Electronic Supplementary Material (ESI) for Chemical Science.

Supplemental Information

Correlating Drug-Target Kinetics and *In vivo* Pharmacodynamics: Long Residence Time Inhibitors of the FabI Enoyl-ACP Reductase

F. Daryaee,[a#] A. Chang,[a#] J. Schiebel,[c,e,f] Y. Lu,[a] Z. Zhang,[a] K. Kapilashrami,[a] S. G. Walker,[b] C. Kisker,[c] C. A. Sotriffer,[e] S. L. Fisher[d]* and Peter J. Tonge[a]*

[a]*Institute for Chemical Biology & Drug Discovery, Department of Chemistry, Stony Brook University, Stony Brook, NY 11794-3400, USA.*

[b]*Institute for Chemical Biology & Drug Discovery, Department of Oral Biology and Pathology Stony Brook University, Stony Brook, NY 11794-3400, USA.*

[c]*Rudolf Virchow Center for Experimental Biomedicine, Institute for Structural Biology, University of Würzburg, D-97080 Würzburg, Germany.*

[d]*The Broad Institute, Cambridge MA 02142, USA.*

[e]*Institute of Pharmacy and Food Chemistry, University of Würzburg, D-97074 Würzburg, Germany.*

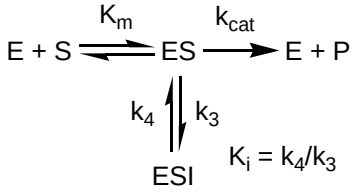
[f]*Present address: Institute of Pharmaceutical Chemistry, Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany.*

#These authors contributed equally to this work.

*Correspondence to: stewfisher@slfisherconsulting.com and peter.tonge@stonybrook.edu

Derivation of a PK/PD model incorporating 1 step uncompetitive inhibition

The mechanistic PK/PD model that we described in Walkup et al.[1] assumes that every enzyme species apart from ES leads to inhibition of bacterial growth. The original model was derived for competitive two-step enzyme inhibition. The saFabI inhibitors operate through a one-step binding mechanism and are uncompetitive inhibitors, thus they bind to ES and not E (**Scheme 1**).



Scheme 1

The first task is to calculate the concentration of ES based on **Scheme 1**. Interconversion between enzyme [E] and substrate [S] and enzyme-substrate complex [ES] is rapid, but formation of the enzyme-substrate-inhibitor complex is slow.

The total enzyme concentration $[E_0]$ is given by Equation S1.

$$[E_0] = [E] + [ES] + [ESI] \qquad \text{Equation S1}$$

Dividing both sides of Equation S1 by $[E_0]$ gives the ratio of each form of the enzyme with respect to the initial total enzyme concentration (Equation S2).

$$1 = \frac{[E]}{[E_0]} + \frac{[ES]}{[E_0]} + \frac{[ESI]}{[E_0]} \qquad \text{Equation S2}$$

If we define $\frac{[E]}{[E_0]}$ as $[E]$, $\frac{[ES]}{[E_0]}$ as $[ES]$, etc. we have Equation S2,

$$1 = [E] + [ES] + [ESI] \qquad \text{Equation S3}$$

Assuming that we have steady-state conditions for enzyme and enzyme-substrate complex formation, we have (Equation S4),

$$[ES] = \frac{[E][S]}{K_m} \qquad \text{Equation S4}$$

Equation S3 becomes Equation S5:

$$1 = [ES] * \frac{K_m}{[S]} + [ES] + [ESI]$$

$$1 - \left(1 + \frac{K_m}{[S]}\right) * [ES] = [ESI]$$

$$M = \frac{K_m}{[S]}; \beta = 1 + \frac{K_m}{[S]}$$

$$1 - \beta * [ES] = [ESI] \qquad \text{Equation S5}$$

Taking derivatives of both sides with the assumption that substrate and inhibitor concentrations, [S] and [I], are not changing over time, we have Equations S6

$$-\beta * \frac{d[ES]}{dt} = \frac{d[ESI]}{dt} \qquad \text{Equation S6}$$

According to **Scheme 1**, there is an equilibrium between [ES] and [ESI], so,

$$\frac{d[ESI]}{dt} = k_3 * [ES] * [I] - k_4 * [ESI] \qquad \text{Equation S7}$$

Combining Equations S6 and S7, we have

$$-\beta * \frac{d[ES]}{dt} = k_3 * [ES] * [I] - k_4 * [ESI] \qquad \text{Equation S8}$$

Replacing [ESI] with the equation containing [ES], Equation S5, and also replacing k_3 with $\frac{k_4}{K_i}$, we have

$$-\beta * \frac{d[ES]}{dt} = k_4 * [ES] * \frac{[I]}{K_i} - k_4 * (1 - \beta * [ES]) \qquad \text{Equation S9}$$

Dividing both sides of Equation S9 by –β and also applying some basic algebraic principles, leads to Equation S10:

$$\frac{d[ES]}{dt} = \frac{-k_4}{\beta} * [ES] * \frac{[I]}{K_i} + \frac{k_4}{\beta} - k_4 * [ES]$$

$$\frac{d[ES]}{dt} = \frac{k_4}{\beta} - \left(k_4 + \frac{k_4}{\beta} * \frac{[I]}{K_i}\right) * [ES]$$

$$k = k_4 + \frac{k_4}{\beta} * \frac{[I]}{K_i}$$

$$\frac{d[ES]}{dt} = \frac{k_4}{\beta} - k * [ES] \qquad \text{Equation S10}$$

Integrating the differential equation yields

$$[ES] = \frac{k_4}{\beta * k} + \left([ES]_0 - \frac{k_4}{\beta * k}\right) * e^{-k*t} \qquad \text{Equation S11}$$

Equation S11 can then be incorporated into Equation S12 which relates the concentration of every enzyme species that is not ES to the rate of bacterial growth, where λ is the logarithmic growth rate, ε is the maximum inhibitor-induced kill rate and *N* is the bacterial cell count in CFU/mL, to give Equation S13:

$$\frac{dN}{dt} = (\lambda - \varepsilon[NotES])N \qquad \text{Equation S12}$$

$$\frac{dN}{dt} = \left(\lambda - \varepsilon\left(1 - \frac{k_4}{\beta * k} - \left([ES]_0 - \frac{k_4}{\beta * k}\right) * e^{-k*t}\right)\right)N \qquad \text{Equation S13}$$

Integration of Equation S13 gives Equation S14

$$[N] = [N_0]exp\left(\left[\lambda - \varepsilon\left(1 - \frac{k_4}{\beta * k}\right)\right]t + \varepsilon\frac{\left([ES]_0 - \frac{k_4}{\beta * k}\right)}{k}(1 - e^{-k*t})\right) \qquad \text{Equation S14}$$

It is important to note that [I] is the concentration of inhibitor at the target (i.e. the intracellular drug concentration). Since we do not have direct knowledge of this value, we introduce a permeability factor ρ_m, which relates the intracellular drug concentration to the concentration of drug in the media in the *in vitro* PAE experiment or to the free fraction plasma drug concentration in the *in vivo* experiments. As we don't have any experimental value for ρ_m, we estimate its value after fitting the *in vitro* PAE data to Equation S14 by choosing a random seed value and letting the value float within a large constraint range to get the best estimate of ρ_m based on the best fit.

$$[I] = [Drug]_{in\ media\ or\ plasma} * \rho_m$$

Calculation of TO_{min} and TO_{max}

To estimate the minimum and maximum target occupancy required for antibacterial activity, a new model, complementary to the original integrated model, has been developed in Mathematica. In this new model equations S10 and S12 are again our key equations.

$$\frac{d[ES]}{dt} = \frac{k_4}{\beta} - k * [ES] \qquad \text{Equation S10}$$

$$\frac{dN}{dt} = (\lambda - \varepsilon[NotES])N = \big(\lambda - \varepsilon(1 - [ES]_t)\big)N \qquad \text{Equation S12}$$

However, to estimate the minimum and maximum target occupancy an extra condition is introduced in which all target occupancy levels (1-$[ES]_t$) with values less than TO_{min} result in no effect and values higher than TO_{max} produce the maximum obtainable efficacy (Equation S15). In addition, a linear correlation between occupancy and effect is assumed to exist between TO_{min} and TO_{max} (Equation S16).

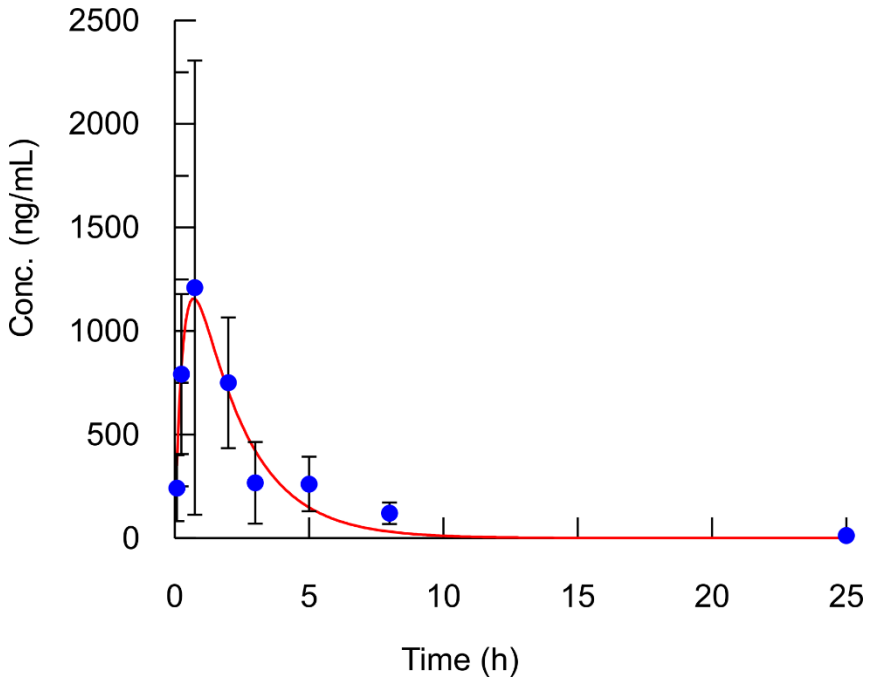
$$\frac{dN}{dt} = \lambda * N - \varepsilon * (\text{If}[(1 - [ES]_t) < TO_{max}, \text{If}[(1 - [ES]_t) > TO_{min}, 1/(TO_{max} - TO_{min}) * (1 - [ES]_t) - TO_{min}/(TO_{max} - TO_{min}), 0], 1]) * N \quad \text{Equation S15}$$

$$0 < \frac{(1-[ES]_t)-TO_{min}}{TO_{max}-TO_{min}} < 1 \quad \text{Equation S16}$$

Fitting of the experimental PAE data obtained at each drug concentration to Equation S15 yields values of TO_{min} and TO_{max}, as well as the value of $[ES]_t$ at the beginning of PAE phase.

Pharmacokinetic Data for PT55 and PT119

a)



b)

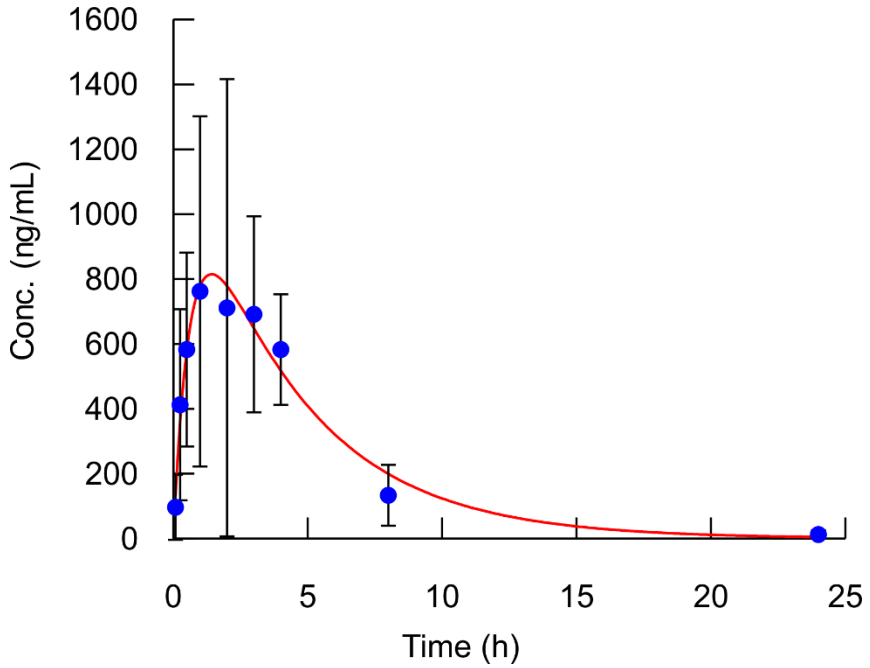
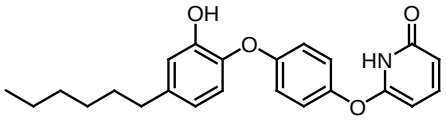


Figure 1. Pharmacokinetics (PK) of a) PT55 and b) PT119. Experimental data points represent mean values from triplicate and error bars represent 1 s.d. Solid lines are the result of fitting the experimental values to a one-compartment PK model. The PK parameters are given in Table 3.

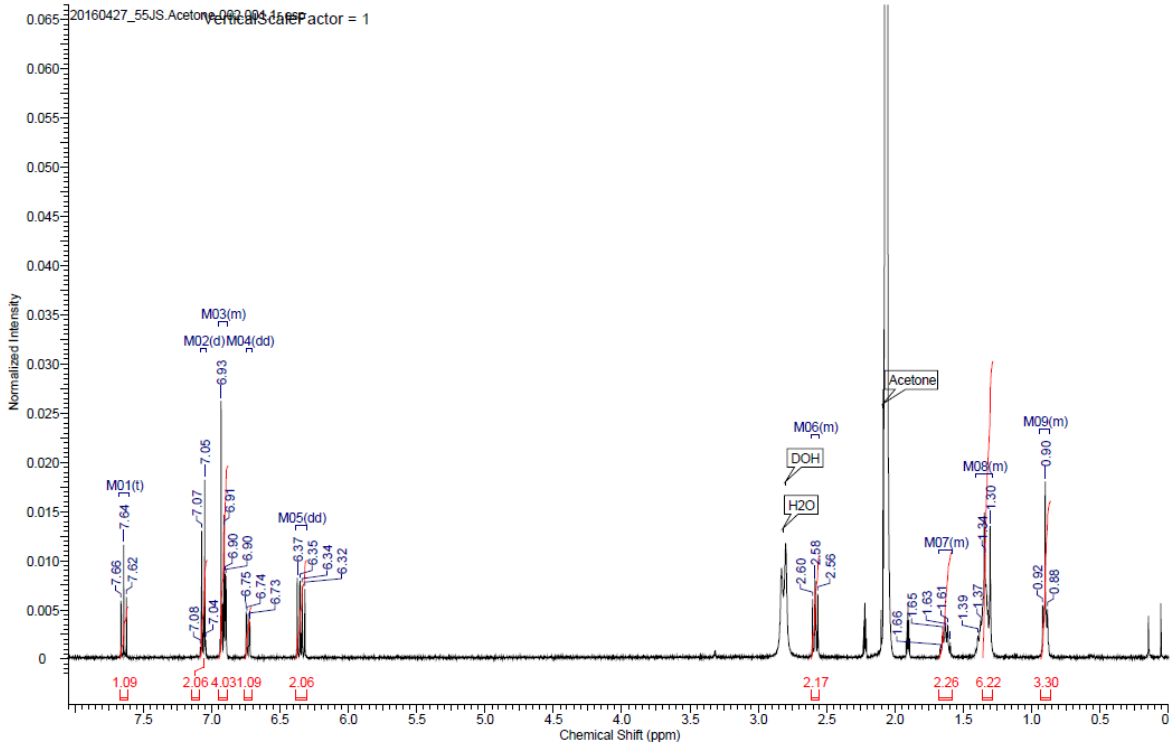
Analytical Data for SKTS1



SKTS1

Chemical Formula: $C_{23}H_{25}NO_4$
Exact Mass: 379.17836

Acquisition Time (sec)	3.9846	*Comment*	1H	*Date*	27 Apr 2016 10:00:32				
Date Stamp	27 Apr 2016 10:00:32			*File Name*	E:\20160427_55JS\2\pdata\1\1r			*Frequency (MHz)*	399.83
Nucleus	1H	*Number of Transients*	16	*Origin*	spect	*Original Points Count*	32768	*Owner*	zhuzhang
Points Count	65536	*Pulse Sequence*	zg30	*Receiver Gain*	65.76	*SW(cyclical) (Hz)*	8223.68	*Solvent*	Acetone
Spectrum Offset (Hz)	2469.1111	*Spectrum Type*	STANDARD			*Sweep Width (Hz)*	8223.56	*Temperature (degree C)*	25.151



^{1}H NMR (400 MHz, Acetone) d ppm 0.87 - 0.94 (m, 3 H) 1.28 - 1.41 (m, 6 H) 1.58 - 1.68 (m, 2 H) 2.56 - 2.61 (m, 2 H) 6.35 (dd, *J*=13.87, 7.84 Hz, 2 H) 6.73 (dd, *J*=8.16, 2.01 Hz, 1 H) 6.89 - 6.95 (m, 4 H) 7.06 (d, *J*=9.16 Hz, 2 H) 7.64 (t, *J*=7.91 Hz, 1 H). ESI-MS (*m/z*): calc for $C_{23}H_{25}NO_4$ $[M - H]^-$ 378.2; found 378.1 $[M - H]^-$. HRMS (m/z): calc for $C_{23}H_{25}NO_4$ $[M + H]^+$ 380.1856; found 380.1855 $[M + H]^+$; calc for $C_{23}H_{25}NO_4$ $[M - H]^-$ 378.1711; found 378.1717 $[M - H]^-$.

References

1. G. K. Walkup, Z. You, P. L. Ross, E. K. Allen, F. Daryaee, M. R. Hale, J. O'Donnell, D. E. Ehmann, V. J. Schuck, E. T. Buurman, A. L. Choy, L. Hajec, K. Murphy-Benenato, V. Marone, S. A. Patey, L. A. Grosser, M. Johnstone, S. G. Walker, P. J. Tonge and S. L. Fisher, *Nat Chem Biol*, 2015, **11**, 416-423.

COS-13-A005-73 in CDCl3
File No : 20130527_26

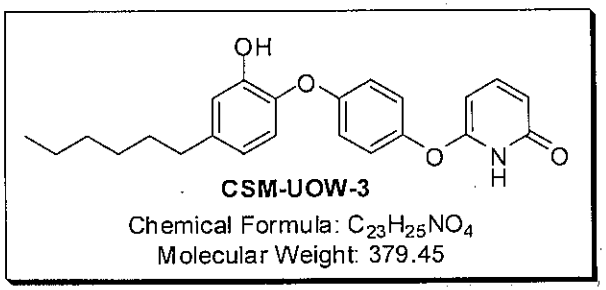
Sample Name:

Data Collected on:
localhost.localdomain-vnmrs400
Archive directory:

Sample directory:

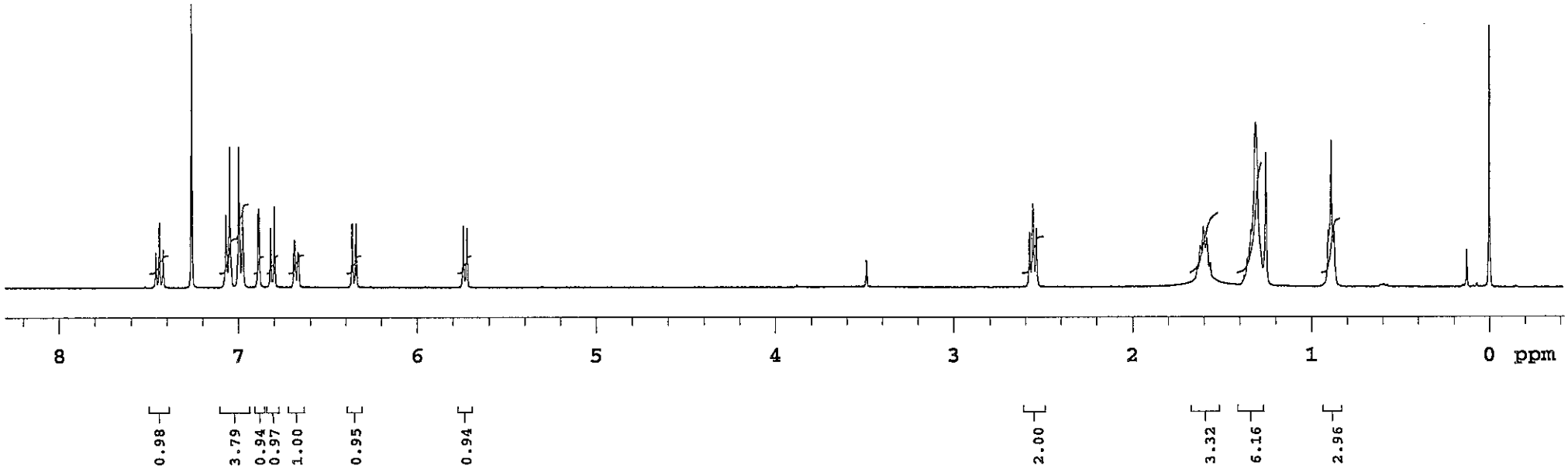
FidFile: PROTON

Pulse Sequence: PROTON (s2pul)
Solvent: cdcl3
Data collected on: May 27 2013

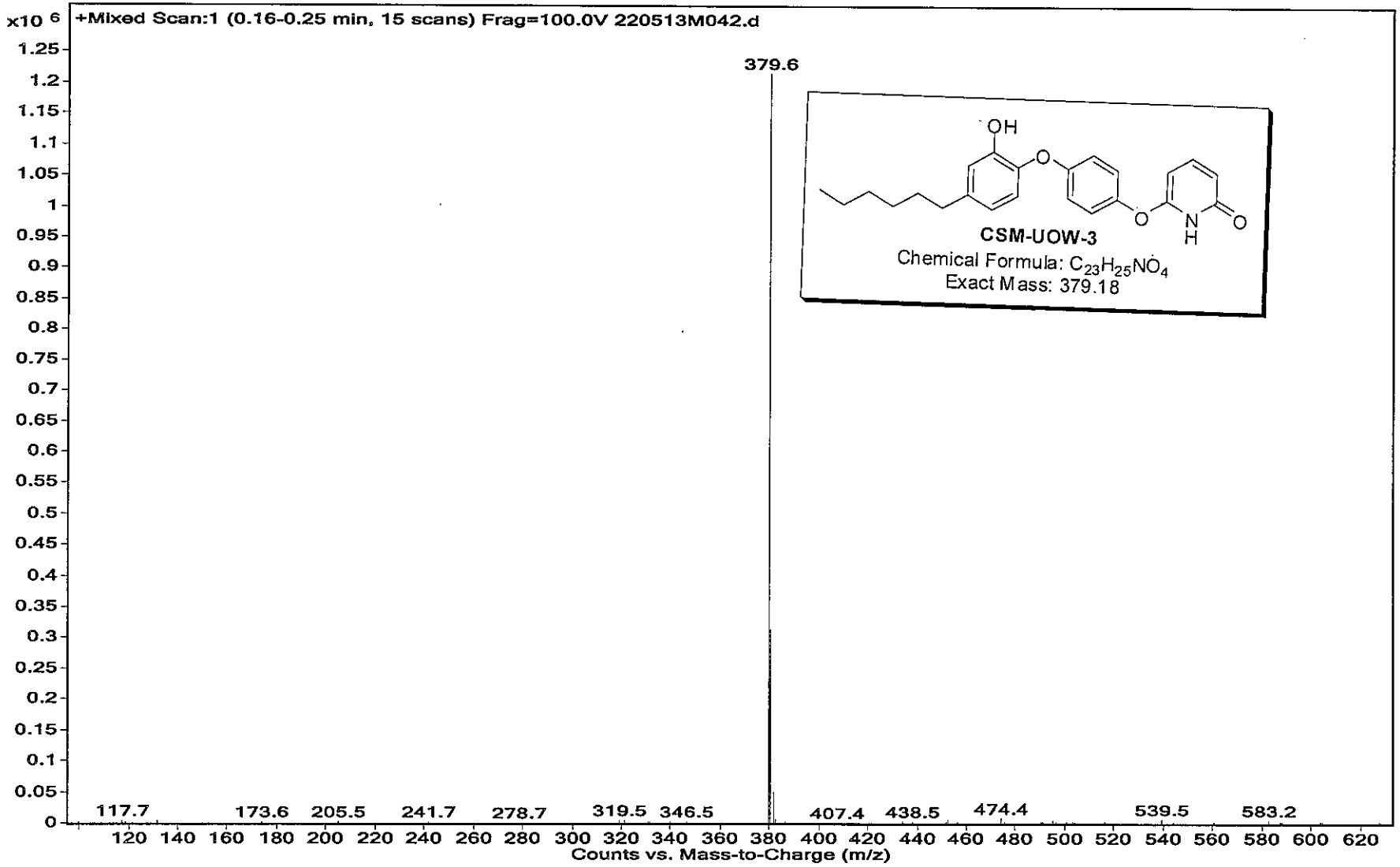


CSM-UOW-3

Chemical Formula: $C_{23}H_{25}NO_4$
Molecular Weight: 379.45



Sample Name	COS-13-A005-73	**Position**	Vial 1	**Instrument Name**	Instrument 1	**User Name**	
Inj Vol	0.5	**InjPosition**		**SampleType**	Sample	**IRM Calibration Status**	Not Applicable
Data Filename	220513M042.d	**ACQ Method**	ILS.m	**Comment**		**Acquired Time**	5/22/2013 3:25:16 PM



22.05.13

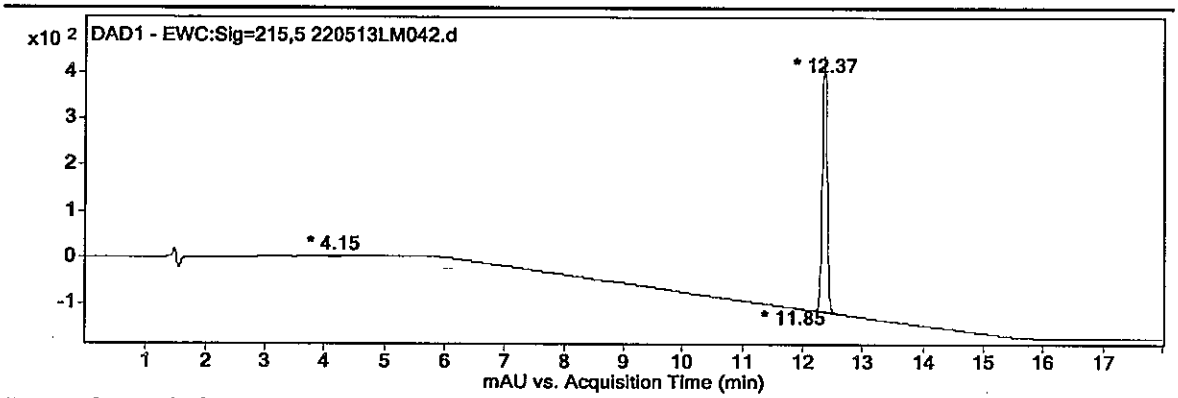
COSMIC Discoveries@ILS

LC-MS Analysis Report

Data Filename	220513LM042.d	**Sample Name**	COS-13-A005-73
Sample Type	Sample	**Position**	Vial 1
Instrument Name	Instrument 1	**User Name**	
Acq Method	ILS LM-2.m	**Acquired Time**	5/22/2013 4:57:45 PM
IRM Calibration Status	Not Applicable	**DA Method**	Default.m
Comment			

Sample Group **Info.** XBridge C18 150*4.6mm 5µm

User Chromatograms

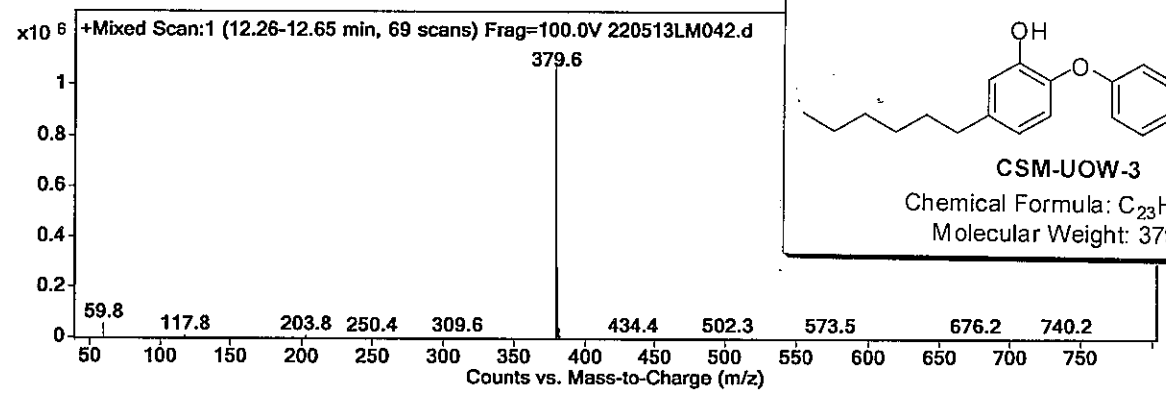


Integration Peak List

Peak	RT	Area	%Area
1	4.15	6.805	0.22
2	11.85	1.721	0.06
3	12.37	3019.536	99.72

User Spectra

Fragmentor Voltage 100 **Collision Energy** 0 **Ionization Mode** Mixed



CSM-UOW-3

Chemical Formula: $C_{23}H_{25}NO_4$

Molecular Weight: 379.45

--- End Of Report ---

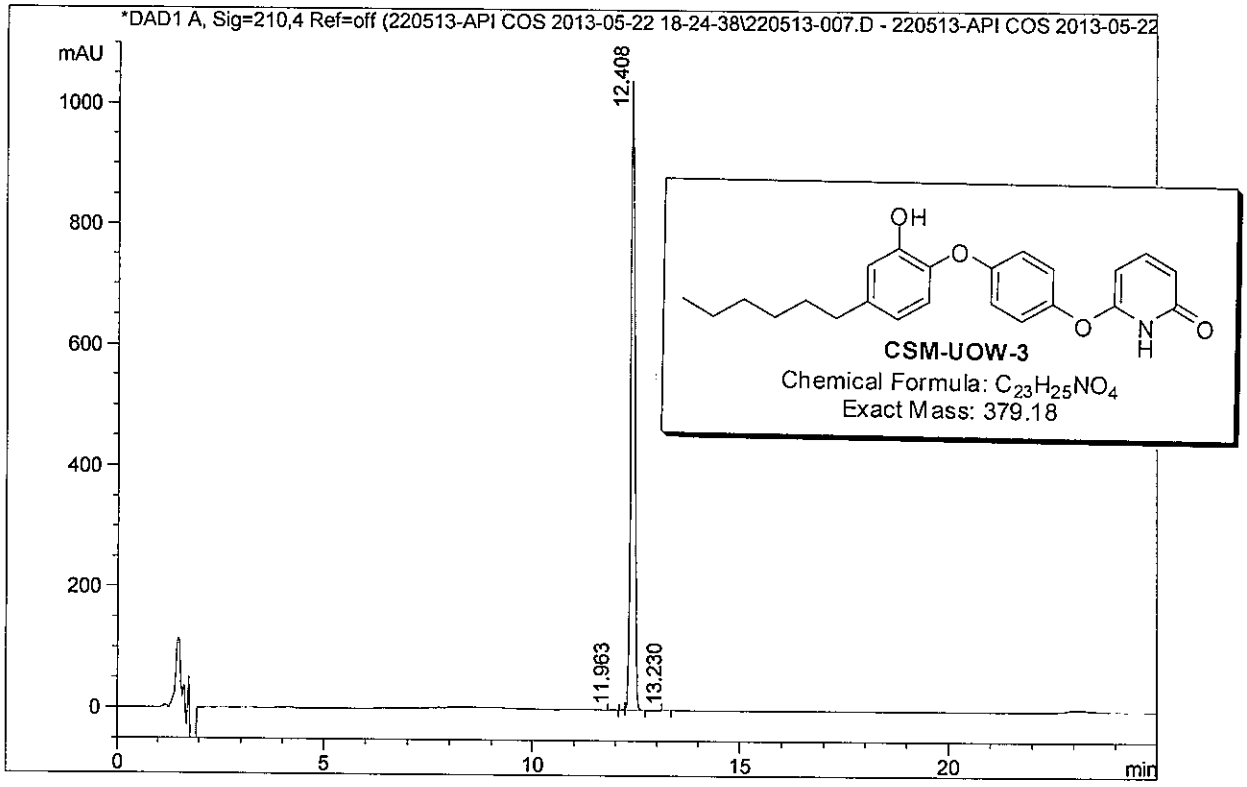
22.05.13

Printed at: 5:20 PM on: 5/22/2013

COSMIC DISCOVERIES @ ILS

HPLC ANALYSIS REPORT

		Seq Line :	7
Injection Date	: Wed, 22. May. 2013	Location :	Vial 53
Sample Name	: COS-13-AOO5-73	Inj. No. :	1
Acq Operator	: RADHA	Inj. Vol. :	10 µl
Acq. Method	: D:\chem32\1\DATA\220513-API COS 2013-05-22 18-24-38\->		
Analysis Method	: D:\CHEM32_002\1\METHODS\AMD COS.M		
Method Info	: Column: X-BRIDGE C-18 150*4.6mm 5µm		
	Mobile phase: A) 0.1% TFA in Water B) ACN		
	T/%B:0/40,3/40,13/95,20/95,22/40,25/40		
	Flow:1.0ml/min, Diluent: MEOH		



==========

Signal 1: DAD1 A, Sig=210,4 Ref=off

Peak #	RT [min]	Area	Area %
1	11.963	7.412	0.101
2	12.168	8.706	0.119
3	12.408	7306.644	99.734
4	13.230	3.403	0.046

==========

*** End of Report ***