Visceral Leishmaniasias (VL) – Optimization of 2-Substituted Quinolines

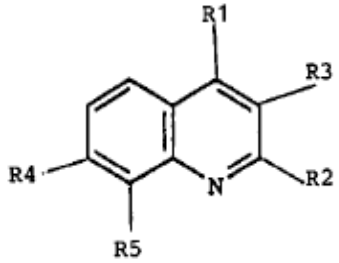
Agenda

- Why 2-substituted quinolines?
- Known SAR: 2-substituted quinolines.
- Optimization strategy: 2-substituted quinolines.
- SAR results
- DMPK work

Basis for quinoline series - First patent (1991-1992)

(54) **Quinoléines 2-substituées pour le traitement des leishmanioses.**

(57) L'Invention a pour objet des quinoléines substituées, de formule générale (I)



(I)

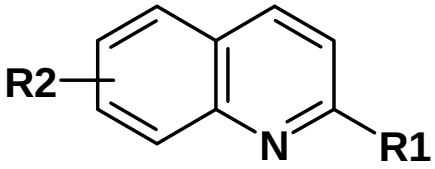
dans laquelle R_1, R_4 et R_5 représentent un atome d'hydrogène, ou un groupe méthoxy; et R_2 représente un groupe alkyle, alkényle ou époxyalkyle en C_3 à C_5, ou bien un groupe phényle, un groupe phénéthyle, méthylènedioxyphénéthyle, diméthoxyphénéthyle, ou bien R_2 et R_3 forment ensemble un cycle furane, ainsi que leurs sels et leurs dérivés, pour l'utilisation comme médicaments.

« evanta » *Galipea longiflora* Kr (Rutaceae)

Uses in traditional medicine: cutaneous leishmaniasis, fever, malaria, amoebiasis

Fournet A., Angelo Barrios A., Mu–oz V., Hocquemiller R., Roblot F., Bruneton J., Richomme P., Gantier J. C. Quinol˘i nes 2-substitu˘e s pour le traitement des leishmanioses. PCT/FR92/00903

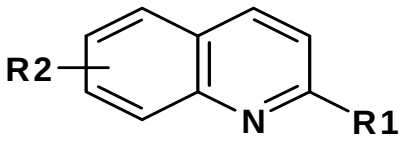
Known SAR: 2-substituted quinolines



Known SAR:

- Unsaturation at R1 required for potential putative chemical reactions
- 2-n Propyl Quinoline may have active antileishmanial metabolites
- Optimal length seems to be 5 carbons
- Limited modification on R1 chain can be done to effect vitro activity.
- Not much known about R2 substitutions
- Oral efficacy of some derivatives has been established

VL - Optimization Program: 2-substituted quinolines

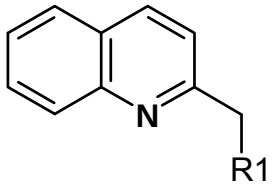


US 2005/0165052, July 28, 2005

38 specific examples

Mostly 2-substituted derivatives

A few 8-substituted derivatives



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Bioorg. Med. Chem. **2003**, *11*, 5013 – 5023

2-substituted derivatives, 50 examples

Antimicrob. Agents Chemother. **2005**, *49*, 4950-56

2-substituted derivatives, 9 examples

Compound 5:

IC_{50} low μM

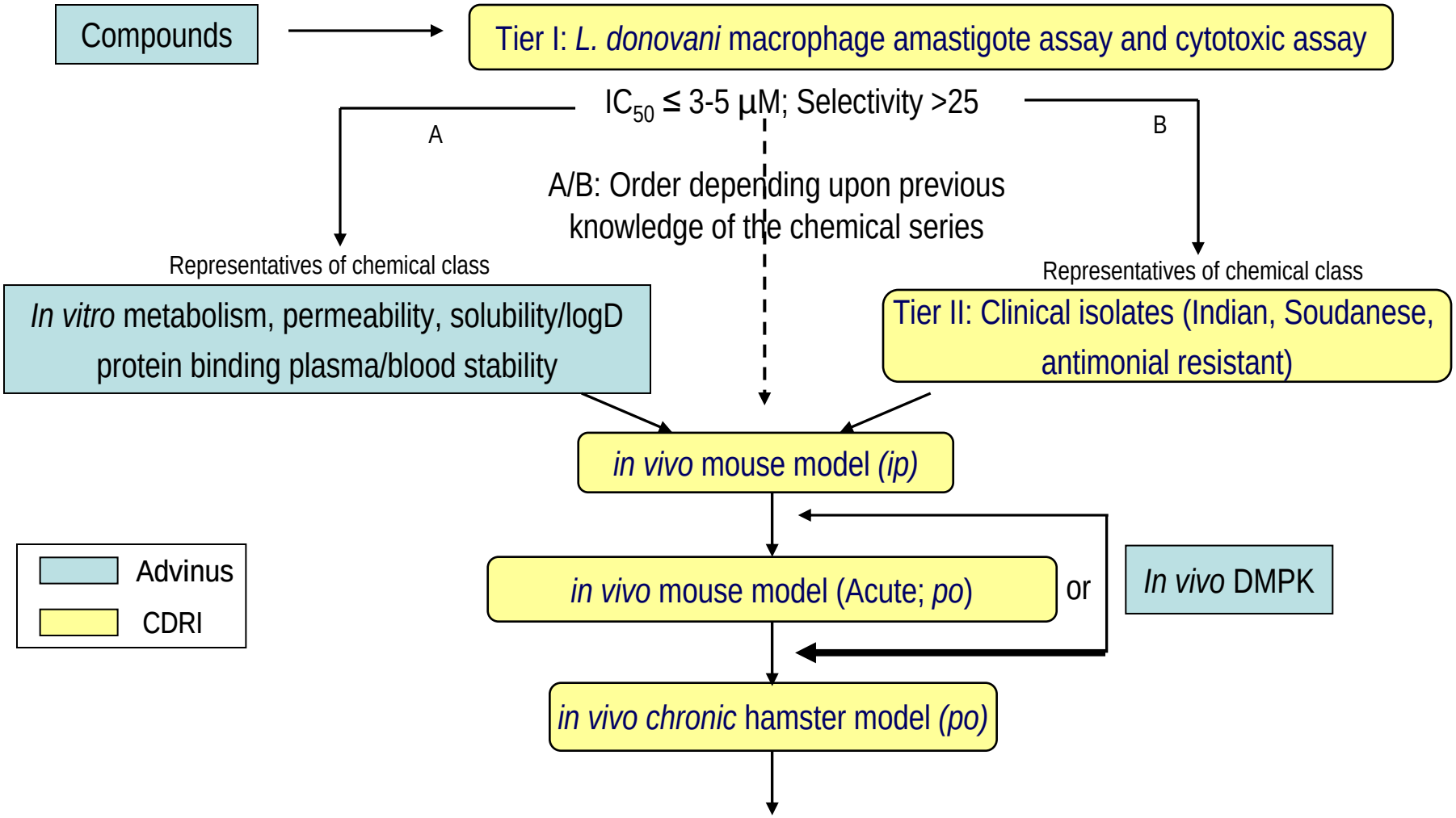
Has other activities

Orally active at 12.5 mpk in mouse model

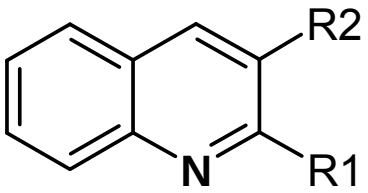
Clinical candidate profile

IP	Patentability	Yes
in vitro	In vitro antileishmanial assays	1. IC_{50} < 1 μM 2. IC_{50} < 1 μM mouse macrophase based assay
	Selectivity over mammalian isoform	> 10 fold
ADMET	Metabolic stability (HLM, RLM, DLM, MLM)	intrinsic clearance of less than 5 mL/min/g liver
	CYP inhibition (3A4, 2D6, 2C9, 2C19, 1A2)	IC_{50} > 10 μM (HLM)
	Oral bioavailability	> 30% in rat and dog
	hERG binding/EP	IC_{50} > 50 μM
	Caco2/MDCK permeability	>10 nm/sec
	Cytotoxicity	ND up to 50 μM
	Mutagenicity	None
	14 days toxicity in rat and dog	Exposure multiple to NOAEL > 10X
in vivo	In vivo efficacy in mouse	ED_{90} 10 mg/kg in 2 days

Screening cascade

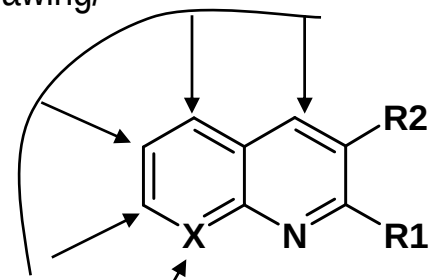


Ongoing optimization studies with DNDi-VLQ-005



DNDi-VLQ-005 (parent)
IC_{50} (*L. Donovani*) = 10μM
CC_{50} (macrophage) = 2 μM

Electron donating/withdrawing/
bulky substituents

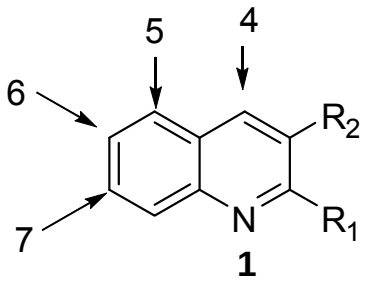


R1 and R2 constant

X = C/N
(N at different ring positions)

Optimization results of DNDi-VLQ-005

	Position					
SNo.	4	5	6	7	IC_{50}	CC_{50}
1	OMe		Cl		2.6	3.7
2	OMe		Cl	F	1.65	26.06
3		F	Cl			
4		Cl	Cl		3.12	7.14
5			F	F	21.4	20.69
6		F	F		11.24	14.26
7	Cl				2.54	
8			Cl		<1.56	



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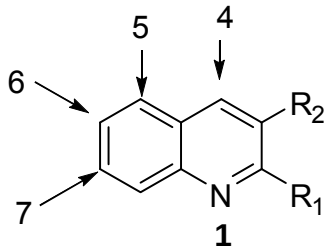
Conclusions from SAR studies with DNDi-VLQ-005 derivatives

- Methoxy group is not contributing for activity if it is at 5 and 6 position alone.
- Di-substitution at 5 and 6 position- reduces activity
- Chloro group in 6th position contributing for activity.
- Fluoro group at 7th position is a good combination with 6-chloro when there is electron donating group present at 4th position.



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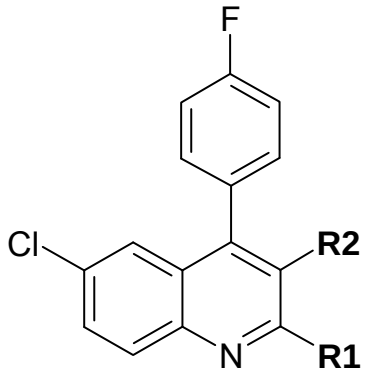
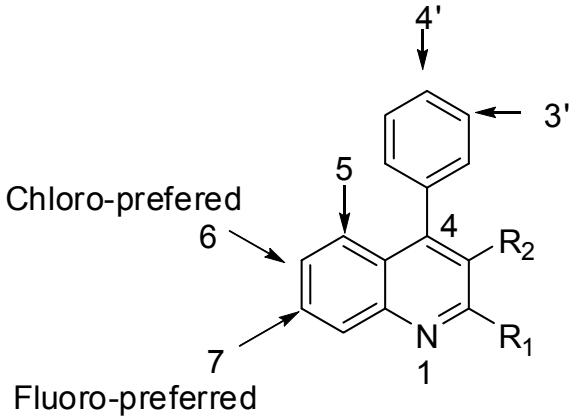
Based on this observation 4-fluoro-phenyl group was inserted at 4th position to obtain DNDi-VLQ-065

DNDi-VLQ-065



CDRI								
In vitro activity against L. donovani								Cytotox
100 μM	50 μM	25 μM	12,5 μM	6.25 μM	3.12 μM	1.56 μM	IC50	CC50
95.6*	100.9*	97.5*	92.9*	91.15*	88.0#	65.2	<1.56	2.02

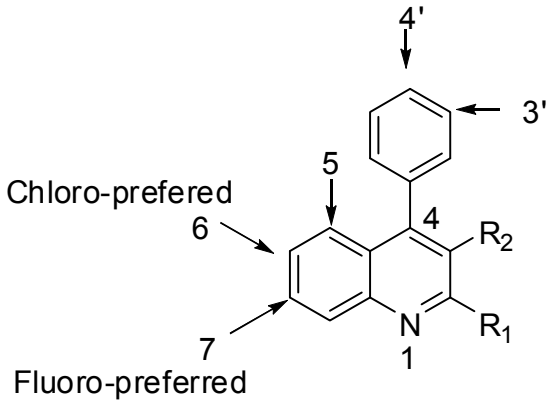
Optimization results of DNDi-VLQ-065



	Position					
SNo.	6	7	4'	3'	IC_{50}	CC_{50}
1	Cl		Cl		0.46	3.5
2	Cl	F	F		1.43	4.48
3	Cl	F		F	1.79	4.58
4	Cl	F	OMe		1.21	7
5	Cl		F		<1.56	2.02
6	F	Cl	F		2.97	6.04
7	Cl		OMe			28.83
8	Cl			F	3.86	63.99

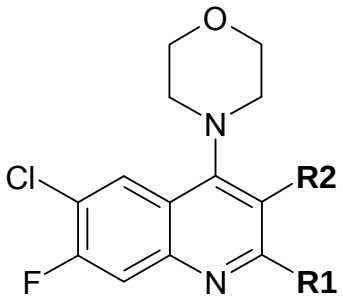
Conclusions from SAR studies with DNDi-VLQ-065 derivatives

- Di-substitution at 6 and 7 position- preferred for activity as well as selectivity
- Chloro group in 6th position preferred for activity.
- Fluoro group at 7th position is a good combination with 6-chloro when there is electron donating group present at 4th position.
- More optimization of substituents on phenyl ring at 4th position needs to be done.



Based on this 4-phenyl group replaced with morpholine and Piperidine at 4th position to obtain DNDi-VLQ-0133

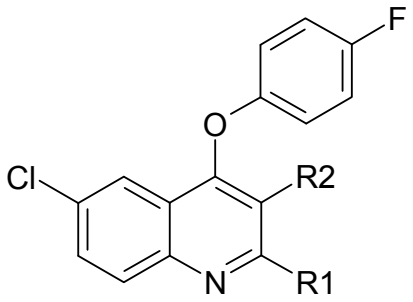
DNDi-VLQ-0133



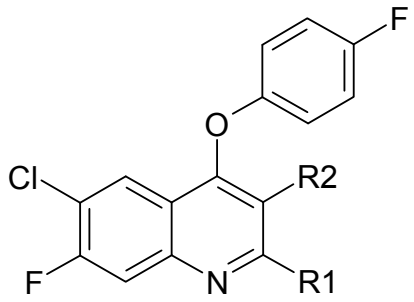
Percent Inhibition at µM Conc.							*IC_{50}	CC_{50}
3.12	**1.56**	**0.78**	**0.39**	**0.195**	**0.097**	**0.048**	**(µM)**	**(µM)**
80.84$	74.82$	66.59$	59.94$	49.17$	44.1$	19.71$	0.21	22.12

$ - Macrophage Cells normal

Insertion of o-aryl group at 4-position



DNDi-VLQ-0131

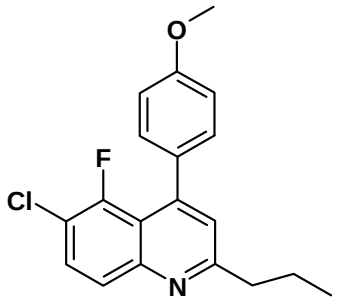


DNDi-VLQ-0132

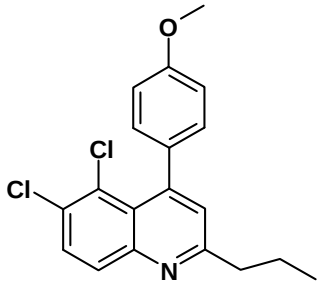
Compound	Percent Inhibition at µM Conc.							*IC_{50}	CC_{50}
Code No.	3.12	1.56	0.78	0.39	0.195	0.097	0.048	(µM)	(µM)
DNDi-VLQ-0131	87.99#	31.4#	NI$	15.11$	2.69$	20.08$	NI$	1.96	15.41
DNDi-VLQ-0132	86.47*	NI$	NI$	NI$	NI$	NI$	NI$	2.56	11.35

$ - Macrophage Cells normal

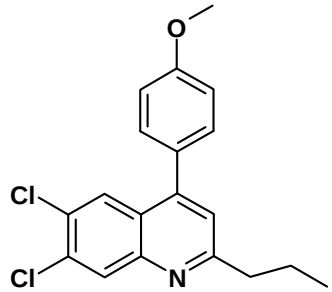
2-*n*-propyl quinoline analogues



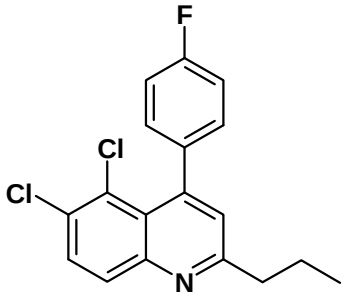
PCADV05-0008
(ADV-B-235-93)
IC_{50} = 17 µM
IC_{50} macrophage = 104 µM



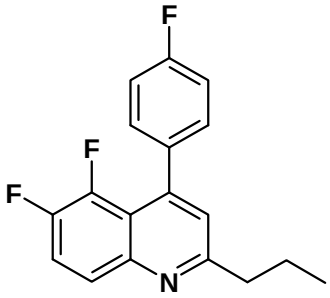
PCADV05-0028
(ADV-B-299-55)
IC_{50} = 21 µM
IC_{50} macrophage = 61µM



PCADV05-0006
(ADV-B-299-11)
IC_{50} = 25 µM
IC_{50} macrophage = 43 µM

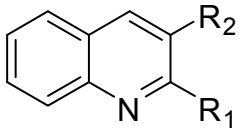


PCADV04-0003
(ADV-B-299-07)
IC_{50} macrophage = 159 µM



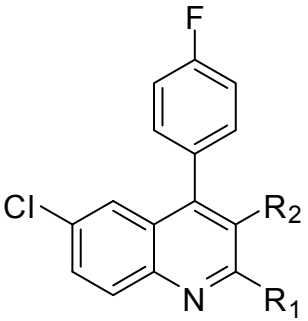
PCADV05-0023
(ADV-B-300-38)
IC_{50} macrophage = 249 µM

SAR and key developments



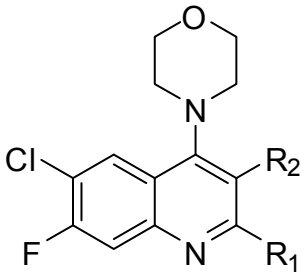
DNDi-VLQ-0005
(ADV-B-235-93)
IC_{50} = 10μM
CC_{50} > 2μM

synthesized different analogs

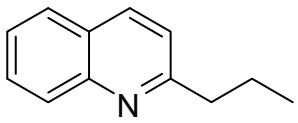


DNDi-VLQ-0065
(ADV-B-269-64)
IC_{50} < 1.56 μM
CC_{50} > 2μM

Structural analogs

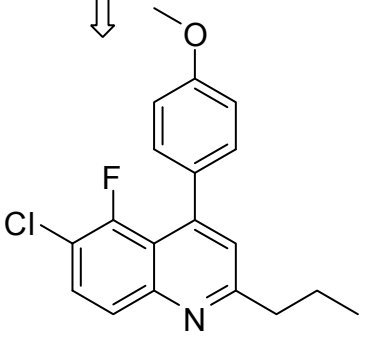


DNDi-VLQ-0133
(ADV-B-330-57)
IC_{50} = 0.21 μM
CC_{50} > 22 μM



Invitro-Inactive
Ivivo-active

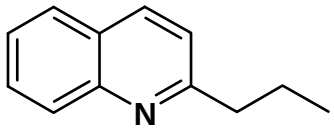
synthesized 21 different analogs



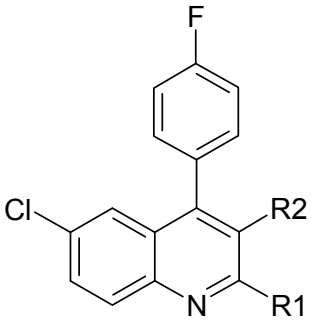
PCADV05-0008
(ADV-B-235-93)
IC_{50}< 16.66 μM
CC_{50} > 200 μM

First time propyl series shows *in vitro* activity

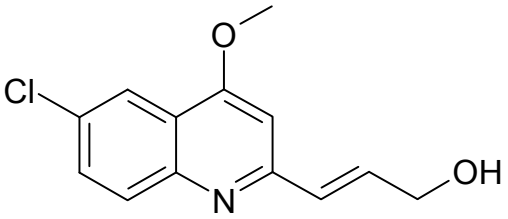
Quinolines: Solubility and metabolic stability in liver microsomes



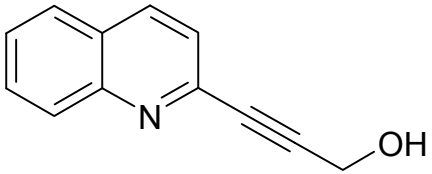
DNDi-VLQ-0125
Solubility (uM) > 100
% Metab in 30 min:
HLM = 98 , MLM = 100



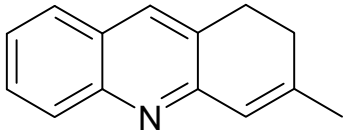
DNDi-VLQ-0065
Solubility (uM) = 10
% Metab in 30 min:
HLM = 25 , MLM = 78



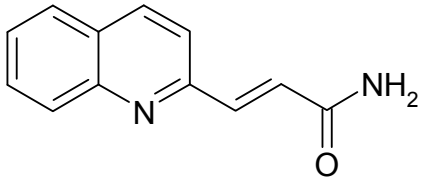
DNDi-VLQ-0064
Solubility (uM) = 60
% Metab in 30 min:
HLM = 59 , MLM = 100



DNDi-VLQ-0010
Solubility (uM) > 100
% Metab in 30 min:
HLM = 22 , MLM = 99

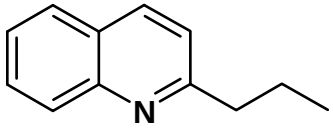


DNDi-VLQ-0031
Solubility (uM) > 100
% Metab in 30 min:
HLM = 100 , MLM = 100

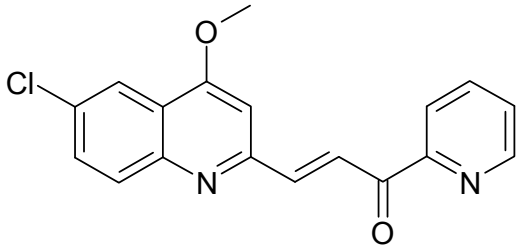


DNDi-VLQ-0059
Solubility (uM) > 100
% Metab in 30 min:
HLM = 44, MLM = 97

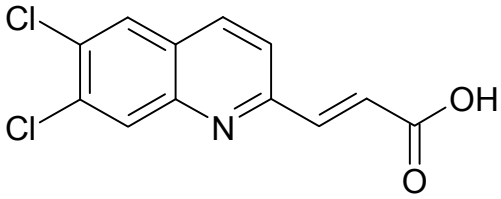
Quinolines: Solubility and metabolic stability in liver microsomes



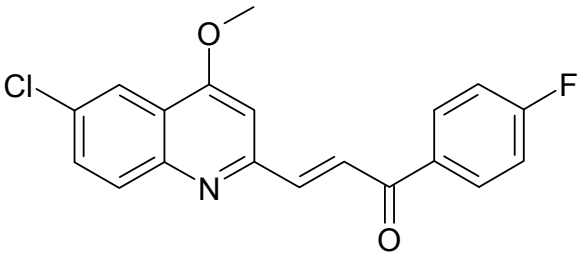
DNDi-VLQ-0125
Solubility (uM) > 100
% Metab in 30 min:
HLM = 98 , MLM = 100



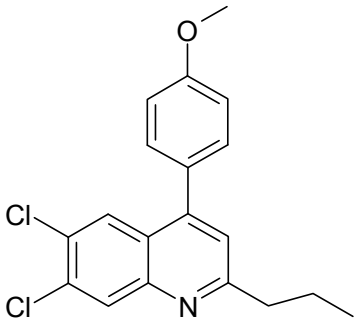
DNDi-VLQ-0074
Solubility (uM) < 10
% Metab in 30 min:
Not reportable



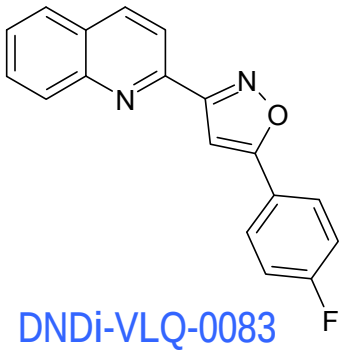
DNDi-VLQ-0052
Solubility (uM) > 100
% Metab in 30 min:
HLM = 80 , MLM = 96



DNDi-VLQ-0064
Solubility (uM) < 5
% Metab in 30 min:
HLM = 95 , MLM = 100

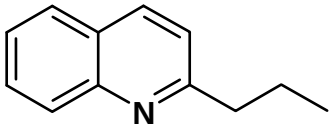


DNDi-VLQ-0095
Solubility (uM) < 10
% Metab in 30 min:
HLM = 5 , MLM = 91

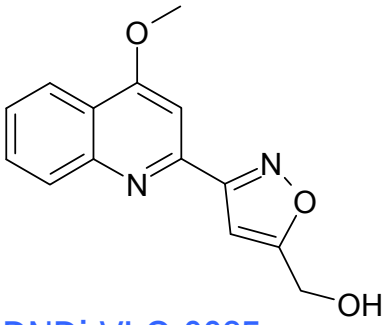


DNDi-VLQ-0083
Solubility (uM) < 10
% Metab in 30 min:
HLM = 27 , MLM = 91

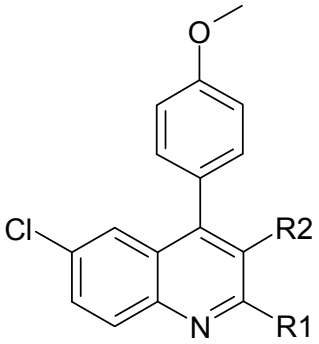
Quinolines: Solubility and metabolic stability in liver microsomes



DNDi-VLQ-0125
Solubility (uM) > 100
% Metab in 30 min:
HLM = 98 , MLM = 100

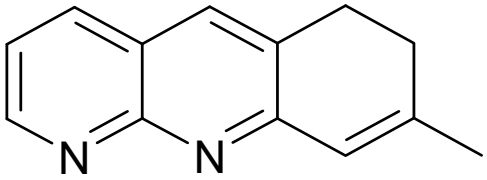


DNDi-VLQ-0085
Solubility (uM) > 100
% Metab in 30 min:
HLM = 46 , MLM = 99

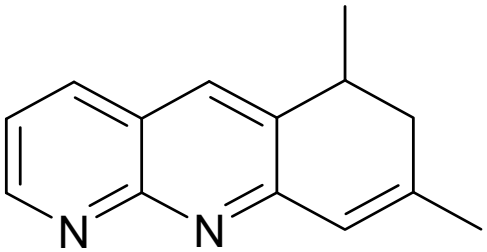


DNDi-VLQ-0088
Solubility (uM) < 10
% Metab in 30 min:
HLM = 8 , MLM = 34

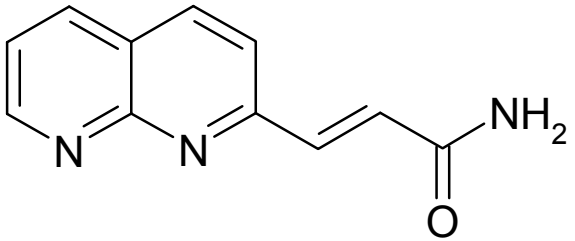
Naphthyridines: Solubility and metabolic stability in liver microsomes



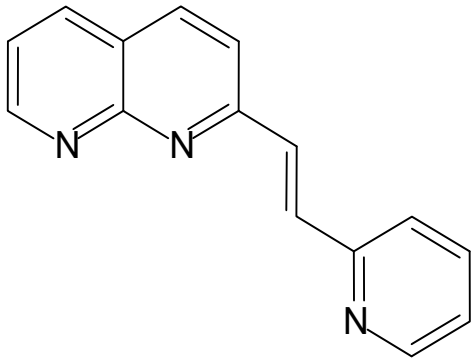
DNDi-VLN-0004
Solubility (uM) > 100
% Metab in 30 min:
HLM = 68 , MLM = 100



DNDi-VLN-0008
Solubility (uM) > 100
% Metab in 30 min:
HLM = 34 , MLM = 97



DNDi-VLN-0022
Solubility (uM) > 100
% Metab in 30 min:
HLM = 2 , MLM = 77



DNDi-VLN-0037
Solubility (uM) > 100
% Metab in 30 min:
HLM = 40 , MLM = 100

DMPK Summary

- Results for various quinoline and naphthyridine analogues suggests that metabolic stability is an issue that needs to be resolved
    - Compound DNDi-VLQ-0088 is relatively stable in human and mouse liver microsomes
    - Generally speaking compounds are more stable in human microsomes

Clinical candidate profile

IP	Patentability	Yes
in vitro	In vitro antileishmanial assays	1. IC_{50} < 1 μM 2. IC_{50} < 1 μM mouse macrophase based assay
	Selectivity over mammalian isoform	> 10 fold
ADMET	Metabolic stability (HLM, RLM, DLM, MLM)	intrinsic clearance of less than 5 mL/min/g liver
	CYP inhibition (3A4, 2D6, 2C9, 2C19, 1A2)	IC_{50} > 10 μM (HLM)
	Oral bioavailability	> 30% in rat and dog
	hERG binding/EP	IC_{50} > 50 μM
	Caco2/MDCK permeability	>10 nm/sec
	Cytotoxicity	ND up to 50 μM
	Mutagenicity	None
	14 days toxicity in rat and dog	Exposure multiple to NOAEL > 10X
in vivo	In vivo efficacy in mouse	ED_{90} 10 mg/kg in 2 days

Summary

- 220 diverse analogues of 2-substituted quinolines synthesized so far
- Activity is in nanomolar range
- More than 100 fold selectivity observed
- What's next?
  - Druggability
  - *In vivo* efficacy and safety

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