Zentner et al. Supplemental Information

MATERIALS AND METHODS

Analytical data for compound 14.

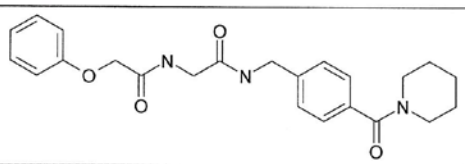
Enamine

CERTIFICATE of ANALYSIS

ISSUE / EXPIRY DATE: 12.03.2012 / 12.03.2013

REREFERENCE (CATALOGUE ID): **T5872535**
BATCH/Weight (mg) **R1973198 / 100**

1. CHEMISTRY

1.1 Structure	
1.2 Chemical Name	N^2-(phenoxyacetyl)-N^1-[4-(piperidin-1-ylcarbonyl)benzyl]glycinamide
1.3 Formula	C23H27N3O4
1.4 Formula weight	409.48

2. PHYSICAL DATA

2.1 Appearance	Light yellow crystal powder
2.2 Melting point, °C	N/A
2.3 Boiling point, °C	N/A

3. ANALYSIS TESTS and RESULTS

3.1	^{1}H NMR	File name	T5872535
		Date of Analysis	06.03.2012
		Identity	Agrees with the structure
		Solvent	DMSO
		Frequency, MHz	500
3.2	LC/MS	File name	T5872535
		Date of Analysis	07.03.2012
		UV Area, %	98.85
		Ret. Time	0.964
		Mol. Ion	410
		Comments	
		Experimental Details	

4. Data of elemental analysis if applicable

4.1 Element	C	H	N			
4.2 Calculated, %						
4.3 Found, %						

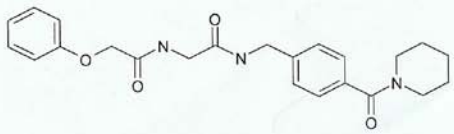
5. Purity data

5.1 Assigned purity, %	95
5.2 Found impurities, comments	

Date of Certification: **12.03.2012**
Certification Signature:

Officer of Quality Control Department

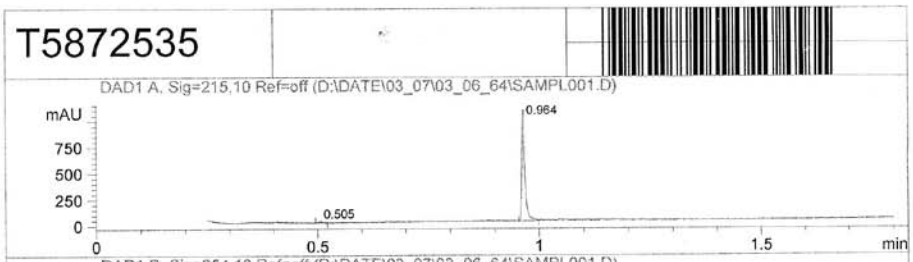
T5872535

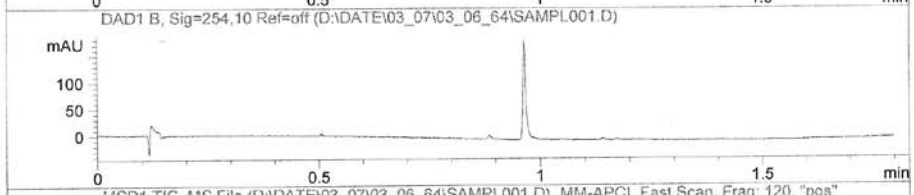


File name: T5872535	Operator: root□	SF: 500.0680 MHz	NSC: 1	PW: 0.00 usec, RG: 24	SI: 32768
Date: 06-Mar-2012	Solvent: DMSO	SW: 8503 Hz	TE: 0 K	AQ: 1.93 sec, RD: 0.00 sec	Parameter file, XWIN-NMR□□Version 3.5□

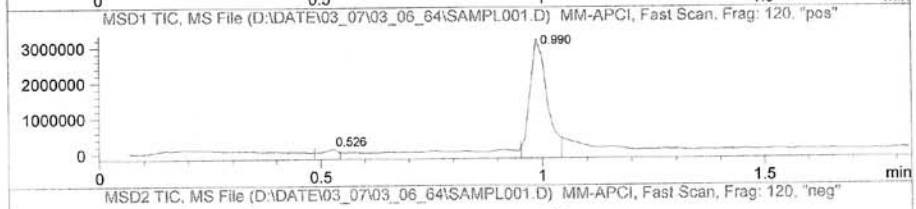
MaxPeak: 98.85%
Ret_Time: 0.964 min

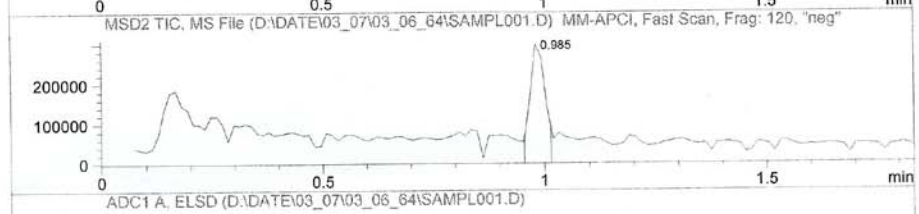
T5872535

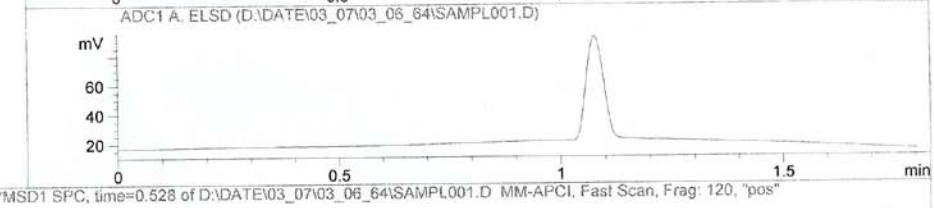




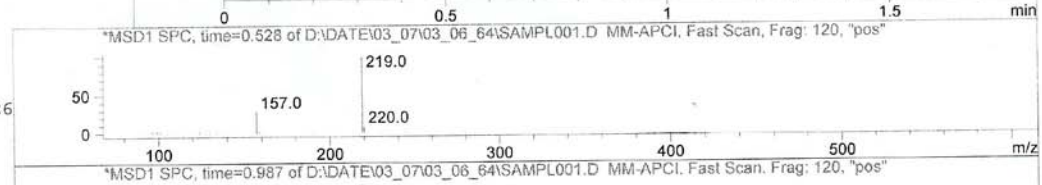
#	Time	Area%
1	0.505	1.15
2	0.964	98.85



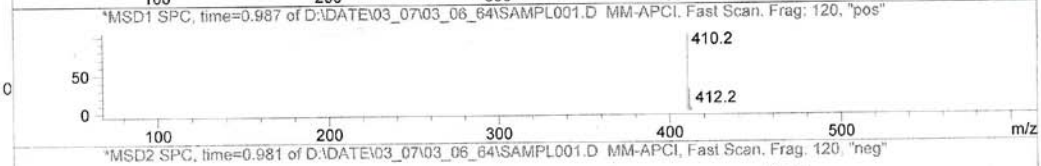




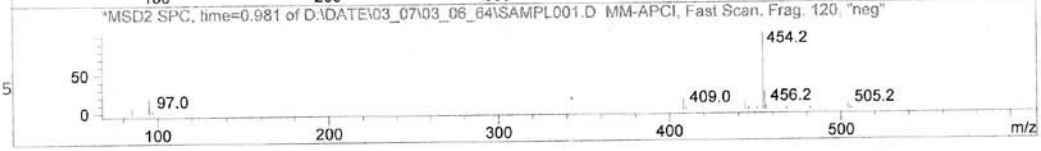
RT 0.526



RT 0.990



RT 0.985



Determination of the antiviral spectrum of compound 14. To determine the spectrum of antiviral activity of compound 14, we evaluated it in cytopathic effect (CPE) assays against a panel of viruses from different families.[1] Compound specificity against Japanese encephalitis virus (JEV, strain 14-14-2; Nepal JEV Institute), yellow fever virus (YFV, strain 17-D; United States Army Medical Research Institute for Infectious Disease [USAMRIID]), Chikungunya (CHIKV, strain 181-25, USAMRIID), dengue-2 (DENV2, strain New Guinea C; University of Texas Medical Branch [UTMB]), dengue-1 (DENV1, strain TH-S-MAN; UTMB), dengue-3 (DENV3, strain H87; UTMB), respiratory syncytial virus (RSV, strain A2; Functional Genetics, Gaithersburg, MD), vaccinia (VACCV, strain NYCBH; USAMRIID), dengue-4 (DENV4, strain H241; UTMB), influenza H1N1 (INFV, strain A/PR/68; Charles River Laboratories, Wilmington, MA) was assessed at Integrated Biotherapeutics, Inc. (Gaithersburg, MD). Briefly, Vero cells (for DENV, JEV, RSV, CHIKV, and YFV), BSC-40 cells (for VACCV), or Madin-Darby canine kidney cells (for INFV) were seeded for infection in 96-well plates at 10^4 cells per well in minimal essential medium, Dulbecco's modified minimal essential medium, or UltraMDCK (supplemented with 1 µg/mL tosyl phenylalanyl chloromethyl ketone [TPCK]-treated trypsin), respectively, and containing 2 mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, and fetal bovine serum (Invitrogen, Carlsbad, CA; 5% for BSC-40 cells, 1% for Vero cells, and 0% for UltraMDCK). Cells were incubated at 37°C in humid incubator containing 5% CO_2. Dose-response curves were generated by measuring CPE at a range of compound concentrations. Eight compound concentrations (100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 µM) were used to generate inhibition curves suitable for calculating the EC_{50} from virus-induced CPEs. Compound dilutions were prepared in DMSO prior to addition to the cell culture medium. The final DMSO concentrations in all samples were 0.1%. Cells were infected with approximately 0.1 plaque-forming units per cell approximately 1 h after addition of compound. At a virus-dependent 4 to 6 days after infection, cultures were fixed with 5% glutaraldehyde and stained with 0.1% crystal violet in 5% methanol. Virus-induced CPE was quantified spectrophotometrically by absorbance

at 570 nm. EC_{50}s were calculated by fitting the data to a four-parameter logistic model to generate a dose-response curve using XLfit 5.2 (equation 205, IBDS, Emeryville, CA). The linear correlation coefficient squared (R^2) for fitting data to this model was typically > 0.98%. From this curve, the concentration of compound that inhibited virus-induced CPE by 50% was calculated. As controls, uninfected cells and cells receiving virus without compound were included on each assay plate, as well as the reference agent ribavirin (Sigma) when applicable.

The effects of compound 14 on herpes simplex virus-1 (strain HF evaluated in Vero cells; virus and cells obtained from the American Type Culture Collection) were assessed as described previously.[2]

Supplemental Table 1. Antiviral spectrum of compound 14.

Virus strain*	IC_{50} (µM)	TC_{50} (µM)	Antiviral index (TC_{50}/IC_{50})
HSV-1	> 100	>100	NA
DENV (serotypes 1-4)	> 100		NA
RSV	> 100		NA
YFV	> 100		NA
JEV	> 100		NA
H1N1	> 100		NA
Vaccinia	> 100		NA
Chikungunya	> 100		NA

HSV-1 = herpes simplex virus 1; DENV = Dengue virus; RSV = respiratory syncytial virus; YFV = yellow fever virus 17D vaccine strain; JEV = Japanese encephalitis virus 14-14-2; H1N1 = influenza strain A/PR/8/34; NA = not applicable.

References

1. Kortagere, S.; Madani, N.; Mankowski, M. K.; Schon, A.; Zentner, I.; Swaminathan, G.; Princiotto, A.; Anthony, K.; Oza, A.; Sierra, L. J.; Passic, S. R.; Wang, X.; Jones, D. M.; Stavale, E.; Krebs, F. C.; Martin-Garcia, J.; Freire, E.; Ptak, R. G.; Sodroski, J.; Cocklin, S.; Smith, A. B., 3rd. *J. Virol.* **2012,** *86*, 8472.

2. Ptak, R. G.; Gallay, P. A.; Jochmans, D.; Halestrap, A. P.; Ruegg, U. T.; Pallansch, L. A.; Bobardt, M. D.; de Bethune, M. P.; Neyts, J.; De Clercq, E.; Dumont, J. M.; Scalfaro, P.; Besseghir, K.; Wenger, R. M.; Rosenwirth, B. *Antimicrob. Agents Chemother.* **2008,** *52*, 1302.