$ 1. Type in the compound structure
2. Search	Advanced Search F Display Summary Tools: Signature All: 1 Rule of 5: 1	Preview/Index History Clipboard Details Show 20 Sort By Send to Links: Related Structures, BioAssays, BioSystems, Literature, Other Links ? Related Structures, BioAssays,
3. Obtain Compound	ID _	Indole-3-carbinol; INDOLE-3-METHANOL; 3-Hydroxymethylindole IUPAC: 1H-indol-3-ylmethanol
4. Next slide		MW: 147.173860 g/mol MF: CgHgNO Tested in BioAssays: All: 258, Active: 1; BioActivity Analysis ⊗

http://pubchem.ncbi.nlm.nih.gov/assay/assay.cgi?p=bioactivity



5. Output of 280 assays this compound appeared in

Bioavailability

Lipinski et al., Advanced Drug Delivery Reviews 46:3-26, 2001.

Lipinski's rule of 5 says that, in general, an orally active drug has no more than one violation of the following criteria:

Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms) Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms) A molecular weight under 500 daltons An octanol-water partition coefficient log P of less than 5

Liu et al., Drug Metabolism and Disposition 32:132–139, 2004. Molecular polar surface area and blood brain barrier

molinspiration

http://www.molinspiration.com/cgi-bin/properties







Output: **Bioavailability and Drug** Likeliness Parameters Molinspiration drug-likeness

GPCR ligand	0.13
Ion channel modulator	-0.10
Kinase inhibitor	-0.08
Nuclear receptor liga	nd -0.81
<u>Get data as text</u> (for	copy / paste)

Get 3D geometry BETA

miLogP	1.426
TPSA	36.019
natoms	11
MW	147.177
nON	2
nOHNH	2
nviolations	0
nrotb	1
volume	137,841
Accessing the MLPCN





Molecular Libraries Probe Centers Network

http://mli.nih.gov/mli/mlpcn/

NIH Chemical Genomics Center (NCGC) Broad Institute Comprehensive Screening Center Sanford Burnham Center for Chemical Genomics The Scripps Research Institute Molecular Screening Center Johns Hopkins Ion Channel Center University of New Mexico Center for Molecular Discovery Southern Research Specialized Biocontainment Screening Center Kansas Specialized Chemistry Center Vanderbilt Specialized Chemistry Center for Accelerated Probe Development

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Important MLPCN Resources

http://mli.nih.gov/mli/mlpcn/

Welcome to the Melcome to the Defense to the Defens			
Access MLPCN Resources	Access MLPCN Resources		
MLPCN Centers Center Capabilities Access to Technical Assistance (MLPCN) Documents & Definitions User Login Required Login MLP CARS CARS Training	SubMenus: MLPCN Centers Center Capabilities Technical Assistance Documents & Definitions The MLPCN resource can be accessed by any member of the scientific community in a number of ways, If you already have an HTS-ready assay (in 96, 384, 1536-well plate or flow cytometry), then you can: • Obtain technical assistance in developing a high throughput screening (HTS) plan by clicking here • Apply through R03 Assay Implementation for peer review organized by CSR If you have a potentially HTS-compatible assay (in test tube or 96-well plate) • Obtain technical assistance in developing a high throughput screening (HTS) plan by clicking here • Apply through R03 Assay Implementation for peer review organized by CSR		
Assay Wiki	If you are a PL of an existing assay grant (R21, R01 etc) you can		
Assay Annotation Search type, hit enter	 Obtain technical assistance in developing a high throughput screening (HTS) plan by clicking here. Fast Track Entry of Assay Projects Apply for Fast Track entry to MLPCN click: Fast Track Entry Request. Fast Track Entry of Medicinal Chemistry Projects Please contact Enrique Michelotti (michelottiel@mail.nih.gov) prior to completing the application, found here: Chemistry Fast Track Template (September 2010). 		

Requirements for entering the NCGC

Criteria	Biochemical	Cell-based
	96-well or higher density plate	96-well or higher density plate
Plate Format*	NCGC: 1536-well format	NCGC: 1536-well format
	Assay volume 2-6 ul	Assay volume 4-6 ul
	=10 steps with 96-well plate.	=10 steps with 96-well plate.
Assay Steps	Steps include, reagent additions, timed incubations, plate transfers to incubator, reading, etc.	Steps include, reagent additions, timed incubations, plate transfers to incubator, reading, etc.
Minimum time increments and maximum assay duration	Minimum assay window is 5 min. (i.e., earliest time point after last reagent	<24 hr is ideal; max 48 hrs.
	addition)	Minimum assay window is 5 min.
Reagent Addition Steps	4 maximum (4 unique reagents max; more if pre-mixed)	4 maximum (4 unique reagents including cells max; more if pre-mixed)
Reagent removal steps*	No plate coating steps	Aspiration steps*
Temperature	Between RT and 37°C	Between RT and 37°C
Demonstrated DMSO Tolerance*	0.5 – 1% DMSO	0.5 – 1% DMSO
Signal : Background Ratio	=3-fold	=3-fold
Day-to-Day variation of control (e.g., IC ₅₀ , EC ₅₀)	<3-fold	<3-fold
Reagent stability @ final	=8 hrs @ RT or on ice bath;	=8 hrs @ RT or on ice bath;
working concentration	No on-line thawing	No on-line thawing
Validation run reagent supply	10 – 96-well plate equivalents	10 – 96-well plate equivalents
Protocol	Complete detailed protocol. All steps, equipment used, all vendor & catalog # for reagents. Data from 96-well or high density plate tests.	Complete detailed protocol. All steps, equipment used, all vendor & catalog # for reagents. Detailed cell culture procedure, passage # .Data from 96- well or high density plate tests.
	PE ViewLux (Top reading only: FI, TRF, FP, Abs, Luminescence)	PE ViewLux (Top reading only: FI, TRF, FP, Abs, Luminescence)
Detectors	PE Envision (bottom reading FI, ALPHA)	PE Envision (bottom reading FI, ALPHA)
	Acumen Explorer (fluorescent laser cytometry)	Acumen Explorer (fluorescent laser cytometry)
Special	For unique reagents either investigator prepares sufficient quantity for HTS or identifies a reliable 3rd party vendor.	Cells must be certified micoplasma-free by direct culture assay and cell-DNA fluorochrome staining.

Acknowledgments

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