A Comparative Analysis of the Measured Thermodynamic Properties of Azithromycin, Bacitracin and Levofloxacin with In Silico Methods

Anita Elizabeth Luana Mae Perez[1], Geneva Hiromi Mackey[1], Victor Hugo Ruiz[1], Basanth Babu Eedara[1], David Encinas-Basurto[1], and Heidi M. Mansour[1,2]

[1]*R. K. Coit College of Pharmacy, Skaggs Pharmaceutical Science Center, The University of Arizona, Tucson, AZ, USA.*
[2]*College of Medicine, Department of Medicine, The University of Arizona, Tucson, AZ, USA.*

THE UNIVERSITY OF ARIZONA R. Ken Coit College of Pharmacy

THE UNIVERSITY OF ARIZONA College of Medicine Tucson

Pharmacy

Background

- Antibiotics can be described as medications that inhibit or destroy the growth of microorganisms and considered life saving development in the medical world. The antibiotics, azithromycin, bacitracin, and levofloxacin, act by inhibiting protein synthesis, cell wall synthesis and DNA synthesis, respectively.
- Molecular modelling analytical software program is utilized to predict a molecule's chemical and pharmacokinetic properties.
- Differential scanning calorimetry (DSC) is an analytical technique that utilizes small amounts of a drug sample and heat to analyse the physical properties and thermal transitions of a pharmaceutical material.
- Hot stage microscopy (HSM) is a combination of thermal and polarized light microscopy technique that is used to observe the presence or absence of birefringence characteristic of crystallinity.
- Using analytic data, molecular modelling and pharmacokinetic/ pharmacodynamic (PK/PD) predictive software we can further understand both physicochemical properties and how such drugs may interact in the body.

Objectives

- The primary purpose of this project is to identify the molecular and physicochemical properties of azithromycin, bacitracin and levofloxacin and investigate how they compare to *in vitro* derived findings.

Methods

- Data collection was obtained by using molecular modeling software SwissADME®, ChemDraw19.1® and Chem3D version 19.1® CambridgeSoft, Cambridge MA, USA.
- DSC was performed on a TA Q1000 differential scanning calorimeter (DSC) (TA Instruments, New Castle, DE, USA) equipped with T-Zero® technology, RSC90 automated cooling system, auto sampler and calibrated with indium.
- HSM studies used a Leica DMLP cross-polarized microscope (Wetzlar, Germany) equipped with a Mettler FP 80 central processor heating unit and Mettler FP82 hot stage (Columbus, OH, USA). The images were digitally captured using a Nikon Coolpix 8800 digital camera (Nikon, Tokyo, Japan) under 10x optical objective and 10x digital zoom.

Results

Azithromycin (Zithromax®) (Figure 1,2,3)

Fig. 1. Ball and stick model of azithromycin via Chem3D 19.1™

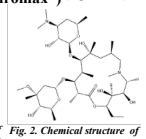

Fig. 2. Chemical structure of azithromycin via ChemDraw19.1™

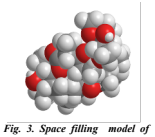

Fig. 3. Space filling model of azithromycin via Chem3D 19.1™

Bacitracin (Bacilim®) (Figure 4,5,6)

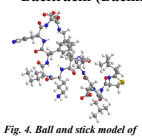

Fig. 4. Ball and stick model of bacitracin via Chem3D 19.1™

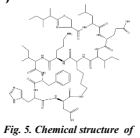

Fig. 5. Chemical structure of bacitracin via ChemDraw19.1™

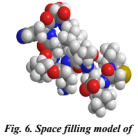

Fig. 6. Space filling model of bacitracin via Chem3D 19.1™

Levofloxacin (Levaquin®) (Figure 7,8,9)

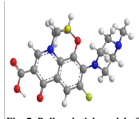

Fig. 7. Ball and stick model of levofloxacin via Chem3D 19.1™

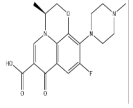

Fig. 8. Chemical structure of levofloxacin via ChemDraw19.1™

Fig. 9. Space filling model of levofloxacin via Chem3D 19.1™

*Table 1. Retrieval of pharmacokinetic and physicochemical properties of azithromycin (Azith), bacitracin (Bac) and levofloxacin (Levo) using SwissADME® and ChemDraw™; Data obtained from * Thermo Fisher Scientific Inc, **ChemSpider™*

Pharmacokinetic Properties	SwissADME®			ChemDraw®		
	Azith	Bac	Levo	Azith	Bac	Levo
Molecular Weight (g/mol)	748.98	134.64	361.37	749	137.62	361.37
tPSA (Å²)	180.08	556.17	75.01	180.08	428.37	73.32
LogP	2.13	-2.01	1.15	2.64	2.79	-0.51
LogS	-6.55	-3.10	-1.99	-4.08	-10.8	-3.85
H Bond Donor	5	17	1	--	--	--
GI Absorption	Low	Low	High	--	--	--
BBB Perm	No	No	No	--	--	--
Lipinski Rule of 5	No, 2 Violations	No, 3 Violations	Yes, 0 Violations	--	--	--
Boiling Point (°C)	--	--	--	717*	571.5**	699.73
Melting Point (°C)	--	--	--	126*	224**	520.92

Results Continued

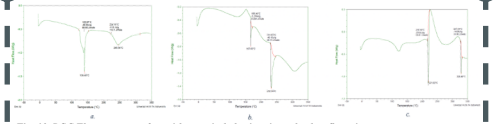

Fig. 10. DSC Thermograms of a. azithromycin b. bacitracin and c. levofloxacin

Table 2. Thermogram analysis of azithromycin, bacitracin and levofloxacin (n=3, mean ± standard deviation)[4]

	Max Peak (°C)	Enthalpy (J/g)
Azithromycin	137.67 ± 1.07	86.15 ± 15.95
Bacitracin	168.78 ± 16.44	1.64 ± 3.82
Levofloxacin	173.82 ± 51.65	30.82 ± 19.74

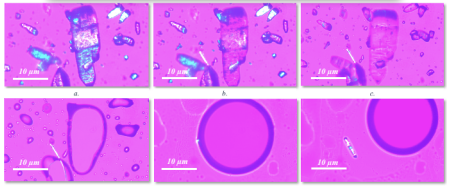

Fig. 11. HSM micrograph of azithromycin at a. 37 °C b.75 °C c.125 °C d. 130 °C e. 230 °C f. 245 °C.

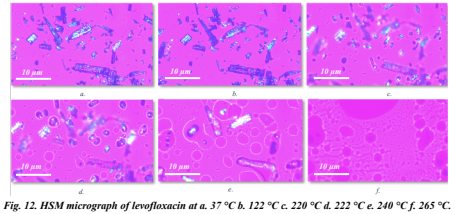

Fig. 12. HSM micrograph of levofloxacin at a. 37 °C b. 122 °C c. 220 °C d. 222 °C e. 240 °C f. 265 °C.

Discussion

- Pharmacokinetic and pharmacodynamic parameters obtained from molecular modelling software are shown in Table 1. These values help predict how a compound may behave in the body. Azithromycin and bacitracin indicate low gastrointestinal absorption as reflected by their tPSA values. The three drugs demonstrate an increased potential of lipophilicity as reflected by their logP values that are less than 5.
- The standard deviation as represented in Table 2. of azithromycin, bacitracin reflected low variation from the samples runs conducted. However, levofloxacin displayed the highest variation and that could have been due to various possible errors while conducting the experiments.
- Phase transition temperatures and enthalpies are summarized in Table 2. The first and second peaks in the DSC thermograms of each antibiotic may be indicative of a solid state transition (melting and charring) that is endothermic. This shows the energy /heat is being absorbed by the system. The enthalpy of the systems are overall positive. This indicates that the systems are absorbing heat.
- Representative images for the HSM studies are displayed in Figures 11 and 12. Azithromycin and levofloxacin exhibited birefringence confirming their crystallinity. Both azithromycin showed a solid state to liquid state transition at about 125 °C and levofloxacin at 222 °C, corresponding to their melting points predicted with molecular modelling software. These images were in good agreement with the DSC data previously described.

Acknowledgements

We would like to sincerely thank Dr. Mansour, Dr. Ruiz, Dr. Eedara, and Dr. Encinas for their mentorship, guidance and support throughout this project. We would also like to thank Neftali Ortega-Alarcon for his assistance in the lab and with this project. Both authors shared equal contribution in the completion of this project.

References

1. Spink CH. Differential scanning calorimetry. Methods in Cell Biology. 2008; 84:115–41.
2. Muralidharan P, Acosta M, Hayes D, Black S et al. Solid- state physicochemical characterization and microscopy of particles in dry powder inhalers. Inhalation. 2016; 10:20-7.
3. Acosta M, Abrahamson M, Encinas-Basurto D, Fineman J et al. Inhalable nanoparticles/ microparticles of AMPK and Nrf2 activator for targeted pulmonary drug delivery as a dry powder inhaler. The AAPS Journal. 2021;23:1-14.
4. Furey, Edward "Standard Deviation Calculator" from Calculator Soup.com, http://www.calculatorsoup.com