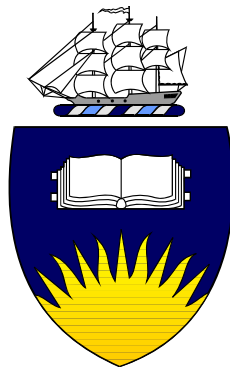


# **PHYSICOCHEMICAL DETERMINANTS OF THE NON- SPECIFIC BINDING OF DRUGS TO HUMAN LIVER MICROSOMES**

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Thesis submitted for the degree of Doctor of Philosophy  
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June 2008



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## **DECLARATION**

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I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university and that to the best of my knowledge and belief, it does not contain any material previously published or written by another person except where due reference is made in the text.

James A McLure

11 June 2008

## ACKNOWLEDGEMENTS

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I would like to thank God, my family; the late Oma and Opa and Gran and Grandpa, India, Jet, Rocks, Mum, Dad, Fiona, Anthony, Samuel, Molly, Luke, Andrew, Cameron, Trent, Alisdair, Corinne, Oliver, Lewis, Claudia, Siobhan, Michael, Thomas, Anabelle, Phoebe, Patrick, Suzanne, Edvardas, Sophia, Benjamin, and Sarah for their love, belief and support.

To Professor Donald Birkett, thank you for your guidance, willingness to teach, encouragement, and support. To Professor John Miners thank you for your support, understanding, and friendship. Thank you both for believing in me.

To our Tuesday night Grow group, thank you everyone for your understanding, encouragement, genuinity, and support.

To Dr Gill thank you for helping to piece my life back together.

To all my team mates and friends at the Woodville West Torrens Football Club, Goolwa-Port Elliot Football Club, Callington United Eagles Football Club, and Henley Football Club a sincere thank you for your support.

My thanks, appreciation, and extended friendship go to Heather Aubert, Sally Coulter, Dr Andrew Stone, Dr Samuel Boase, Kushari Bowalgaha, Andrew Rowland, Dr Thomas Polasek, Dr Matthew Sykes, Benjamin Lewis, and David Elliot. I really enjoyed working with all of you.

I would like to thank the Department of Clinical Pharmacology, Flinders University, for their support from 2000 – 2002 and 2004, Flinders University for their support in 2003, and the Australian Society of Clinical and Experimental Pharmacologists and Toxicologists (ASCEPT) for travel grants in 2002 and 2004. I would also like to

thank the Flinders Medical Centre Foundation for a travel grant in 2002. A further thank you to the Australian Government for their support over the past four years.

I could not have completed this thesis without all of the aforementioned support. I am sincerely grateful to everyone who has been there helping me to learn, held my hope (Dad), or offered friendship.

James A McLure

11 June 2008

## **PUBLICATIONS AND AWARDS**

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### **Publications**

McLure JA, Sorich MJ, Miners JO, Smith PA, Birkett DJ 2002, 'Determinants of the membrane binding of drugs in microsomal *in vitro* systems', *Proceedings of the Australian Health and Medical Research Congress*, Poster Abstracts 2118.

McLure, JA, Birkett, DJ, Miners, JO 2004, 'Determinants of the non-specific binding of drugs to human liver microsomes', *Clinical and Experimental Pharmacology and Physiology* 31, PO-209.

McLure, James, Miners, John, Birkett, Donald 2006, 'The development of a fluorescence technique for measuring the non-specific binding of drugs to human liver microsomes' *Acta Pharmacologia Sinica* July; Supplement 1, p. 221, P170084.

McLure, JAL 2006, 'Development of an 8-anilinonaphthalene-1-sulfonate (ANS) fluorescence technique for measuring the non-specific binding of drugs to human liver microsomes' *Proceedings of the Australian Health and Medical Research Congress* ISSN 1447-6610, p. 416, Abstract # 1364.

### **Awards**

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## ABSTRACT

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Accurate determination of the *in vitro* kinetic parameters  $K_m$  (Michaelis constant) and  $K_i$  (inhibition constant) is critical for the quantitative prediction of *in vivo* drug clearance and the magnitude of inhibitory drug interactions. A cause of inaccuracy *in vitro* arises from the assumption that all drug added to an incubation mixture is available for metabolism or inhibition. Many drugs bind non-specifically to the membrane of the *in vitro* enzyme source.

The aims of this thesis were to: 1) investigate the comparative importance of lipophilicity (as  $\log P$ ), and  $pK_a$  as determinants of the non-specific binding of drugs to human liver microsomes; 2) develop and validate an ANS fluorescence technique for measuring the non-specific binding of drugs to human liver microsomes; 3) characterise the non-specific binding of a large dataset of physicochemically diverse drugs using the ANS fluorescence procedure; 4) evaluate relationships between selected physicochemical characteristics and the extent of non-specific binding of drugs to human liver microsomes and; 5) computationally model the non-specific binding of drugs to discriminate between high binding ( $f_{u(mic)} < 0.5$ ) and low binding ( $f_{u(mic)} \geq 0.5$ ) drugs.

The comparative binding of the basic drugs atenolol ( $\log P = 0.1$ ;  $f_{u(mic)} = 1.00$ ), of propranolol ( $\log P = 3.1$ ;  $f_{u(mic)} = 0.36 - 0.84$ ), and imipramine ( $\log P = 4.8$ ;  $f_{u(mic)} = 0.42 - 0.82$ ) suggested that lipophilicity is a major determinant of non-specific binding. In contrast, the comparative binding of diazepam ( $pK_a = 3.3$ ;  $f_{u(mic)} = 0.69 - 0.80$ ), a neutral compound; and the bases propranolol ( $pK_a = 9.5$ ;  $f_{u(mic)} = 0.36 - 0.84$ ) and lignocaine ( $pK_a = 9.5$ ;  $f_{u(mic)} = 0.98$ ), indicated that  $pK_a$  was not a determinant of the extent of non-specific binding. The non-binding of lignocaine, a relatively



lipophilic base, was unexpected and confirmed by the non-binding of the structurally related compounds bupivacaine and ropivacaine. These results implicated physicochemical characteristics other than lipophilicity and charge as important for the non-specific binding of drugs to human liver microsomes.

An assay based on 1-anilinonaphthalene-8-sulfonate (ANS) fluorescence was developed using the seven drugs employed in the initial study. Non-specific binding data from equilibrium dialysis and the ANS fluorescence methods were compared and a linear correlation ( $r^2 = 0.92$ ,  $p < 0.01$ ) was observed at drug concentrations of 100 and 200  $\mu\text{M}$ . The approach was further validated by characterising the microsomal binding of nine compounds (bupropion, chloroquine, chlorpromazine, diflunisal, flufenamic acid, meclofenamic acid, mianserine, triflupromazine, and verapamil) using both binding methods (i.e. equilibrium dialysis and ANS fluorescence). A significant logarithmic relationship ( $r^2 \geq 0.90$ ) was demonstrated between  $f_{u(\text{mic})}$  and the modulus of ANS fluorescence for all drugs and for basic drugs alone at concentrations of 100 and 200  $\mu\text{M}$ , while the acidic/neutral drugs showed a significant linear relationship ( $r^2 \geq 0.84$ ) at these two concentrations ( $p < 0.01$ ). The non-binding of bupropion provided further evidence that physicochemical properties other than  $\log P$  and charge were important for non-specific binding of drugs to human liver microsomes.

The ANS fluorescence technique was then used to characterise the non-specific binding of 88 physicochemically diverse compounds. In general, acids and neutrals bound to a 'low' extent ( $f_{u(\text{mic})} \geq 0.5$ ) whereas bases bound the full  $f_{u(\text{mic})}$  range (0.0001 – 1). Statistically significant relationships were observed between the non-specific binding of bases and  $\log P$ , the number of hydrogen bond donors and hydrogen bond acceptors per molecule, and molecular mass.

Preliminary *in silico* modeling of the dataset generated by the ANS fluorescence technique, using the program ROCS, provided discrimination of all but one (itraconazole) of the ‘high’ binding bases. However, there were 14 false positives, resulting in low overall prediction accuracy.

Taken together, the studies conducted in this thesis provide important insights into the physicochemical factors that determine the non-specific binding of drugs to human liver microsomes.

## ABBREVIATIONS

---

ADME	Absorption, Distribution, Metabolism, Excretion
ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicology
ANS	1-anilino-8-naphthalene sulfonate
$B_{\max}$	maximum binding capacity
Caco-2	intestinal cell line from human colorectal cancer
Chol	cholesterol
CL	clearance
$C_B$	concentration of bound drug
$C_F$	concentration of free drug
$CL_H$	hepatic clearance
$CL_{\text{int}}$	intrinsic clearance of drug metabolising enzyme(s)
$C_0$	initial concentration at time zero
$CL_S$	systemic clearance
$C_{SS}$	concentration of drug in plasma at steady state
$C_t$	drug concentration at time t after the dose
CYP	cytochrome P450
D	drug
$D_F$	free drug
DMSO	dimethylsulfoxide
$D_N$	dispersion number
$E_H$	hepatic extraction ratio
$f_m$	fraction of dose metabolised along pathway of interest
$f_u$	fraction of drug unbound in blood
$f_{u(\text{inc})}$	fraction of drug unbound in an incubation

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$f_{um}$	fraction of drug unbound in an incubation medium
$f_{u(mic)}$	fraction of drug unbound in microsomes
$f_{u(T)}$	fraction of drug unbound in tissue
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HPLC	high performance liquid chromatography
I	inhibitor
$I_u$	unbound inhibitor
IND	Investigational New Drug
k	elimination rate constant
$K_D$	dissociation constant
$K_i$	inhibition constant
$K_m$	Michaelis constant (substrate concentration at half maximal velocity)
$K_{m(app)}$	apparent Michaelis constant
$\log P$	$\log$ of the concentration of drug in the lipid phase / concentration of drug in the aqueous phase
M	metabolised
MM	molecular mass
MW	molecular weight
NDA	New Drug Application
NMR	nuclear magnetic resonance
NNN'N' TMED	NNN'N' tetramethylethylenediamine
NSAID	non steroidal anti-inflammatory drug
PB	potassium phosphate buffer, 0.1M, pH 7.4

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PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PS	phosphatidylserine
PSA	polar surface area
$Q_H$	liver blood flow
QSAR	Quantitative Structure-Activity Relationship
$R_c$	ratio of the areas under the plasma drug concentration time curves in the presence and absence of inhibitor
ROCS	Rapid Overlay of Chemical Structures
S	substrate
SD	standard deviation
SM	sphingomyelin
t	time
$t_{1/2}$	half life of a drug dose
UGT	UDP-glucuronosyltransferase
$V, v$	velocity or rate of metabolite formation
$V_d$	volume of distribution
$V_{max}$	maximal velocity of a reaction at a saturating substrate concentration
$V_P$	plasma volume
$V_T$	tissue volume