
Samples in DMSO: What an end user needs to know

Christopher A. Lipinski
Adjunct Senior Research Fellow
Pfizer Global R&D
Groton New London Labs
Christopher.a.lipinski@pfizer.com

Who is an end user

- ◆ **Stores HTS plates in lab for a few weeks to 3 months**
- ◆ **Room temperature chemical degradation**
 - **5% at 3 months**
 - **10% at 6 months**
- ◆ **By analogy to $p = 0.05$ store samples for up to 3 months then dispose**
- ◆ **CAVEAT**
 - **trifluoroacetic acid (TFA) contamination**

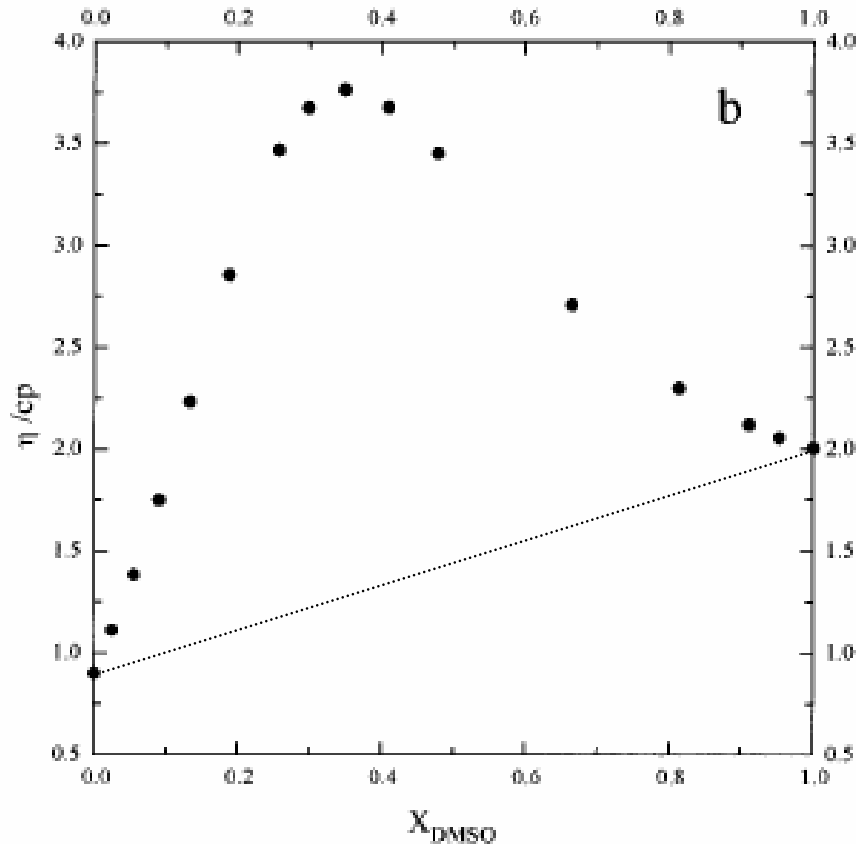
Why is DMSO hygroscopic?

- ◆ Liquid A in equilibrium with vapor of liquid B
- ◆ Ideal behavior case
 - liquids have no interactions
- ◆ Sealed container molar composition depends on:
 - starting molar concentrations of A and B
 - relative vapor pressures of A and B
 - 20⁰ C vapor pressure ratio is 41.66 H₂O to 1 DMSO
- ◆ DMSO and water have pronounced interactions
- ◆ Non ideal behavior
- ◆ Driver for DMSO hygroscopic behavior

DMSO and water, non-ideal behavior

- ◆ **DMSO plus water exhibits very non ideal behavior**
 - change maximum at 33% by weight water
 - corresponds to molar ratio 1 DMSO : 2 H₂O
 - liquid phase is very structured
 - ice-1 like lattice structure
 - more H-bonding, more order than in pure water
- ◆ **Melting point declines from 18 deg C to -73 deg C**
- ◆ **Viscosity increases as water is added to DMSO**
- ◆ **Solution gets colder as water is added to DMSO**
- ◆ **DMSO is very hygroscopic**
 - (J Org Chem 2001, 66, 5846-5852)

Viscosity and water addition to DMSO



High

Viscosity of
the solution

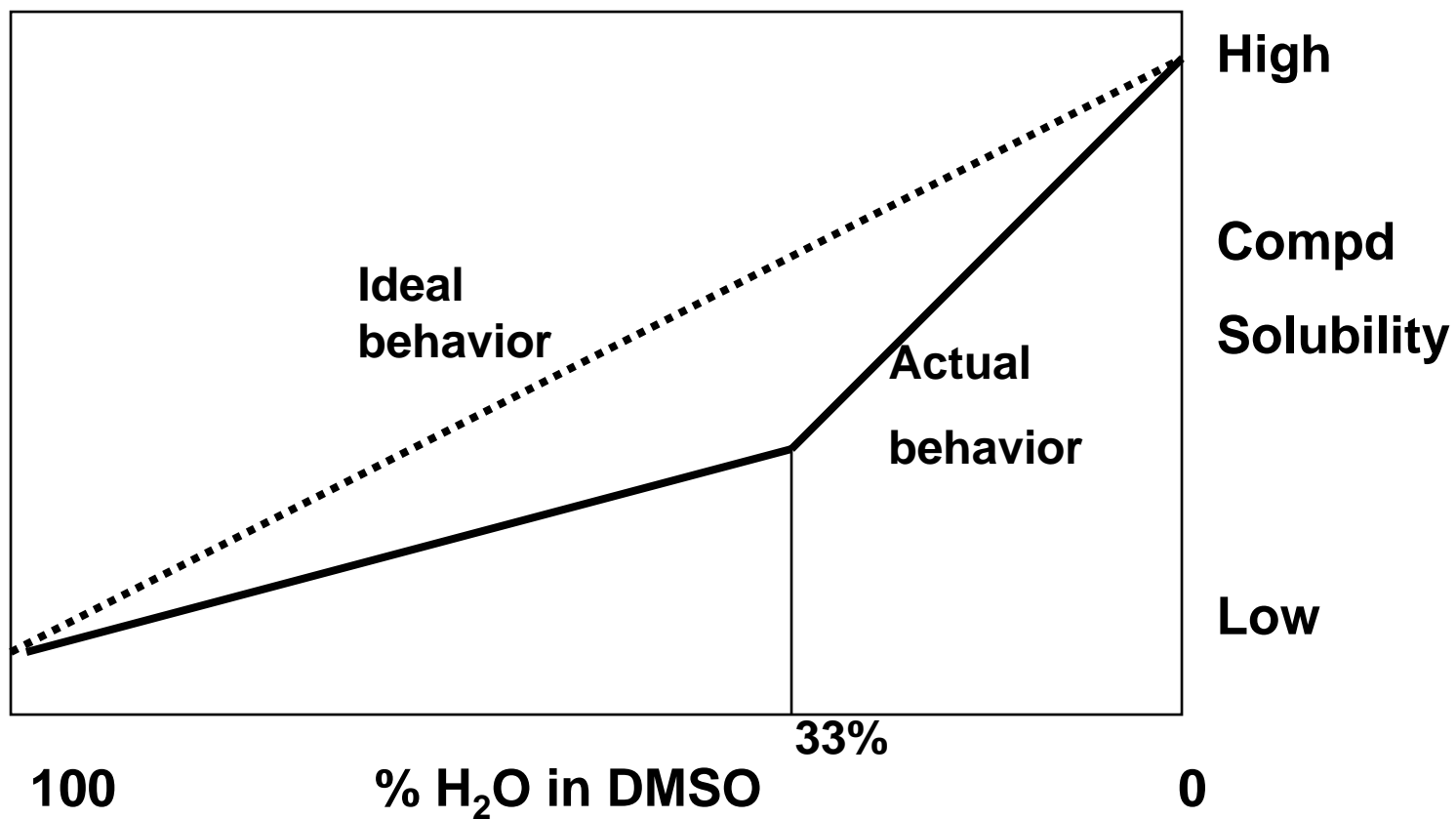
Low

Mole fraction of DMSO

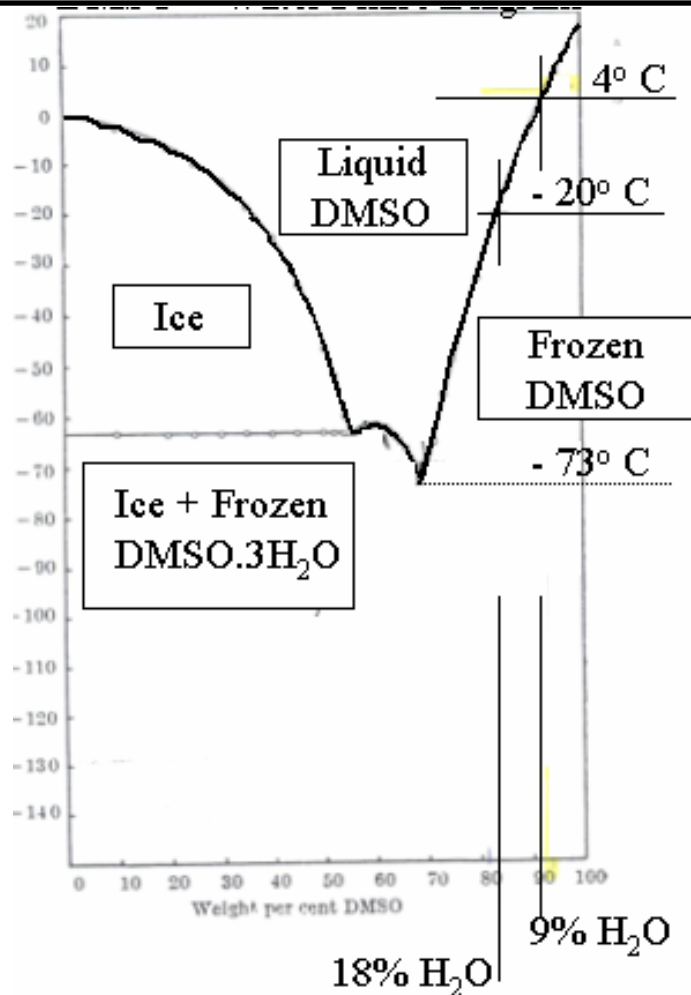
Compound solubility in DMSO and H₂O

- ◆ As H₂O is added solvent is more structured
- ◆ More difficult to form a cavity
- ◆ More difficult to dissolve a large lipophilic compound
- ◆ Solubility non ideality is expected to be worst at 33% by weight H₂O
- ◆ Chaotropic compound will be less sensitive to the H₂O effect
- ◆ PEGS show a similar behavior to DMSO
- ◆ Keep DMSO dry! Single use tubes or inert atmosphere storage modules

DMSO solubility dependence on H₂O



DMSO - water phase diagram



DMSO containing 9% water is unfrozen in the typical lab refrigerator

Very bad practice. Typical biology procedure of storing samples in DMSO in the non-freezer part of a lab refrigerator

Nature (1969), 220, 1315-1317

Storage Pod to keep DMSO plates / tubes dry



StoragePod™

4titude™

<http://www.4ti.co.uk>

Compound storage and compound concentration

- ◆ Nobody knows compound concentration
- ◆ 2 - 4 x concentration error is insignificant in HTS
- ◆ 10 – 100 x concentration error is significant
- ◆ IC-50 orders get scrambled
- ◆ Selectivity panels are inaccurate
- ◆ Compounds precipitate from DMSO
- ◆ Compounds precipitate from buffer
- ◆ LLQ issue in buffer quantitation

Streamlined System for Purifying and Quantifying a Diverse Library of Compounds and the Effect of Compound Concentration Measurements on the Accurate Interpretation of Biological Assay Results. *Anal. Chem.* (2004), 76(24), 7278-7287.

Improving quality by analytical

- ◆ **Compound identity**
- ◆ **Compound purity**
- ◆ **Compound concentration**
- ◆ **Adding analytical quantitation to HTS is the single greatest HTS quality advance possible**
- ◆ **Technically requires 100-1000 x improvement in technology efficiency**
 - **CLND detection**
 - **ELSD detection**
 - **reduced solvent suppression MS**

Matrical / Pfizer freeze thaw data

High Throughput Sonication: Evaluation for Compound Solubilization

Kevin Oldenburg¹, Douglas Pooler¹, Kurt Scudder¹, Christopher Lipinski² and Michele Kelly². *

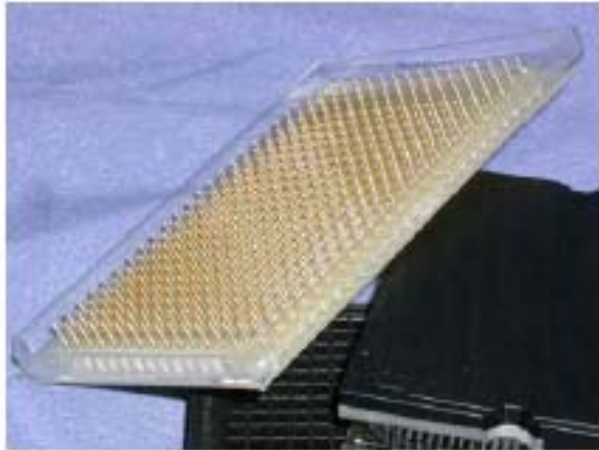
¹*Matrical, Inc., Spokane, WA*, ²*Pfizer Global Research and Development, Groton, CT, USA*

Combinatorial Chemistry & High Throughput Screening, 2005, 8, 499-512

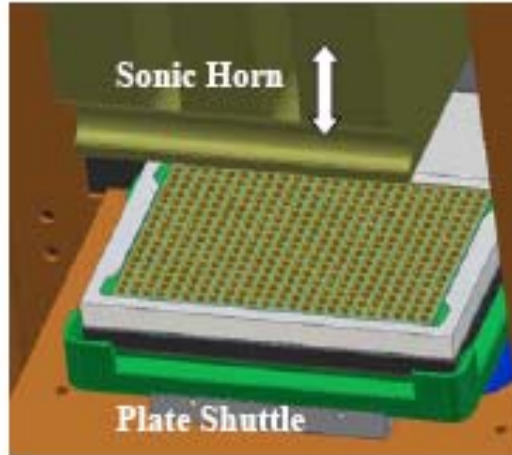
The adverse effect of freeze thaw cycles on DMSO stock solutions stored in plate format as a result of cherry picking operations has led to the gradual replacement of plate-based storage with tube-based storage so as to minimize the number of freeze thaw cycles. Compound solubility in DMSO is markedly decreased by uptake of small quantities of water

Finally, we demonstrate that precipitation of compound from DMSO stock solutions is synergistically enhanced by water uptake into DMSO compound stock solutions as well as by increasing the number of freeze thaw cycles.

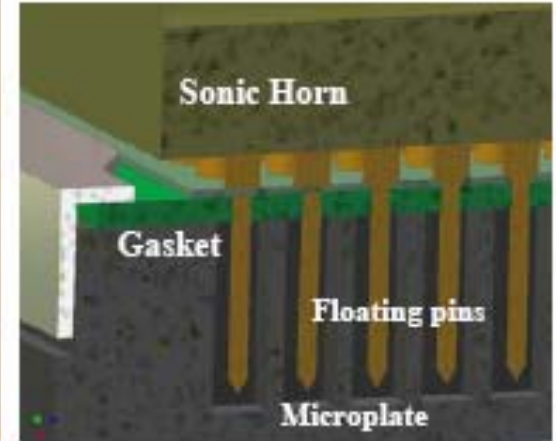
Sonication puts 50% of solids into solution



Disposable 384-sonication lid



Sonic horn transfers energy directly to samples via lid



Gasketed lid design ensures airtight seal during sonication

<http://www.matrical.com/SonicMan.htm>

Sonic / Acoustic effects on re-dissolution

- ◆ **Expected effects with contact sonication**
 - excellent mixing
 - efficient cell lysis
 - speed up equilibration time
 - minor effect - may induce precipitation
- ◆ **Unexpected beneficial effects**
 - 50% dissolving of precipitated samples
 - light scattering data
 - rescue of expired HTS plates
 - similar dissolving with contact-less acoustics
- ◆ **Theory of re dissolving is speculative**

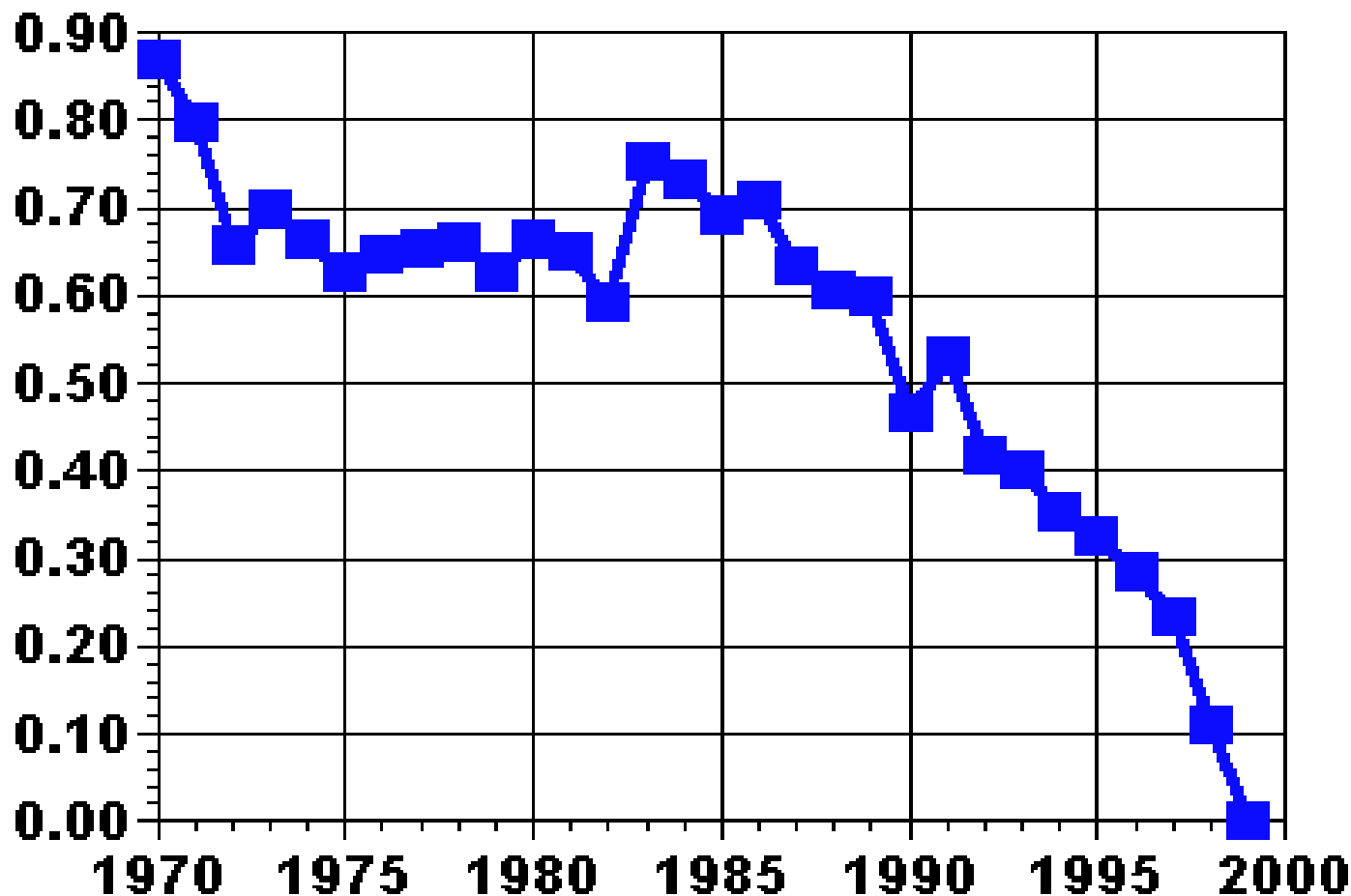
Sonication, why does it work?

- ◆ **Redissolution from both contact and external sonication**
- ◆ **Redissolved material is thermodynamically more stable, less soluble so dissolution is counter to thermodynamics**
 - modest bulk temperature increase, Temp < 60 °C
 - no chemical degradation by HPLC
- ◆ **Explanation?**
 - cavitation - local temperature, pressure gradients?

Synergy between water uptake and freeze thaw

- ◆ **Super saturation in DMSO is high**
 - possible to see freeze thaw effect
- ◆ **Super saturation in DMSO is low to moderate**
 - difficult to see freeze thaw effect
- ◆ **Water in DMSO increases super saturation**
- ◆ **Water in DMSO greatly decreases solubility**
- ◆ **A little water in DMSO decreases compound solubility a whole lot**

Fraction of Pfizer Groton compounds having melting point field information



Compounds differ in aqueous and DMSO solubility based on crystalline form

- ◆ **Ostwalds “rule of stages”**
- ◆ **Sequence of compound batch isolation proceeds towards thermodynamically most stable form**
 - 1 - amorphous - highest energy solid form
 - 2 - highest energy crystalline polymorph
 - 3 - lowest energy crystalline polymorph
- ◆ **Amorphous is the highest energy form**
 - most soluble in water and DMSO
- ◆ **Ostwald ripening**
 - amorphous suspension turns crystalline
 - inhibited by insoluble co-phase

Dimension of the solubility problem

- ◆ 40% of compounds have poor aqueous solubility
- ◆ Half the problem due to size / lipophilicity
- ◆ Half the problem due to crystal packing
- ◆ DMSO no better than water for compounds insoluble due to crystal packing
- ◆ Suggests an upper limit of 20% compounds insoluble in dry DMSO at 10uM
 - supported by NCI Frederick, MD studies
- ◆ Will this limit ever be achieved?
- ◆ Depends on whether a nucleation event occurs
- ◆ Depends on number of freeze thaw cycles

Taking advantage of amorphous states

- ◆ **Amorphous state enhances dry DMSO solubility**
 - negligible problems at 60 mM
- ◆ **Keep the DMSO dry**
- ◆ **Avoid cooling in less than 3 months storage**
- ◆ **No freeze thaw cycles**

Solubility and compound purity

- ◆ **Pressure on chemistry to increase output**
 - crystallization has disappeared
- ◆ **Combinatorial compounds are now being purified by automated procedures**
 - 85-95% pure by ELSD or UV
- ◆ **Compounds “appear” more soluble**
 - amorphous state
 - impurities enhance solubility
 - crystal growth retardation
- ◆ **Compressed samples (multiplexing)**
 - multiple compounds in a well
 - solubility is enhanced

Pro's and con's of amorphous compounds

- ◆ **Pro:**
- ◆ **Amorphous DMSO solubility is always higher than when compound is crystalline**
- ◆ **Amorphous compounds from combichem or medchem initially easily dissolve in DMSO**
 - **allows easy preparation of DMSO stocks**
- ◆ **Con:**
- ◆ **Sets stage for later precipitation problems**
 - **if the DMSO gets wet**
 - **if there are freeze thaw cycles**
 - **if samples are cooled while liquid**

Timing factor in compound DMSO solubility

- ◆ **Once a compound crystallizes from DMSO it will not easily re-dissolve**
 - crystallized compound is in a lower energy, higher melting point, less DMSO soluble form
- ◆ **Narrow working window (time window) for keeping most compounds dissolved in DMSO**
 - 1 to 2 days at room temperature
 - explains why compounds are active when freshly made but not when stored
- ◆ **Freeze thaw cycles increase the probability of crystallization**

Alternatives to DMSO?

- ◆ **Organic solvent – evaporate store as film**
 - **chemical stability issues**
 - **poor control of plasticity – crystallization**
 - **wet-ability issues – glycerol in DMSO**
- ◆ **Additive in DMSO to retard crystallization from H₂O**
 - **pluronic copolymer**
 - **block ethylene /propylene oxide copolymer**
 - **very low CMC**
 - **two solubilization mechanisms are possible**
 - **micellar solubilization**
 - **kinetic retardation of crystal growth**

<https://worldaccount.basf.com/wa/NAFTA/Catalog/ChemicalsNAFTA/pi/BASF/Brand/pluronic>

Functionality most sensitive to precipitation

- ◆ **Carboxylic acids**
 - 4 x more likely to precipitate from wet DMSO
 - DMSO poor solvation for anions
- ◆ **Zwitterionic compounds**

Beware of trifluoroacetic acid (TFA)

Trifluoroacetate, a contaminant in purified proteins,
inhibits proliferation of osteoblasts and chondrocytes

J. CORNISH,¹ K. E. CALLON,¹ C. Q.-X. LIN,¹ C. L. XIAO,¹
T. B. MULVEY,² G. J. S. COOPER,^{1,2} AND I. R. REID¹
*¹Department of Medicine and ²School of Biological Sciences,
University of Auckland, Auckland 1001, New Zealand*

Am. J. Physiol. 277 (Endocrinol.Metab. 40): E779–E783, 1999.

10 nM TFA is cytotoxic in a 24 hour cell culture

Dry DMSO maximises stability with TFA present

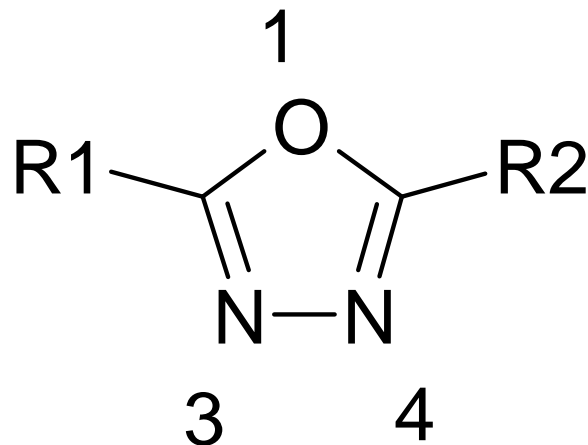
Studies of the Relative Stability of TFA Adducts vs Non-TFA Analogues for Combinatorial Chemistry Library Members in DMSO in a Repository Compound Collection

Jill Hochlowski,* Xueheng Cheng, Daryl Sauer, and Stevan Djuric

*Abbott Laboratories, 100 Abbott Park Road,
Abbott Park, Illinois, 60064*

J Combinatorial Chemistry, vol 5, no 4, July/August 2003

Heterocycle sensitive to TFA acid hydrolysis



2,5-substituted-1,3,4-oxadiazole

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gratefully acknowledged**