FACULTY OF MEDICINE

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF PHARMACOLOGY

PHPH 3101

Rational Drug Design

COURSE OUTLINE

SESSION 2, 2009

CONTENTS **PAGE**

COURSE INFORMATION 1
Objectives of the Course 1
Course Coordinator and Lecturers 1
Approach to Learning and Teaching 2

ASSESSMENT PROCEDURES 2

TEXTBOOK AND READING LIST 3

GENERAL INFORMATION 4
Attendance Requirements 4
WWW Teaching Resources 6
Student Rights and Responsibilities 6
Missed Assessment Items and Practical Classes 6
Special Consideration 7
Plagiarism 8

LECTURE and PRACTICAL OUTLINES FOR RATIONAL DRUG DESIGN 9

TIMETABLE 15

INSTRUCTIONS FOR GROUP ASSIGNMENT 17

PRACTICAL CLASS EXPERIMENTAL PROCEDURES 25
Molecular Modelling: Drug Target Visualisation 26
Drug-Receptor Interactions: Mass Spectrometry 27
Lead Identification and Screening: Radioligand Binding 29
Lead Modification: SAR 37
INSTRUCTIONS FOR LAB REPORT 45
Preclinical Toxicology: Ames Test 50

PAST EXAM QUESTIONS 56

PHPH3101 Course Information

Rational Drug Design (PHPH3101) is a 3rd year Science Course worth Six Units of Credit (6 UOC). The course is usually undertaken as part of a major in Pharmacology for the Bachelor of Science or Bachelor of Medical Sciences. The course will build on the information you have gained in Pharmacology (PHPH2011, PHPH3102 & PHPH3251) and Physiology (PHPH2101 & PHPH2201).

OBJECTIVES OF THE COURSE

This course will explore the process of drug development, from lead discovery to final drug registration. It will present drug development as a process involving target selection, lead discovery using computer-based methods and combinatorial chemistry/high-throughput screening. Safety evaluation, bioavailability, clinical trials, and the essentials of patent law will also be discussed. Along the way you will learn about molecular recognition, computer-aided drug design, and toxicology as applied to the development of new medicines

COURSE CO-ORDINATOR and LECTURERS:

Course Coordinator:
A/Prof. Renate Griffith
Rm M206 Wallace Wurth Building ph: 9385 1912
Consultation times: Wednesday 11-12am

Students wishing to see course coordinator outside consultation times should make an appointment *via* email.

Lecturers in this course:

Dr Trudie Binder	w.binder@unsw.edu.au
Dr Angela Finch	angela.finch@unsw.edu.au
Dr Ross Grant	r.grant@unsw.edu.au
A/Prof. Renate Griffith	r.griffith@unsw.edu.au
Prof. Peter Gunning	p.gunning@unsw.edu.au
Dr John Langlands	j.langlands@.unsw.edu.au
Dr Trevor Lewis	t.lewis@unsw.edu.au
Dr Lu Liu	lu.liu@unsw.edu.au
Dr Tim Murphy	tim.murphy@unsw.edu.au
A/Prof. Laurence Wakelin	l.wakelin@unsw.edu.au

COURSE STRUCTURE and TEACHING STRATEGIES

Learning activities occur on the following days and times:

- Lectures: Tuesday 5-6 pm, Wednesday 12-1 pm; **Weeks 1-12**
- Tutorials: Tuesday 3-4 pm or* Wednesday 10-11 am; **Weeks 3-9, 11-13**
- Practicals: Monday 3-6 pm; **Weeks 2-8, 12+13**
- *: Once enrolled in one of the two sessions, students cannot change.

Students are expected to attend all scheduled activities for their full duration (2 hours of lectures per week and up to 4 hours of practical and tutorial sessions per week). Students are reminded that UNSW recommends that a 6 units-of-credit course should involve about 125-150 hours of study and learning activities. The formal learning activities are approximately 60 hours throughout the semester and students are expected (and strongly recommended) to do at least the same number of hours of additional study.

Lectures will provide you with the concepts and theory essential for understanding the processes involved in drug development. To assist in the development of research and

analytical skills practical classes and tutorials will be held. These classes and tutorials allow students to engage in a more interactive form of learning than is possible in the lectures. The skills you will learn in practical classes are relevant to your development as professional scientists.

APPROACH TO LEARNING AND TEACHING

The learning and teaching philosophy underpinning this course is centred on student learning and aims to create an environment which interests, challenges, and enthuses students. The teaching is designed to be relevant and engaging in order to prepare students for future careers.

Although the primary source of information for this course is the lecture material, effective learning can be enhanced through self-directed use of other resources such as textbooks and Web based sources. Your practical classes will be directly related to the lectures and it is essential to prepare for practical classes before attendance. It is up to you to ensure you perform well in each part of the course; preparing for classes; completing assignments; studying for exams and seeking assistance to clarify your understanding.

STUDENT LEARNING OUTCOMES

PHPH3101 will develop those attributes that the Faculty of Science has identified as important for a Science Graduate to attain. These include; skills, qualities, understanding and attitudes that promote lifelong learning that students should acquire during their university experience.

Graduate Attributes

A. Research, inquiry and analytical thinking abilities
B. The capability and motivation for intellectual development
C. Ethical, social and professional understanding
D. Effective communication
E. Teamwork, collaborative and management skills
F. Information Literacy – the skills to locate, evaluate and use relevant information.

On completion of this course students should:

1. be able to describe the process of drug discovery and development
2. be able to discuss the challenges faced in each step of the drug discovery process
3. have gained a basic knowledge of computational methods used in drug discovery
4. be able to organise information into a clear report
5. be able to demonstrate their ability to work in teams and communicate scientific information effectively

ASSESSMENT PROCEDURES

- Progress exam (40 min duration): short and long answer questions **10%**
- Practical assessment (1 report) **10%**
- Group assignment (10%) and presentation (5%) **15%**
- End of session examination (3 hours duration): short and long answer q. **65%**
  (50% on lectures and tutorials, 15% on practicals)

The *practicals and tutorials* are provided to support lecture material and practise analytical skills. The practical classes and tutorials help you to develop graduate attributes A, C, D & E. During the practical course you will be required to submit a written report covering three of the practical sessions. The report itself should be in the form of a scientific communication comprising aims, results and discussion (see pages 45-49). Reports must be as concise as possible, and are limited to a maximum of 4 pages of writing (excluding tables, figures and computer traces). The report will be due Monday, September 28 (week 10). Written

assessment tasks must be accompanied by a signed plagiarism form and placed in the locked box in room MG14. The report also has to be submitted electronically *via* My eLearning VISTA, through Turnitin. A penalty will apply for late submissions (10% per day). Material covered in the Practical Classes will be examined.

Students will work in teams to research the drug discovery process of a given drug. They will submit a *written assignment* (due Monday, 14th September) and give a *presentation* (Tutorial session Week 13) on their findings. This assessment task will allow you to develop your research, information literacy, communication and time management skills, as well as allowing you to demonstrate your ability to work in a team and collaborate successfully (Graduate attributes A, D, E &F). The marking criteria and instructions are on pages 17 to 23. Written assessment tasks must be accompanied by a signed plagiarism form and placed in the locked box in room MG14. They also have to be submitted electronically *via* VISTA, through Turnitin. A penalty will apply for late submissions (10% per day).

The *progress examination* will be held during the lecture session on the 25th of August. This exam will give you feedback on how you are succeeding in the course. The *progress examination* and *end of session examination* will test not only your knowledge of the process of drug design and development but also your ability to apply the knowledge you have acquired from multiple lectures to drug development scenarios. The examination will be in the format of short and long answer questions. The questions will be based on the material covered in the lectures, practical classes and tutorials. Material covered prior to the progress exam may be again examined in the final exam. The examinations will address graduate attributes A and B. The end of session examination will be held during the official examination period.

TEXTBOOK AND READING LIST

Recommended Primary Text:

- Drug Discovery and Development; Technology in Transition. HP Rang. Elsevier Ltd 1st edition 2006.

This textbook will be available at the UNSW bookshop.

Additional reading suitable as Secondary Resources:

- Instant Notes: Medicinal Chemistry. G. Patrick. BIOS Scientific Publishing, Oxford UK, 1st edition, 2001
- An introduction to medicinal chemistry. G. L. Patrick. 4th Edition Oxford UK, Oxford University Press, 2009.
- Medicinal chemistry: an introduction. G. Thomas. Chichester UK: John Wiley, 2000.
- Textbook of Drug Design. Krogsgaard-Larsen, Liljefors and Madsen (Editors), Taylor and Francis, London UK, 2002
- Drug Discovery Handbook S.C. Gad (Editor) Wiley-Interscience Hoboken USA, 2005

These textbooks are available from the UNSW library.

Other Resources:

The following electronic journals are accessible via the UNSW library.

- Nature Reviews: Drug Discovery. In particular the Article series
- Drug discovery and development
- Drug discovery today.
- Science online special “Drug discovery” http://www.sciencemag.org/sciext/drugdisc/

Additional articles of interest will be placed on the course pages on VISTA.

COURSE EVALUATION AND DEVELOPMENT

Each year feedback is sought from students about the courses offered in the Department of Pharmacology and continual improvements are made based on this feedback. The Course and Teaching Evaluation and Improvement [CATEI] Process of UNSW is the way in which student feedback is evaluated and significant changes to the course will be communicated to

subsequent cohorts of students. Also a staff-student liaison group will be set up and students will be invited to become class representatives to seek feedback from their colleagues and meet with academic staff to discuss any issues that arise. Several improvements to PHPH3101 have been made based on feedback given in 2006 to 2008. These changes include: a new textbook, increased tutorial support and changes to lecture and practical content.

GENERAL INFORMATION

The Department of Pharmacology is part of the School of Medical Sciences and is within the Faculty of Medicine. It is located on the lower ground, 2nd and 3rd floors of the Wallace Wurth building. General inquiries can be made at the School of Medical Sciences Student enquires counter, located on the Ground Floor of the Wallace Wurth (MG14). Office hours are 9.00 am - 5:00 pm.

Professor Margaret Morris is Head of Department and appointments may be made through the Administrative Assistants in Room MG14.

There is an honours program conducted by the School. The Honours program is coordinated by Dr Angela Finch Room M207 (ph: 9385 1325). Any students considering an Honours year should discuss the requirements with the coordinator. Outstanding students may be considered for scholarships offered by the University and School and these are offered annually.

Postgraduate research degrees
The Department of Pharmacology offers students the opportunity to enter into the following graduate program:

Doctorate (***Ph.D)***: In Pharmacology. For further information contact the co-ordinator Dr Pascal Carrive (p.carrive@unsw.edu.au)

Departmental Vacation Scholarships: The Department of Pharmacology supports several summer vacation scholarships each year to enable good students to undertake short research projects within the department. For further details contact the Administrative Officer.

The School Teaching Administrator
Ms Carmen Robinson is able to provide additional information on any courses offered by the School. Student Enquires Counter MG14 Wallace Wurth, ph:9385 2464,
Email: Carmen.Robinson@unsw.edu.au

OFFICIAL COMMUNICATION BY EMAIL

All students in the course PHPH3101 are advised that e-mail is now the official means by which the School of Medical Sciences at UNSW will communicate with you. All e-mail messages will be sent to your official UNSW e-mail address (e.g. z1234567@student.unsw.edu.au) and, if you do not wish to use the University e-mail system, you MUST arrange for your official mail to be forwarded to your chosen address. The University recommends that you check your mail at least every other day. Facilities for checking e-mail are available in the School of Medical Sciences and in the University library. Further information and assistance is available from DIS-Connect, ph. 9385 1777. Free e-mail courses are run by the UNSW Library.
When contacting a lecturer with a query, it is essential that the following information is provided as a minimum: student name, student number, course number, course name.

ATTENDANCE REQUIREMENTS

Attendance at practical classes is compulsory, and must be recorded in the class roll ON THE DAY OF THE CLASS. It is your responsibility to ensure that the demonstrator

records your attendance and no discussions will be entered into after the completion of the class. Satisfactory completion of the work set for each class is essential. It should be noted that non-attendance for other than documented medical or other serious reasons, or unsatisfactory performance, **for more than 1 practical class during the session** may result in an additional practical assessment exam or ineligibility to pass the course.

BEHAVIOUR IN PRACTICAL CLASSES

The practical class is an opportunity for students to develop graduate attribute C by behaving in an ethical, socially responsible and professional manner within the practical class.

- Punctual arrival is expected.
- Turn off mobile phones before entering the class.
- A lab coat must be worn to all practical classes
- Enclosed shoes are compulsory.

Students must take due care with biological and hazardous material and make sure all equipment is left clean and functional. Those who don't adhere to these basic laboratory rules will be marked absent.

PRACTICAL CLASSES

The experimental procedure for each practical is given in this course outline, Students are required to familiarise themselves with the experimental procedure before attending each class.

In the interests of safety, special attention should be paid to any precautionary measures recommended in the notes. If any accidents or incidents occur they should be reported immediately to the demonstrator in charge of the class who will record the incident and recommend what further action is required.

Animal Experimentation

The procedures used in the laboratory classes involving *the use of animals* have been approved by Animal Care and Ethics Committee (registration number ACE04/54B). All experiments undertaken in the Department of Pharmacology adhere to the NHMRC code of conduct for animal experimentation.

NOTICEBOARDS

Noticeboards for this course can be found on the 2nd floor of the Wallace Wurth building. Current timetables and information relevant to you will be displayed here and on the course page on My eLearning VISTA. It is your responsibility to check these regularly.

WWW TEACHING RESOURCES

The Department of Pharmacology has chosen to use the University's central My eLearning Vista service to provide teaching material for all of its courses.

- To access these materials, either point your browser to: http://vista.elearning.unsw.edu.au/ or go to the School's home page at: http://medicalsciences.med.unsw.edu.au/ then select "Current Students" from the menu bar and click on My eLearning Vista, under "Quicklinks" in the left column.
- You will need to click through the "UNSW" at the left, then click the "Log on" button and enter your Unipass credentials (zStudentNo. and password).
- After logging on to My eLearning Vista, look for the course PHPH3101. You should have access to it if you are properly enrolled.

Browser Settings that are needed for My eLearning Vista:
My eLearning Vista makes extensive use of "pop-up" windows. Most browsers now block such pop-ups so you will need to allow pop-ups on this site for it to work properly for you.
The My eLearning Vista service recommends the use of the "Firefox" browser when accessing My eLearning Vista. This will probably not be necessary in order to make use of the site for courses provided by the Dept. of Pharmacology.

You can make use of Lectopia (formerly ilectures) recordings taken of the lectures that are available on My eLearning Vista. Lecture notes will also be made available on My eLearning Vista.

HANDWRITING

Students whose writing is difficult to understand will disadvantage themselves in their written assessment. Make every effort to write clearly and legibly. Do not use your own abbreviations.

STUDENT RIGHTS AND RESPONSIBILITIES

Refer to Student Central @:
https://my.unsw.edu.au/student/academiclife/StudentCentralKensington.html.

MISSED ASSESSMENT ITEMS

If in any circumstances you unavoidably miss an examination, progress exam or cannot hand in an assessment task on time, **you must inform the course coordinator and you must lodge a special consideration request**, supported by a medical certificate or other documentation to Student Central (see web address above) within **3 DAYS**.
Your request for consideration will be assessed and a deferred exam may be granted. You cannot assume you will be granted supplementary assessment.
If necessary, a supplementary final examination will be held in the week starting 7th December 2009.
Normally, if you miss an exam (without valid reasons) you will be given an absent fail. If you arrive late for an exam no time extension will be granted. It is your responsibility to check timetables and ensure that you arrive with sufficient time.

MISSED PRACTICAL CLASSES

Students who miss practical classes due to illness or for other reasons must submit a copy of medical certificates or other acceptable documentation to the course coordinator in Room M206. **Certificates should be lodged no more than 3 days after an absence.**

SPECIAL CONSIDERATION

Please note the following Statement regarding Special Consideration.

If you believe that your performance in a course, either during session or in an examination, has been adversely affected by sickness or for any other reason, you should notify the Registrar and ask for special consideration in the determination of your results. Such requests should be made as soon as practicable after the problem occurs. ***Applications made more than three days after an examination in a course will only be considered in exceptional circumstances****.*

*When submitting a request for special consideration you should provide all possible supporting evidence (eg medical certificates) together with your registration number and enrolment details. Consideration request forms are available from Student Central. In exceptional circumstances further assessment may be given****. If you believe you might be eligible for further assessment on these grounds, you should contact the Course Coordinator as soon as possible.***

REPEATING STUDENTS

Practical class exemptions may be granted to repeat students but students **must** check with the course co-ordinator whether they have exemption **prior** to their first practical class. All students must be familiar with the material covered in the practical classes.

STUDENT SUPPORT

Those students who have a disability that requires some adjustment in their teaching or learning environment are encouraged to discuss their study needs with the course coordinator prior to, or at the commencement of, their course. Issues to be discussed may include access to materials, signers or note-takers, the provision of services and additional exam and assessment arrangements. Early notification is essential to enable any necessary adjustments to be made.

APPEAL PROCEDURES

Refer to Student Central @:
https://my.unsw.edu.au/student/academiclife/StudentCentralKensington.html.

GRIEVANCE RESOLUTION OFFICER

In case you have any problems or grievance about the course, you should try to resolve it with the Course Organizer (A/Prof. Renate Griffith ph:9385 1912) or the Head of Department (Prof Margaret Morris ph: 9385 1560). If the grievance cannot be resolved in this way, you should contact the School of Medical Sciences Grievance Officer, Dr P.Pandey (9385 2483, P.Pandey@unsw.edu.au)

PLAGIARISM

The School of Medical Sciences will not tolerate plagiarism in submitted written work. The University regards this as academic misconduct. Evidence of plagiarism in submitted assignments, etc. will be thoroughly investigated and may be penalised by the award of a score of zero for the assessable work. Evidence of plagiarism may result in a record being made in the Central Plagiarism Register and the Faculty Students Ethics Officer being notified.

What is Plagiarism?
Plagiarism is the presentation of the thoughts or work of another as one's own.* Examples include:

- direct duplication of the thoughts or work of another, including by copying material, ideas or concepts from a book, article, report or other written document (whether published or unpublished), composition, artwork, design, drawing, circuitry, computer program or software, web site, Internet, other electronic resource, or another person's assignment without appropriate acknowledgement;
- paraphrasing another person's work with very minor changes keeping the meaning, form and/or progression of ideas of the original;
- piecing together sections of the work of others into a new whole;
- presenting an assessment item as independent work when it has been produced in whole or part in collusion with other people, for example, another student or a tutor; and
- claiming credit for a proportion a work contributed to a group assessment item that is greater than that actually contributed.†
- For the purposes of this policy, submitting an assessment item that has already been submitted for academic credit elsewhere may be considered plagiarism.
- Knowingly permitting your work to be copied by another student may also be considered to be plagiarism.
- Note that an assessment item produced in oral, not written, form, or involving live presentation, may similarly contain plagiarised material.
- The inclusion of the thoughts or work of another with attribution appropriate to the academic discipline does *not* amount to plagiarism.
- The Learning Centre website is the main repository for resources for staff and students on plagiarism and academic honesty. These resources can be located via:www.lc.unsw.edu.au/plagiarism

The Learning Centre also provides substantial educational written materials, workshops, and tutorials to aid students, for example, in:

- correct referencing practices;
- paraphrasing, summarising, essay writing, and time management;
- appropriate use of, and attribution for, a range of materials including text, images, formulae and concepts.

Individual assistance is available on request from The Learning Centre.

Students are also reminded that careful time management is an important part of study and one of the identified causes of plagiarism is poor time management. Students should allow sufficient time for research, drafting, and the proper referencing of sources in preparing all assessment items.

* Based on a document proposed to the University of Newcastle by the St James Ethics Centre. Used with kind permission from the University of Newcastle
† Adapted with kind permission from the University of Melbourne.

RATIONAL DRUG DESIGN
LECTURE and PRACTICAL OUTLINES

The course timetable is appended at the end of these notes and can also be found on My eLearning VISTA.

The course is divided into 6 main themes covering the drug development process from bench to bedside.

1. Introduction to Drug Design and Development
2. Target Selection
3. Lead Identification and Modification
4. Computer-Aided Drug Design
5. Drug Delivery
6. Pre-clinical and Clinical Testing

1. Introduction to Drug Design and Development

Drug Discovery as a Process

This lecture stresses the important realisation that drug discovery is a lengthy, expensive, and complicated process, that requires the collaboration of a large number of research scientists with skills ranging from computational and structural chemistry, through synthetic organic chemistry, molecular cell biology, genomics, proteomics, physiology, pharmacology, toxicology, and clinical biochemistry, amongst others.

Target Identification and Validation

In this lecture the role of genomics and bioinformatics in target selection and drug design and development will be explored. The use of genetic approaches to identify target candidates such as the mapping of disease loci, genomics and proteomics will be covered. Also covered will be the role of bioinformatics in the analysis of nucleic acid sequence, protein sequence and structure, expression databases and functional pathway data contained in databases.

Molecular Modelling Practical: Visualisation

Students will learn how to use molecular visualisation software to examine protein structures, protein/ligand interactions, and DNA/ligand interactions pertinent to structure-based drug design.

Structure Determination

A fundamental requirement of rational drug design is knowledge of the 3-dimensional structure of the receptor, generally a protein, sometimes a nucleic acid, occasionally a protein-nucleic acid complex. In this lecture we will explore the experimental methods available for determining these structures, focussing on X-ray crystallography, NMR spectroscopy, and the computational force fields that are used to refine such structures. These force fields are also important for determining ligand conformations and the energies of ligand-receptor binding – topics that will be taken up in later lectures.

2. Target Selection

Targets: DNA

DNA, messenger RNA, and ribosomal RNA are important molecular targets for cancer, viral, and microbial chemotherapy. Drugs that bind to these targets inhibit DNA replication, the transcription of mRNA, and its translation into proteins. In this lecture we will focus on how structure-based approaches have been applied to the rational design of DNA groove binding

agents that recognise specific nucleotide sequences, and how this provides the opportunity for the development of gene-specific inhibitors of transcription – a holy grail of many molecular pharmacologists.

Drug-Receptor Interactions Practical

DNA is a major target for cancer drugs, and agents that bind irreversibly by alkylating the bases are clinically important. The effective biological lesion is an inter-strand crosslink that interferes with DNA replication and transcription resulting in tumour cell death by apoptosis. In this practical we will use mass spectrometry methods to probe how an experimental cancer drug binds covalently to DNA. MALDI-TOF methods will be used to investigate the nature of the covalent linkages between a minor groove targetted aniline mustard and a DNA dodecanucleotide. The alkylated bases will be individually identified, and the nature of the inter- and intra-strand crosslinks delineated.

Targets: RNA

In the “Targets: DNA” lecture we focussed on DNA-binding agents that specifically inhibit the transcription of designated genes, here, in the “Targets: RNA” lecture we will consider the development of agents that selectively block mRNA so as to inhibit gene expression at the level of translation. We will discuss three different approaches: (1) the development of anti-sense oligonucleotides, (2) the design of ribozymes that selectively cleave designated mRNAs, and (3) the use of small inhibitory RNAs, known as siRNAs, in post-transcriptional gene silencing.

Targets: Receptors

Over 50% of all current drug targets are receptors and the majority of these receptors are membrane proteins. The role of the major families of both soluble and membrane bound receptors as drug targets will be examined. Newly identified receptor families and their potential as drug targets will also be discussed. The lecture will cover the challenges faced in the drug design and development process when transmembrane receptors are the target; including receptor selectivity, the difficulty in obtaining crystal structures for membrane proteins and performing high throughput screening.

Targets: Ion Channels

Ion channels are a large and important class of drug targets, which encompasses ligand-gated ion channels, voltage-gated ion channels, osmolyte or mechanosensitive channels and gap junction channels. This topic will explore why ion channels are a good target, the main mechanisms by which drugs can act on ion channels, the structural information currently available on ion channels and the techniques available for the screening of drug action.

Targets: Enzymes

Many cellular process involved in disease are mediated or controlled by the specific action of enzymes. A number of disease processes can therefore be reduced or eliminated by manipulating the activity of specific enzymes. This lecture will briefly outline how selected enzymes are identified as drug targets and then validated. Examples of how several drugs exert their therapeutic effects by interacting with these enzymes will also be given.

Drug Target Validation

Validation of a drug target involves the demonstration that successful 'targeting' will indeed produce the desired outcomes. Experimental approaches to validation increasingly use genetic manipulation of the target to identify the functional consequences of compromising the target. In this interactive lecture validation approaches will be presented with practical examples of both strengths and weaknesses intrinsic to current approaches.

3. Lead Identification and Modification

Biological Assays: Lead Identification and High-throughput Screening

Before computational drug discovery was introduced drugs were discovered by chance in a trial- and error-manner. The introduction of new technologies, such as high throughput screening (HTS) can experimentally test hundreds of thousands of compounds a day, which have resulted in a more successful identification of promising drug candidates or reduced drug development costs. The outline of the lectures:

- Lead identification: the new technologies eg. genomics, combinatorial chemistry and HTS used to search new compounds
- Lead optimization: HTS used for validation runs; laboratory biological assays for testing binding properties, activities and selectivity of new compounds.

Lead Identification and Screening Practical: Radioligand Binding

Radioligand binding experiments are easy to perform, and provide useful data in many fields, including drug screening for lead compound identification. Through the analysis of the raw binding data obtained from COS-7 cell line expressing the human β_2 adrenoceptor, students will gain an understanding of the concepts of kinetic, saturation and competition binding, and will learn to apply various data analytical methods for addressing different binding parameters, eg. the dissociation constant (K_D) and maximum binding capacity (B_{max}) of a radioligand; IC_{50} and inhibitory constants (K_I) of competitors.

Lead Modification Practical: Structure Activity Relationships

This practical will explore the relationship between the structure of a compound and its activity. Using the contraction/relaxation of guinea-pig trachea as the screening assay, drug discovery teams (groups of 3-4 students) will test a lead compound and several compounds based on the lead compound to determine their biological activity.
In the class the following week the data generated will be analysed and the effect of the substituents and stereochemistry of the compounds on activity will be examined.

Combinatorial Chemistry

The last 15 years has seen a revolution in medicinal chemistry, with the introduction of combinatorial methods of general organic synthesis. These approaches make it possible to generate tens of thousands of compounds in a few days, in a form suitable for evaluation in high-throughput biological assays. In this lecture we will review the two principal approaches to these methods: the use of parallel array synthesis, and the split-and-mix approach of library synthesis. We will focus on the logical structure of combinatorial libraries, rather than the specific chemical methodologies.

4. Computer-Aided Drug Design

Molecular Modelling

Computer-aided drug design methods are widely used today in academic and industrial environments. This lecture will explain the basics on how the structures of molecules can be entered into a computer and manipulated *in silico*. This includes methods for geometry optimisation, molecular dynamics simulation, and conformational searching.

Ligand-based Drug Design

To improve the properties of a potential drug, structure activity relationships are established to identify structural moieties that contribute to the binding and activity of a compound. Computational methods will be discussed in this lecture which can be used to model and predict these properties, and to screen databases for new leads. These methods include quantitative structure-activity relationship (QSAR) and pharmacophore determination. A pharmacophore defines the structural features and geometry of a drug that impart biological activity.

Structure-based Drug Design

Where the detailed three-dimensional structure of the protein target is available, so called structure-based computer-aided drug design methods can be utilised to identify and modify lead compounds. If the protein structure is not available, then computer models, based on structures of similar proteins, can be prepared and are suitable for structure-based drug design. This lecture will introduce structure-based drug design and protein modelling methods.

5. Drug Delivery

Bioavailability

Pharmacokinetics is the study of what the body does to a drug once it is within the body. A clinically important outcome of the body's treatment of a drug is how much drug is finally available in the body to bind to its intended therapeutic target (bioavailability). A brief outline will be given in this lecture on how ADME processes (Absorption, Distribution, Metabolism and Excretion) impact on a drug's bioavailability.

Pro-drugs and Drug Delivery

An inactive derivative of a known active drug may be called a prodrug and requires transformation within the body in order to release the active drug. Prodrugs can provide improved physiochemical properties such as solubility and enhanced delivery characteristics and / or therapeutic effect. This lecture outlines barriers to drug action, pro-drugs as drug delivery systems, and the application of pharmacokinetics and pharmacodynamics in drug delivery.

6. Pre-Clinical and Clinical testing

Pre-clinical Toxicology: *In Vitro*

From this lecture students will understand:
- the role of *in vitro* toxicity tests in establishing the safety of new drugs
- *in vitro* toxicity tests required by the world's regulatory bodies; tests for genotoxicity, cytotoxicity and others as required by chemical class
- the theory and methodology underlying various *in vitro* toxicology tests
- the role of Good Laboratory Practice in performing these tests

Pre-clinical Toxicology: *In Vivo*

From this lecture students will understand:
- the role of *in vivo* toxicity tests in establishing the safety of new drugs
- *in vivo* toxicity tests required by the world's regulatory bodies; genotoxicity, acute and short-term toxicity tests, tests for carcinogenic potential, Q-T prolongation and others as required by chemical class.
- the theory and methodology underlying various *in vivo* toxicology tests
- the ethics of *in vivo* toxicity testing and the potential for replacement by *in vitro* models

Pre-clinical Toxicology Practical: Ames Test

Chemicals which damage or mutate DNA and chromosomes are called mutagens. Damage to genetic material may lead to unregulated, cancerous growth of cells and tissues; indeed 80% of known carcinogens (cancer-causing chemicals) are also mutagens. Therefore one of the early, key tests performed on new chemicals or pharmaceuticals is a test for mutagenicity. The standard *in vitro* test for mutagenicity is known as the Ames test, named for its' inventor Professor Bruce Ames. It is a bacterial reverse mutation assay. In this practical specifically developed, mutant strains of *Salmonella typhimurium* are used, which

are unable to synthesize the essential amino acid histidine. Thus the *S. typhimurium* strains will only grow in medium including histidine as an added supplement. Mutagenic chemicals damage the bacterial DNA, causing the strains to revert (reverse-mutate) to the 'wild-type' state in which growth is independent of histidine. In the practical the *S. typhimurium* strain TA98 will be grown in culture with a growth-limiting concentration of histidine. Various test chemicals will be added to the cultures to assess their mutagenic potential. The number of bacterial colonies which form on the culture plates indicates the growth rate; the greater the number of colonies, the greater the mutagenic potential of the chemical.

Clinical Trials

The regulation of therapeutic products and the phases (I-IV) of clinical trial that a drug must pass through before registration will be covered in this lecture.

Clinical Trial Design

This lecture will cover clinical trial design. The components of clinical trial design to be discussed will be: aims, design, controls and placebo, blinds, randomisation procedures, sample size, statistics, endpoints and ethics (ethics will be covered later in the course).

Ethics of Human and Animal Experimentation

Testing of drugs in animals and humans is under strict regulation to limit any harm and distress to the research subjects. In this lecture we will discuss the ethical conduct of biomedical research, including the policies governing biomedical and animal research in Australia. The role of institutional human ethic committees and what constitutes informed consent will be discussed. The general principles for the care and use of animals for scientific purposes and the 3 R's, replacement, reduction and refinement will be covered and the role of institutional animal ethics committees will be covered.

Intellectual Property

The basic principles underlying the protection of intellectual property will be discussed, focussing on the legal issues relevant to the patenting of pharmaceutical agents. We will discuss the types of patents available and what can be protected, the notions of disclosure, prior art, innovation, challenges, and what needs to be included in a patent application.

Commercial Considerations of Drug Development

The lecture will cover commercial considerations in drug development from target discovery, indication selection and lead identification, through safety assessments, clinical trials and marketing. It will look at what drives decisions (Go/No-Go), time-scales, program planning issues and the interactive perspectives of different groups in small through to large pharma companies.

Wk		Practical Mon 3-6 WW202	Lecture 1 Tue 5-6 Mat C	Tutorial-2 time slots; both WW LG seminar room Tue 3-4, Wed 10-11	Lecture 2 Wed 12-1 Biomed E
1	20/7		Introduction & course overview/ Drug discovery as a process R. Griffith/L. Wakelin		Target identification and validation R. Griffith
2	27/7	Assessment in RDD A. Finch	Structure determination L. Wakelin		Targets - DNA L. Wakelin
3	3/8	Visualisation of drug-target interactions R. Griffith	Targets - RNA L. Wakelin	Nucleic acids L. Wakelin	Targets – receptors A. Finch
4	10/8	Drug-receptor interactions: mass spectrometry L. Wakelin	Targets – ion channels T. Lewis	Targets A. Finch	Targets – enzymes R. Grant
5	17/8	Drug-receptor interactions: mass spectrometry L. Wakelin	Biological assays: lead identification L. Liu	Drug target validation (interactive lecture) P. Gunning	Biological assays: high-throughput screening L. Liu
6	24/8	Lead identification and screening: radioligand binding L.Liu	**Test**	Lead identification & screening L. Liu	Combinatorial chemistry L. Wakelin
7	31/8	Lead modification: SAR A. Finch	Molecular Modelling R. Griffith	Drug discovery as a process R. Griffith	Ligand-based drug design R. Griffith
	7/9	**Mid-semester break**	**Mid-semester break**	**Mid-semester break**	**Mid-semester break**
8	14/9	Lead modification: SAR data analysis A. Finch	Structure-based drug design R. Griffith	Computer methods R. Griffith	Bioavailability R. Griffith
9	21/9		Pro-drugs and drug delivery T. Binder	Computer-aided drug design R. Griffith	Pre-clinical toxicology – *in vitro* J. Langlands
10	28/9		Pre-clinical toxicology – *in vivo* J. Langlands		Clinical trials A. Finch
11	5/10	**Holiday**	Clinical trial design A. Finch	Exam preparation R. Griffith	Ethics of human and animal experimentation A. Finch
12	12/10	Preclinical toxicology:Ames test 1 T. Murphy	Intellectual property L. Wakelin	Clinical trial design A. Finch	Commercial considerations of drug development J. Langlands
13	19/10	Preclinical toxicology:Ames test 2 T. Murphy		**Group presentations**	

Assignment due Monday, week 8. Lab report due Monday, week 10.

Group Assignment and Presentation

Each group will research the drug design and development process of a given drug and present the information in the form of an assignment (4000 words) and oral presentation (10 minutes).

The group will comprise of four members. Each member will research one stage of the drug design and development process. These stages are: (A) Target Selection, (B) Lead Discovery, (C) Preclinical Development and (D) Clinical Trials.

Assignment

- Each team member will write 1000 words on their chosen stage of the drug design and development process, the group will then produce an integrated assignment which covers the journey that the drug took from bench to bedside.
- The final document has a word limit of 4000 words, excluding tables, figures legends and references.
- The assignment should be referenced using in-text referencing in the style of the British Journal of Pharmacology.
- The front cover should indicate which student (via student number) researched each stage.
- Written assessment tasks must be accompanied by a signed plagiarism form (signed by each member of the group) and placed in the locked box in room MG14. An electronic version must also be submitted via my eLearning Vista.
- The assignment is to be submitted by 5pm on the 14th of September 2009. A penalty will apply for late submissions.

Oral presentation

- The group will produce a presentation of the drug design and development process of their drug.
- As a group they will produce a script and slides.
- One member of the group will be chosen to give the presentation and the other members of the team will be called upon to answer questions after the presentation.
- The oral presentation will be a maximum of 10 minutes in length and will be followed by 5 minutes of questions.
- The maximum number of slides allowed for each presentation is 10.

Assessment

- The assignment and oral presentation will be worth 15% of your total grade.
- The assignment will be assessed by one of the members of the PHPH3101 lecturing staff.
- The oral presentation will be assessed by two members of the PHPH3101 lecturing staff as well as two groups of your peers (see attached form)
- You will also give a peer/self assessment of the members of your group (see attached form).

STAGES OF DRUG DESIGN AND DEVELOPMENT PROCESS

A. Target Selection:

In "Target selection" you should cover the information that the drug design and development team needed to know to start the process *i.e.* the disease they want to treat, what is the patho-physiological basis of it, why was the target chosen (are there other possible targets), what is known about the target (*i.e.* structure, signalling pathways etc), what physiological processes is the target involved in and can this predict possible side effects.

B. Lead discovery:

Lead discovery is the next step in the process and includes topics such as lead discovery, lead modification, rational drug design (*i.e.* pharmacophores and QSAR) and screening assays. To research this stage you will need to search for information on the compounds that lead to the final development of your drug of interest.

C. Pre-clinical development:

Pre-clinical development includes a comprehensive account of *in vitro* and *in vivo* studies conducted on your drug. These will include studies in animal models of disease states. Your research should cover how the studies were done, what the drug was compared to *i.e.* placebo or current treatment and the results of these studies. This stage of development also includes toxicology studies and non-human pharmacokinetics and metabolism studies

D. Clinical trials:

The journey from bench to bedside of your drug will end with clinical trials. It may be difficult to find any information on phase 1 and 2 trials as they are often not published. However you may find information on the pharmacokinetics of the drug (this information is generated in phase 1 and sometimes phase 2 trials). Your research should cover; what trials were done (how many, where, for what), what were they comparing (*i.e.* drug *vs.* placebo or drug *vs.* current treatment), the types of patients recruited (the diseases they had, sometimes the one drug is trialled in the treatment of a few different conditions/syndromes etc.), what were the outcomes (*i.e.* was the drug 10 times better, the same but with fewer side effects etc), what side effects were reported in the trials.

GROUP ASSIGNMENT MARKING CRITERIA PHPH3101 S2 09

Student name/number:____

Group number & Drug:____

Stage of the drug design and development process

A) Target Selection ☐

(B) Lead Discovery ☐

(C) Pre-clinical development ☐

(D) Clinical trials

SECTION	COMPONENT PARTS	COMMENTS
Content & structure		
Introduction		
The introduction gives an overview of the whole Stage	**Orientation to topic**. ▪The topic and why it is of interest? **Clearly stated purpose.** ▪ What is the overall purpose of the paper.. **Outline-preview** ▪ How is the report going to be organised? **/5**	
Body of Essay		
Background information	**Clearly introduce**- the pharmacological issues, methods and procedures that related to this stage of the drug design and development process and how it relates to the overall development process. **/30**	
Evaluation of the issues identified from the sources	**Critical evaluation** of the key issues identified and supported by your chosen sources. ▪A balanced and logical presentation that explores the steps taken, challenges faced and outcomes achieved to progress your drug through this phase of the drug design and development process. **/30**	
End of Essay		
Conclusion	**Re-state key findings** and how they relate to the overall drug design and development process. **/5**	
Group work		
	F**orms part of an integrated assignment**. ▪Information given in other stages is not duplicated. ▪The relationship of this stage of the drug design and development process for this drug to the other three stages is discussed in the introduction and conclusion. ▪Formatting is consistent with that of the other three sections. **/5**	

SECTION	COMPONENT PARTS	COMMENTS
Writing Conventions		
	Overall readability- ▪Sentence structure-correct grammar and word usage. ▪Sentences and paragraphs well connected. ▪Discipline specific – appropriate vocabulary-use of formal not oral language. ▪Has been proof read **/10**	
	Support –sources-evidence BJP **–** **▪In-text citations and reference list follow conventions.** **▪Relevant information selected.** **/10**	
Formatting		
	Title page: Course name & number, Topic, student names and numbers, date. **Assignment Presentation:** ▪Neat, margins, 1.5 spacing, 12 point font. Simple staple. Page numbering **/2**	
	Word Limit- ▪1000 words (1000/ stage 4000 total) **/3**	

Additional comments:

Content & Structure: /75

Writing Conventions & Formatting: /25

Total: /100

PHPH3101 Group Presentations – Academic Assessment Form

Group **Topic**

Group members

Name……………………………………Name……………………………………

Name…………………………………… Name……………………………………

	Mark (/10)
Presentation	__________
Clear explanation of drug design & development process	
Structure of content – introduction, logical flow, conclusions	
Effective use of PowerPoint to deliver presentation	
Question Time	__________
Students understand the questions	
Answers to questions are appropriate	
	__________
Overall impression (team work)	
Total (/60)	

Comments

Strengths

Improvement

Points for clarification (if necessary)

Assessor: …………………………………… (sign). Date: ………………………

Group Presentations - Peer Assessment Form

Group ______________ Topic__________________

Group members

Name……………………………………Name……………………………………

Name……………………………………Name……………………………………

Student Assessors (Group ___)
Name………………………………………………… Sign………………………………

Name………………………………………………… Sign………………………………

Name………………………………………………… Sign………………………………

Name………………………………………………… Sign………………………………

	Mark (/10)
Presentation	__________
Clear explanation of drug design & development process	
Structure of content – introduction, logical flow, conclusions	
Effective use of PowerPoint to deliver presentation	
Question Time	__________
Students understand the questions	
Answers to questions are appropriate	
Overall impression (team work)	
Total (/60)	

Comments:

Group Members - Evaluation Form

Group number __________

Instructions: Use this form to evaluate the members of your group. Write the name of each group member, including yourself, on top of one of the columns, then assign a score of 0 to 10 (0 being the lowest grade, 10 the highest) to each group member for each criterion. Because each group member has different strengths and weaknesses, the scores you assign will differ. At the bottom of this sheet, write down any comments you wish to make.

Criterion	Group Members			
Regularly attends meetings				
Is prepared at meetings				
Meets deadlines				
Contributes good ideas				
Effort given to researching subject				
Submits high-quality work				
Listens to other members				
Gives constructive feedback				
Responds to constructive feedback				
Overall assessment of this person's contribution				
Total (/100)				

Comments:

FACULTY OF MEDICINE

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF PHARMACOLOGY

PHPH 3101

Rational Drug Design

Practical Class Experimental Procedures

SESSION 2, 2009

Molecular Modelling: Drug Target Visualisation

Introduction:

This computer experiment aims to give you an appreciation of the structural elements that constitute a protein or DNA drug target and to develop an understanding of the molecular interactions that result in the non-covalent binding of a small molecule to a protein or DNA fragment.
You will download your coordinate files from the Protein Data Bank and examine them using Word first.
Then, you will use molecular graphics software (Accelrys DS Visualizer 2.0, a free version of a commercial package) to examine the structure of a receptor molecule and the interaction of this receptor with its ligand, either the endogenous agonist or a drug molecule (antagonist).
You will also examine a receptor protein crystallised in two different conformations, one with an inverse agonist bound (inactive form), and one without any ligand bound, which represents an active form.
Lastly, you will visualise a structure of a DNA fragment complexed with a minor groove binder.

Instructions:
Detailed instructions and worksheets will be provided on WebCT Vista.

Report:

Fill in the worksheets and hand them in as your "report" at the end of your laboratory class (not marked, but compulsory).

Note: This experiment is undertaken individually (one person per computer in the laboratory).

Drug-Receptor Interactions introduction: Mass spectrometry

INTRODUCTION

DNA is a major target for cancer drugs, and agents that bind covalently by alkylating the bases are clinically important. The effective biological lesion is an inter-strand crosslink that interferes with DNA replication, resulting in tumour cell death by apoptosis. Most clinical agents form crosslinks by binding to the N7 nitrogen atoms of guanine in the DNA major groove, but much recent effort has been directed towards the development of novel compounds that alkylate DNA by binding to the N3 nitrogen atoms of purines in the minor groove. The intention is to subvert the extensive ability of tumour cells to repair N7 guanine lesions, thereby making it less likely that drug resistance will develop. The traditional way of investigating where and how alkylating agents bind to DNA, is to use the Maxam-Gilbert approaches to DNA sequencing, because alkylating the bases weakens the glycosidic bond linking base to sugar ring, resulting in de-purination and hydrolytic fragmentation when treated with hot alkali. Indeed, the adenine- and guanine-revealing reactions in the Maxam-Gilbert method rely precisely on the alkylation of the purine ring nitrogens followed by depurination and backbone hydrolysis in hot piperidine.

In collaboration with the Auckland Cancer Society Research Centre, we have developed a novel minor groove directed alkylating agent, designated alkamin, designed to crosslink DNA at the N3 position of adenines in AT-tract sequences (1,2). Sequencing experiments have demonstrated that they do alkylate adenines at such sequences, but have shed little, if any, light on the capacity of alkamin to crosslink DNA (1,3). Recently, we have developed mass spectrometry methods using MALDI and electrospray techniques to evaluate, in detail, how alkamin interacts with a series of AT-tract sequences embedded in 12-mer oligonucleotides (4).

EXPERIMENTAL PROCEDURE

In this practical you will prepare oligonucleotide-alkamin complexes, they will be incubated for 24h at 35°C, and Martin Bucknall will collect MALDI mass spectra for you, which you will analyse to reveal how alkamin binds to d(CGCGAATTCGCG)$_2$. The practical will take two weeks. A full description of the development of alkamin, how MALDI-TOF mass spectrometers work, and how alkylated DNA fragments in a MALDI experiment will be given in the first week. You will prepare the complexes at this time, in groups of 6 students per complex, under the direction of Jiaxu Wu, and Martin will generate the MALDI mass spectra the next day and post the spectra on the web. You will collect the data for your complex from the Web and prepare an analysis which we will discuss in the second week. In order to analyse your spectrum you will have to calculate the masses of various DNA fragments and of base-ligand adducts. You will find an appropriate calculator on the Web at the address: http://library.med.utah.edu/masspec/.

DATA ANALYSIS

You are not required to do a full spectral analysis, but are asked to identify particular species in the spectrum that will tell you about how alkamin binds to d(CGCGAATTCGCG)$_2$, A2T2. These species are, starting at the high molecular mass range:

1. Single stranded DNA ligand adducts (eg SS-L-OH, SS-L-A, SS-L-G, Table S1).
2. Single stranded DNA (SS, Table S1)
3. Depurinated single stranded DNAs (eg SS deG, SS deA, Table S1)
4. Right hand fragments (RHFs, Table S1)

5. Base-ligand adducts (eg A-L-OH, A-L-A, G-L-OH, G-L-G, G-L-A, Table S1)

The molecular mass of alkamin as the free base, including its 2 chlorines, is 641.7, and that of CGCGAATTCGCG as the free acid (ie phosphates protonated) is 3646.4. Remember that A2T2 has hydroxyl groups at its 5' and 3' termini, but that the RHFs generally have a phosphate at the 5' terminus. Also remember that DNAs can sometimes form adducts with sodium and potassium ions. Adenine has a mass of 135.1, and guanine a mass of 151.1.

REPORT

No report is required, however, the material covered in this practical will be assessed in the progress exam in week 6, and can be assessed in the final examination.

REFERENCES

1. Prakash, AS, Valu, KK, Wakelin, LPG, Woodgate, PD, Denny, WA, (1991). Synthesis and anti-tumour activity of the spatially-separated mustard bis-N,N'-[3-(N-(2-chloroethyl)-N-ethyl) amino-5-[N,N-dimethylamino)methy l)-aminophenyl]-1,4-benzene-dicarboxamide, which alkylates DNA at adenines in the minor groove. *Anticancer Drug Des* **6,** 195-206.

2. Atwell, GJ, Yaghi, BM, Turner, PR, Boyd, M, O'Connor, CJ, Ferguson, LR, Baguley, BC, & Denny, WA, (1995). Synthesis, DNA interactions and biological activity of DNA minor groove targeted polybenzamide-linked nitrogen mustards. *Bioorg Med Chem* **3,** 679-691.

3. Turner, PR, Ferguson, LR, Denny, WA, (1999). Polybenzamide mustards: structure-activity relationships for DNA sequence-specific alkylation. *Anticancer Drug Des* **14,** 61-70.

4. Abdul Majid, A.M.S., Smythe, G., Denny, W.A. and Wakelin, L.P.G. Structure of the $d(CGCGAATTCGCG)_2$ complex of the minor grove binding alkylating agent alkamin studied by mass spectrometry. *Molecular Pharmacology* (2007) **71**: 1165-1178.

Lead Identification: Receptor Radioligand Binding Practical

AIMS

- To investigate the expression of human β_2-adrenoceptors (β_2-AR) transiently expressed in COS-7 cells using [^{3}H]-dihydroalprenolol ([^{3}H]-DHA), a non-selective β-AR antagonist.
- To characterize and identify ligands for β_2-AR.

INTRODUCTION

Receptors are recognition sites capable of triggering a biological response in a cell. Most pharmacological receptors are membrane-bound proteins which may be studied separately from the processes they control. Receptor studies can be carried out using whole cells, tissue preparations or isolated organs. The gene responsible for a receptor can be identified, cloned and inserted into the fast-growing cells such as yeast, bacterial and immortalized cell lines cells. Chinese hamster ovarian cells (CHO) and COS cells are commonly used in such studies. The receptor under investigation in this practical class is the human β_2-AR, which have been cloned in COS-7 cells. The β_2-AR belongs to the rhodopsin "superfamily" of G-protein coupled receptors, all of which possess 3 extracellular loops, 3 intracellular loops and 7 hydrophobic transmembrane regions. They interact with G-proteins leading to activation of intracellular second messengers.

Radioligand binding helps to identify the "recognition stage" of drug or ligand interaction with the receptor. This technique provides a means of studying receptor characteristics by labelling the receptor with a selective molecular probe. This probe is typically a drug which is readily iodinated ([^{125}I]) or tritiated ([^{3}H]) to a high specific activity, and which exhibits a high degree of **selectivity** for the receptor class under investigation.

There are some basic criteria which must be satisfied before a radioligand "binding site" may be considered a pharmacologically relevant receptor. Binding of the radioligand must be:

(1) **saturable,**

(2) **reversible** (ie. the kinetics of binding must be analysed), and

(3) **characterised as a distinct receptor class** using a wide range of binding competitors.

The reaction between the radioligand [L*] and receptor [R], may be expressed by the equation:

$$[L^*] + [R] = [RL^*]$$

Note that this reaction proceeds in the forward direction (ie. producing the "bound" radioligand [RL*]) until a state of **equilibrium** is attained. At this point the rate of the forward reaction is balanced by the rate of the reverse reaction, so that the concentration of [RL*] is constant at any given time. It is important to note that the subsequent determination of all other binding parameters is conducted under conditions of equilibrium.

In all radioligand binding experiments, the "specific binding" is the term used to describe the interaction of the radioligand with specific receptor sites (ideally about 80-90% of total binding). In cell membranes, there are many different proteins present in addition to the receptor of interest. The radioligand has very high affinity for this receptor and will preferentially bind with it rather than with other proteins. However, a small proportion of the radioligand will bind to sites other than the receptor - this is termed "non-specific binding". "Total binding" represents binding to both receptor sites ("specific binding") and to non-receptor sites ("non-specific binding"). Non-specific binding is defined experimentally by co-incubation of cell membranes with radioligand and with a large excess of unlabelled ligand with high affinity for the receptor (DHA in this case). This unlabelled ligand will occupy all receptor sites. Therefore any binding of radioligand under these circumstances will **not** be to the receptor but to other, non-receptor, sites.

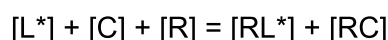
SPECIFIC BINDING = TOTAL BINDING - NONSPECIFIC BINDING

Kinetic studies involve two basic types of experiments examining the association and dissociation of the radioligand to and from the receptor. Today we will describe the **association** phase, or **time-course** of the reaction. An essential feature of the association is that it should be at least as fast as the appearance of corresponding biological response. We would therefore, in most cases, expect the association of the radioligand to be fairly rapid.

Saturation studies are used to determine the affinity (K_D) of a radioligand for a receptor, and the density (B_{max}) of a specific receptor in a given expression system or tissue.

Competition studies are a critical component of drug screening programs, aiming to characterize the receptor profiles of new ligands (ie. whether the ligand interacts with the targeted receptor molecules). Since many ligands for receptors are not available in a radioactive form, the affinity of the unlabeled ligand can be determined indirectly by measuring its ability (IC_{50} or K_i)[1] to compete with a radioligand for the receptor.

The equation below illustrates the principle of a competitor [C] competing with the radioligand [L*] for the receptor [R]:



$$[L^*] + [C] + [R] = [RL^*] + [RC]$$

It must be understood that binding studies are conducted under essentially non-physiological conditions. Consequently, some anomalies may arise when attempting to correlate the functional responses elicited by a particular drug with its characteristics in a binding assay, since the same type of receptor may be controlling many responses in a tissue rather than just the one that is being examined.

Part I. RADIOLIGAND BINDING ASSAYS - VIDEO

Part II. DATA ANALYSIS USING "PRISM"

Open the file "SOMS RECEPTOR BINIDNG DATA". The files can be found in the Practical folder on My eLearning Vista. The file "Experiment Results.xls" contains the results of the binding assays performed in the video.

I. TIME COURSE

1. From **SHEET 1** of "Experiment Results.xls" calculate the mean CPM values for total binding, nonspecific binding, and specific binding (total minus nonspecific) at both temperatures.
2. Open GraphPad Prism 5 via the following path: Class programs \ Physiology and Pharmacology \ Utilities and Office applications \ Graph Pad Prism.
3. Click on File \ New \ New Project File \ Open a file \ Binding Prac \ Time course.

Results of incubation at 37 °C

4. Under folder "Data Tables" click on "Data Table 1_37 degree". Use this template for Table 1 (37 °C) of SHEET 1. Enter the incubation times in the X column, and enter the mean CPM values for total binding, nonspecific binding, and specific binding in the appropriate Y columns. (Note: enter "0" for all X and Y columns on the first row).
5. Under folder "Graphs" click on "Graph 1_37 degree". The data will be depicted graphically.

Results of incubation at 4 °C

6. In Prism, under the folder "Data Tables" click on "Data Table 2_4 degree". Use this

[1] : IC_{50} is the concentration of competitor at which inhibits 50% of maximum specific binding; K_i is the apparent inhibition constant.

template for Table 2 (4 °C) of SHEET 1. Enter the incubation times in the X column, and enter the mean CPM values for total binding, nonspecific binding and specific binding in the appropriate Y columns. (Note: enter "0" for all X and Y columns on the first row).

7. Under folder "Graphs" click on "Graph 2_4 degree". The data will be depicted graphically.
8. Click on Layout 1 under folder "Layouts". The two time course graphs will be displayed on this layout.
9. Copy the graphs and paste into a word document and save on your USB drive or email to yourself. Please note some of the results from this practical class will be used in your report.

II. SATURATION BINDING

1. From **SHEET 2** data sets, calculate the mean total and non-specific binding data and enter in columns 2 and 5, respectively. Calculate the concentration of the radioligand in pM (column 3). Calculate specific binding (column 8).
2. Open GraphPad Prism, click on File \ New \ New Project File \ Open a file \ Binding Prac \ Saturation binding.
3. In Prism, under the folder "Data Tables" click on "Data Table 1_Saturation". Use this template for SHEET 2 data sets. Enter the radioligand concentration (pM) in the X column. Enter the mean total, nonspecific and specific CPM in the appropriate Y columns.
4. In Prism, under the folder "Graphs" click on "Graph 1_Saturation". The data will be depicted graphically.
5. To analyse the data, select "Analyze". Under "XY analyses" select "Nonlinear regression" and click "OK".
6. For one site model analysis, select: "Binding – Saturation", then click on "**One** site – Specific binding with Hill slope".
7. Under "Result" folder click on "Nonlin fit of Data Table 1_saturation". Write down the B_{max} and K_D value shown in Specific binding column (Column C).
8. Analyze "Graph 1_Saturation" again with **two** sites model. Back to "Graph 1_Saturation" \ Analyze \ XY analyses \ Nonlinear regression \ Binding – Saturation \ Two site - specific binding.
9. Check the Result sheet to see whether data fit well with a two-site model of binding.

The graph you have just generated illustrates the relationship between TOTAL, NONSPECIFIC and SPECIFIC BINDING, with increasing concentration of radioligand. You have also obtained the B_{max} and K_D values from the non-linear curve fitting analysis. Alternatively, you can transform specific binding data to a Scatchard plot to calculate K_D and B_{max}.

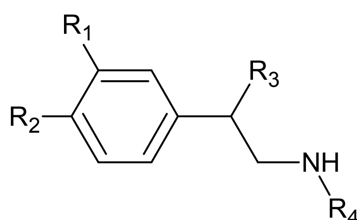
Scatchard Plot

1. Still working on SHEET 2 of "Experiment Results.xls", calculate the "free" radioligand (CPM added minus specific CPM bound) and enter into column 9, then calculate the ratio Bound CPM/Free CPM (to column 10). Then convert the mean specific CPM bound (column 8) to a concentration in pM (enter into column 11).
2. In the Prism file of "Saturation binding". Under folder "Data Tables" click on "Data Table 2_Scatchard Plot". Enter the radioligand concentration (pM) (Col. 11) in the first (X) column. Enter Bound/Free (Col. 10) in the second (Y) column.
3. Under folder "Graphs" click on "Graph 2_Scatchard Plot". Your data should be displayed graphically.

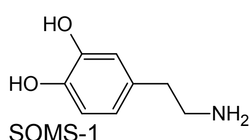
4. To analyse the data, select “Analyse”, then “linear regression”.
5. Click on Layout 1 under folder “Layouts”. The saturation fitting curves and Scatchard plot will be displayed on this layout.
6. Copy the graphs and paste into a word document and save on your USB drive or email to yourself.

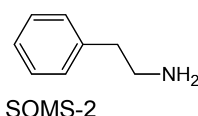
III. COMPETITION BINDING

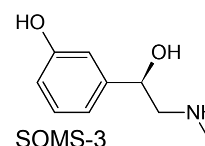
The pharmaceutical company Somsceuticals has a development program that is pursuing new drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD). The chemists from Somsceuticals have synthesized a compound SOMS-1. In preliminary testing SOMS-1 has shown affinity for the β_2-adrenergic receptor. Based on this lead compound a series of compounds (SOMS 2 to 10) has been synthesized.
You are a pharmacologist who is a member of the respiratory disease division of Somsceuticals. Your role in this development process is to determine the affinity of these compounds for the β_2-adrenergic receptor. Your data will allow the development team to determine which functional groups of SOMS-1 are required for optimal receptor binding.
All of the compounds that you will be testing have the basic form of:

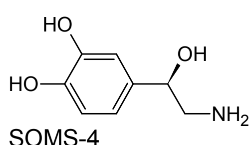


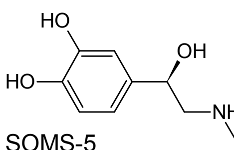
R = functional groups

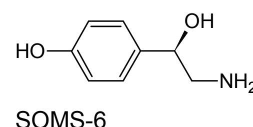


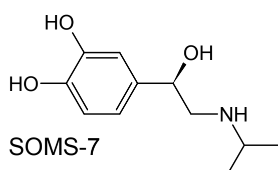


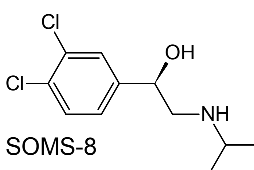


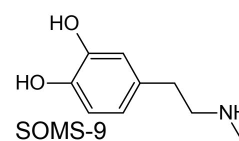












These ligands were used in a competition binding assay. A fixed concentration of radioligand ([^{3}H]-DHA 70 pM) and a range of concentrations of competitors were used in the assay. The data generated can be found in “SOMS RECEPTOR BINIDNG DATA” file in the Practical folder on My eLearning Vista

1. From **SHEET 3** of “Experiment Results.xls” calculate the mean total, nonspecific and specific binding for each set of data. Calculate the mean CPM for each concentration of the competitor, then subtract nonspecific binding CPM from these values. Convert the corrected CPM to a percentage of specific binding CPM for that curve.
2. Open GraphPad Prism, click on File \ New \ New Project File \ Open a file \ Binding Prac \ Competition binding.
3. Under folder “Data Tables” click on “Data Table_Competition”. Use this template for

SHEET 3 data sets. The log competitor concentrations have been entered in the X column. Enter the corresponding % values, in the appropriate Y columns.

4. Under folder “Graphs” click on “Graph_Competition”. The data will be depicted graphically.

5. To analyse the data, select Analyse \ Nonlinear regression \ Binding-Competitive \ One site – Fit logIC50. Try “Two site – Fig logIC50 competition” as well and compare the results.

6. To obtain the Hillslope of each curve, go back to “Graph_Competition”, reanalyze the data with \ Analyse \ Nonlinear regression \ Dose-response – Inhibition \ log (inhibitor) vs. response – Variable slope.

QUESTIONS

Time course experiments

1. At what time did equilibrium occurred? Did binding remained stable thereafter?

2. What differences did you observe between the same experiments carried out at 37 ^{o}C compared with 4 ^{o}C?

Saturation experiments

K_D is the equilibrium dissociation constant of radioligand. B_{max} is the maximum number of [^3H]-DHA binding sites.

1. What are the B_{max} and K_D values obtained from the non-linear fitting curve?

2. From the Scatchard plot calculate K_D and B_{max}.

   K_D =__________ pM (note: K_D is the negative reciprocal of the slope)

   B_{max}=_________pM (note: B_{max} is the intercept on the *x* axis).

3. Compare the values obtained from the two methods.

Competition experiments

1. Fill in the table with the IC_{50} and K_i values for SOMS ligands.

Competitors	IC_{50} (M)	K_i (M)	Hillslope	Affinity relative to SOMS-1
SOMS-1				**100%**
SOMS-2				
SOMS-3				
SOMS-4				
SOMS-5				
SOMS-6				
SOMS-7				
SOMS-8				
SOMS-9				

(Note: IC_{50} is the concentration of competitor at which inhibits 50% of maximum specific binding; K_i is the apparent inhibition constant provided by Cheng and Prusoff equation: $K_i = IC_{50} / (1 + [L]/K_D)$. [L] is the concentration of the radioligand was used, 70 pM).

2. What was the rank order of affinity of these ligands in inhibiting [^{3}H]-DHA binding?

3. What information can you derive from the Hillslope?

4. Explain the results obtained using SOMS-2 as a competitor.

5. Which of the ligands tested are antagonists?

Lead Modification: Structure-Activity Relationships

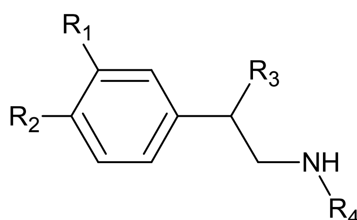
AIMS

To investigate the structural basis of ligand potency and efficacy of a series of compounds (SOMS 1 – 9)

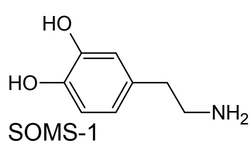
INTRODUCTION

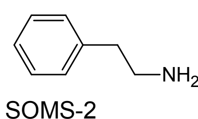
Structure-activity relationships play an important role in the rational design of new pharmaceutical entities. The pharmaceutical company Somsceuticals has a development program that is pursuing new drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD). The chemists from Somsceuticals have synthesized a compound SOMS-1. In preliminary testing SOMS-1 has shown affinity for β_2-adrenergic receptor and in functional studies (relaxation of guinea-pig trachea) has been shown to be a β_2-adrenergic receptor agonist.
Based on this lead compound a series of compounds (SOMS 2 to 10) has been synthesized. It is hoped that from the data collected from the testing of these compounds that it will be possible to generate a pharmacophore and QSAR data. These computer aided drug design tools will then be used facilitate the rational design of new drugs to treat asthma and Chronic obstructive pulmonary disease (COPD).
You are a pharmacologist who is a member of the respiratory disease division of Somsceuticals. Your role in this development process is to generate the pharmacological data that will be used in the production of the pharmacophore and QSAR equation. Your data will allow the development team to determine which functional groups of SOMS-1 are required for optimal binding and receptor activation.

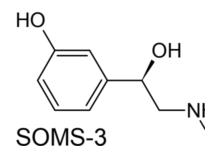
All of the compounds that you will be testing have the basic form of:

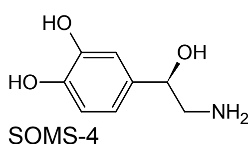


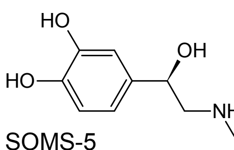
R = functional groups

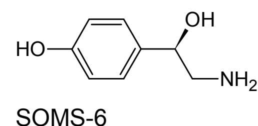


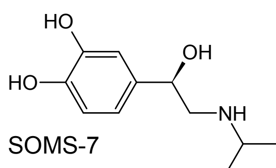


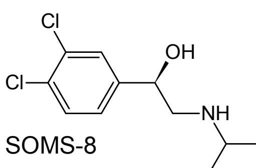


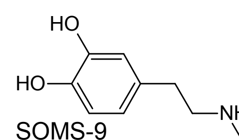












MATERIALS AND METHODS
Materials

Krebs solution NaCl 118mM, KCL 4.7 mM, CaCL2 2.5 mM, MgSO4 1.2 mM, KH2PO4 1.2 mM, NaHCO3 25mM, Glucose 11.7 mM, Ascorbic acid 1.1 mM

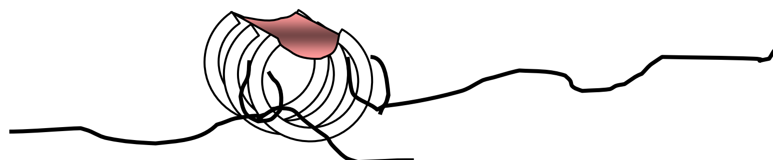
All test compounds are solubilised in ascorbic acid to prevent oxidation.

Methods

Removal of tissue *(this has been already done for you by the demonstrators)*

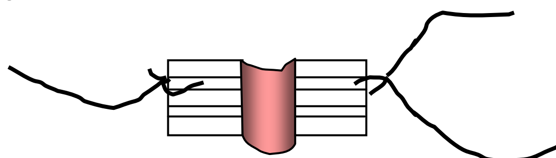
The guinea pig is euthanized by exposure to CO_2 gas. The chest is opened and the trachea is removed, and then cut into rings of tissue.

Setting up preparation *(this has been already done for you by the demonstrators)*
1. The trachea is cut into pieces which are 2-3 rings of cartilage in length.



2. A threaded curved needle is passed through the cartilage about halfway along the length of the tube and a few mm away from the smooth muscle. The ends of the thread are tied to make a small loop close to the tissue
3. Step 2 is repeated on the opposite side of the smooth muscle to the first thread. Making sure that the second thread is placed directly opposite the first thread.
4. The cartilage is cut along the length of the tube between the 2 threads.

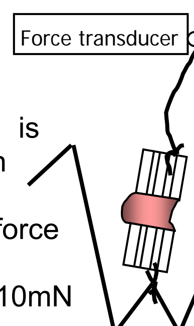
The trachea is now a strip preparation with the smooth muscle lying between the two sections of cartilage.



5. The threads are tied to the peak of the W-hook.
6. Taking care not to stretch the smooth muscle the W-hook with trachea is placed in the organ bath.

The organ bath is filled with Krebs solution, the temperature that is thermostatically controlled and maintained at 32°C. The solution in the bath is aerated with carbogen.

7. The long of thread at the top of the tracheal strip is attached to the force transducer
8. By carefully raising the transducer the resting tension is adjusted to ~10mN tension



Fifteen minutes before the experiment starts add 10uM normetanephrine and 3uM indomethacin. These drugs must be present throughout the experiment and so must be replaced whenever the organ bath is washed out. Normetanephrine is present to inhibit uptake2 (OCT3) and indomethacin prevents the development of spontaneous tone. Tracheal muscle can be contracted with 3uM carbachol to allow the measurement of relaxant responses.

Addition of drugs to organ bath
Add drugs to the organ bath by means the supplied pipettes. Take the required volume of drug solution into the pipette, place the tip just below the surface of the fluid in the organ bath and gently inject the solution into the bath

Wash out procedure

When it is necessary to remove a drug from the organ bath, drain the organ bath and refill, repeat three times.

Experimental procedure

1. Add the following drugs to the organ bath; 10µM normetanephrine (20µL of 5mM) and 3µM indomethacin (30µL of 1mM). Wait for fifteen minutes

2. WITHOUT WASHING OUT THE ORGAN BATH, add 3µM carbachol (30µL of 1mM) and wait until the tissue has contracted to a stable tension (this can take up to 30min)

3. While you are waiting for a stable tension to develop calculate the volume of an appropriate dilution to add to the bath for each concentration in your cumulative concentration-response curve for you first test compound (SOMS 1 - 10). An appropriate starting concentration can be found in Table 1

4. Obtain a *cumulative* concentration-response curve for your first compound SOMS compound.

5. Firstly, add the lowest concentration of SOMS compound (see table). Record the addition of the compound (and concentration) by typing in the comments window and hitting return as the drug is added.

If no response is observed after 2 minutes, add the next concentration WITHOUT WASHING OUT THE ORGAN BATH.

6. As soon as this response has stabilized add the next concentration WITHOUT WASHING OUT THE ORGAN BATH. Continue the cumulative concentration-response curve until no further relaxation is observed. Then washout the preparation for 2 minutes.

7. Repeat steps 1-3.

While you are waiting for a stable tension to develop;

(i) Calculate the volume of an appropriate dilution to add to the bath for each concentration in your cumulative concentration-response curve for your test compound. An appropriate starting concentration can be found in Table 1.

(ii) Collect the data you have obtained for your test compound (for instruction see below) and Calculate $\log EC_{50}$ and EC_{50} using GraphPad Prism

8. WITHOUT WASHING OUT THE ORGAN BATH, obtain a cumulative concentration-response curve for your second test compound (SOMS 1 - 10). Continue doubling the concentrations until no further relaxation occurs. Then washout the preparation (see above for instructions).

9. Collect the data you have obtained for your test compound (for instruction see below) and Calculate $\log EC_{50}$ and EC_{50} using GraphPad Prism

Collecting data from PowerLab

1. Place the marker on the trace at a point just prior to the addition of test drug
2. Measure the relaxation caused by each concentration of drug (measure at the plateau of response) relative to the pre-drug state.
3. Record your data in the tables provided

TREATMENT OF RESULTS

1. Enter your % maximum response data into the data table of GraphPad Prism
2. Click on the analyze button, choose transform, choose X = log X
3. Click on the analyze button, non-linear regression, choose sigmoidal variable slope
4. Generate a graph of the data obtained for each tested compound (use graph folder)
5. Determine the log EC_{50} and EC_{50} values for SOMS-1 and your test drugs. (use data folder)
6. Use these values to calculate the potencies of the test drugs relative to SOMS-1, using the formula: relative potency of test drug = $\log EC_{50}$ (test drug) minus $\log EC_{50}$(SOMS-1).
7. Enter your data onto the class spreadsheet

QUESTIONS

1. What other experimental methods could be used to determine the affinity, potency and efficacy of these compounds?

2. The use of isolated tissues to test drugs is considered low throughput screening. What advantages are gained with high throughput screening?

3. The SOMS-1 targets the β_2-adrenergic receptor, what other targets might Somsceuticals investigate for the treatment of asthma and chronic obstructive pulmonary disease (COPD)? What resources would they use to identify these potential targets?

4. What computer aided drug design techniques could be used in Somsceutical's development process? How would these techniques improve the drug design and development process? What challenges do the computational chemists face with the chosen drug target?

SOMS	Starting Concentration
1	2μM
2	-
3	625 nM
4	2μM
5	160nM
6	2μM
7	5nM
8	-
9	2μM

Test Compound:________________

Concentration in the bath (M)	Volume added to 10mL bath	Change in tension (mN)	% Maximum response

Test Compound:________________

Concentration in the bath (M)	Volume added to 10mL bath	Change in tension (mN)	% Maximum response

DATA ANALYSIS

Open the file "SOMS Data". This file can be found in the Practicals folder on My eLearning Vista.

Calculate % maximum response

For each concentration response curve calculate % maximum response using the following formula:

% Maximum Response = (value x — lowest value)/(highest value — lowest value) x 100

Calculate EC_{50} for each compound

1. Open GraphPad Prism via the following path: Class programs \ Physiology and Pharmacology \ Utilities and Office applications \ Graph Pad Prism
2. Select "start with an empty data table", Choose Graph "points only", for Sub-columns for replicates, choose "enter and plot a single y value".
3. Enter in the X-values column the concentrations of the compound used.
4. Enter % maximum response data into the data table. Use a different column (A-E) for each set of data.
5. Click on the analyze button, select transform, select "transform X values using X = log [X]", click "OK"
6. Click on the analyze button, select non-linear regression, click "OK", select Dose-Response-Stimulation\ log(agonist) vs response, click OK
7. View the log EC_{50} and EC_{50} values for each compound in "view: table of results", in results folder.
8. Open a new data sheet via the following path: File \ New \ Data Table & Graph. Select "start with an empty data table", Choose Graph "points only", for Sub-columns for replicates choose "enter and plot a single y value"
9. Repeat steps 3-7 for each compound tested.

Calculate EC_{50} for each compound

Calculate the mean and standard error (SE) of the log EC_{50} and EC_{50} data collected for each compound. You can use your calculator or Excel spreadsheet.

NB: SE = standard deviation/$\sqrt{(n)}$

Calculate relative potency

Use these values to calculate the potencies of the test drugs relative to SOMS-1, using the formula: mean log concentration ratio (Δ) = logEC_{50} (test drug) — logEC_{50} (SOMS-1). Relative potency as % of SOMS-1 Potency = (antilog Δ x 100%)

Generate graph of mean data for each compound

1. Open a new data sheet via the following path: File \ New \ Data Table & Graph. Select "start with an empty data table", Choose Graph "points only", for Sub-columns for replicates, choose "enter **20** replicate values in side-by-side columns"
2. Enter the concentrations used of the compound in the X-values column.
3. Enter % maximum response data into the data table. Place all data in column A.
4. Click on the analyze button, select transform, select "transform X values using X = log [X]", click "OK"
5. Click on the analyze button, select non-linear regression, click "OK", select Dose-Response-Stimulation\ log(agonist) vs response, click OK
6. Repeat steps 1-5 for each compound tested.
7. Make a record of the sheet numbers that correspond to each set of mean data.

8. Create a new graph sheet via the following path: Insert \ New Graph of Existing Data\ Graph Sheet. Choose one of your sets of non-linear reg (fit) data. Click "OK"
9. Under "Change" menu select "Add Data Sets". Choose, Results- *sheet number* transforms: transformed data" and "Results- *sheet number* Non-linear reg. (fit): Curve" for each set of data.
10. You can change the appearance and scale of the axes by double clicking on them.
11. Label the axes.
12. Copy and paste the final graph into a word document

Calculation of Molecular Properties

1. Open http://www.molinspiration.com/cgi-bin/properties
2. Draw the SOMS-1 using the tools around the edge of the calculator. The buttons along the top give you the basic carbon backbone. To change the atom from carbon click on the atom button to along the left-hand side of the calculator and then click on the carbon you wish to change. Use the Del button to delete any mistakes you make.
3. Click on Calculate properties
4. Record the data for the compound *e.g.*: LogP (miLogP), molecular weight (MW), number of rotatable bonds (nrotb), topological polar surface area (TPSA).
5. Click on "modify this compound"
6. Modify the compound to the structure of SOMS-2
7. Repeat steps 2-6 for each compound
8. Calculate π for each substituent ($\pi_x = \log P_x - \log P_H$)

RESULTS

1. Display; log EC50, EC50, Δ and relative potency in tabular form.
2. Graphically represent the functional data obtained.
3. Graphically represent the QSAR data collected for each compound

QUESTIONS TO CONSIDER

1. What relationship if any exists between each of the functional groups and the potency and affinity of each compound?
2. Does the logP of the compounds relate to their activity or affinity?
3. Is there a relationship between π of specific functional groups and potency and affinity of the compound?
4. Do the compounds have suitable lead-like or drug like qualities?
5. Do you have sufficient data to predict the potency of SOMS-2 and SOMS-8? If so what is the predicted potency of SOMS-2 and SOMS-8? If not what would you require to be able to predict potency of these compounds?
6. Have you identified a suitable drug candidate if so what are the steps you will undertake in this compounds future development?

REPORT

- You are to prepare a report using the data you have collected on the SOMS compounds in the "Lead Identification: Receptor Radioligand Binding" and "Lead Modification: Structure-Activity Relationships" practicals.
- Your written report should take the form of a scientific paper comprising of title, introduction, methods, results and discussion.
- You are to follow the Instructions to Authors provided on the next page.
- Reports must be legible and as concise as possible, and are limited to a maximum of 2000 words (excluding tables, figures and figure legends).
- The report should be referenced using in-text referencing in the style of a scientific journal.
- Written assessment tasks must be accompanied by a signed plagiarism form and placed in the locked box in room MG14 and electronic submission *via* VISTA is also required.
- The report is to be submitted by 5pm, 28th of September 2009. A penalty will apply for late submissions.

INSTRUCTIONS TO AUTHORS (adapted from British Journal of Pharmacology)

Manuscripts must include:

1. Title, Authors & Addresses
2. Introduction
3. Methods
4. Results
5. Discussion and conclusions
6. List of references
7. Tables
8. Figures and Legends

Title, Authors and Addresses

The title should contain no more than 150 characters (including spaces) and should not consist of more than one sentence. It must clearly indicate the subject matter of the paper. Titles should be drafted carefully to indicate broadly what the paper is about and to encourage readership. Cumbersome chemical names, technical details, and unfamiliar abbreviations should be avoided. Following the title the names (and student number) and addresses of the authors should be given.

Introduction

The introduction should give a short and clear account of the background of the problem and the rationale of the investigation. Only previous work that has a direct bearing on the present problem should be cited. The final sentence should summarise the broad conclusions of the paper.

Methods

The methods must be briefly described in sufficient detail to allow the experiments to be interpreted and repeated by an experienced investigator. Where published methods are used, references should be given (*i.e.* “Structure-Activity Relationships” PHPH3101 practical manual 2009).

Results

The description of the experimental results should be succinct, but in sufficient detail to allow the experiments to be analysed and interpreted by an independent reader. The mean results with standard errors, and the number of observations, should be given. The rationale for performing the experiments may be briefly mentioned in the Results section, but conclusions or interpretation of results should not be presented.

Discussion and Conclusions

The purpose of the discussion is to present a brief and pertinent interpretation of the results against the background of existing knowledge. Recapitulation of the results should be avoided. The main conclusions should be conveyed in a final paragraph with a clear statement of how the study advances knowledge and understanding in the field.

References

In the text, references to other work should take the form: (Bolton and Kitamura, 1983) or ‘Bolton and Kitamura (1983) showed that…’ For further details of reference formatting, see the Formatting and Technical Instructions section. References to ‘unpublished observations’ or ‘personal communications’ should be mentioned in the text only, and not included in the list of references.

Tables

Each table should be given on a separate page. Tables should be numbered consecutively with Arabic numerals and the number should be followed by a brief descriptive caption, occupying not more than two lines, at the head of the table. Tables should normally be self-explanatory, with necessary descriptions provided underneath the table.

Figures and Legends

Figure legends should be typed on a separate page. Legends should explain the figures in sufficient detail that, whenever possible, they can be understood without reference to the text. Legends, captions and labels should be consistent with terminology or nomenclature used in the text.

Formatting and Technical Instructions

Text should be in Times New Roman font, size 12, with 1.5 line-spacing throughout the manuscript. Margins at top and bottom and both sides should be 3 cm.

References

The reference list at the end of the manuscript must be arranged alphabetically according to the surname of the first author. When the surnames of first authors are identical, the alphabetical order of the surnames of subsequent authors takes precedence over the year of publication. The authors' names are followed by the year of publication in brackets. If more than one paper by the same authors in one year is cited, a, b, c, etc. are placed after the year of publication, both in the text and in the list of references. All authors should be quoted for papers with up to six authors; for papers with more than six authors, the first six should be quoted followed by *et al*. Entries in the reference list should conform to Harvard style. For example:

Journal Reference

Connor M, Kitchen I (2006). Has the sun set on κ3-opioid receptors? Br J Pharmacol 147: 349–350.

Book Reference

McGrath, JC, Daly CJ (2005). Imaging adrenergic receptors and their function: the use of fluorescent ligands and receptors to visualize adrenergic rceptors. In: Perez DM (ed). *The Adrenergic Receptors, in the 21st Century.* Humana Press: New Jersey, pp 65–72.

Tables

Each table should have a short title. Each column should have a heading and the units of measurement should be given in parentheses in the heading. Numbers up to four digits should be shown without spaces; longer numbers should be spaced in 3 digit groupings, without commas. Additional information should be given below the table and 'call outs' are superscript letters (not symbols).

Units and Symbols

SI units and symbols should be used for physicochemical quantities.

PRACTICAL REPORT: MARKING CRITERIA

	Exemplary (>8.5)	**Very Good** (8.4-7.5)	**Good** (7.4-6.5)	**Satisfactory** (6.4-5.0)	**Unacceptable** (<5.0)
Title and Formatting ___________ **x 0.5**	Title clearly indicates the subject matter of the paper. Name and student number and departmental address given. Times roman, 12 font, 1.5 line-spacing, Margins 3 cm. Word count 2000 ± 200.	Title indicates the subject matter of the paper. Name and student number and departmental address given. Minor errors in formatting. Word count 2000 ± 200.	Title indicates the subject matter of the paper. Name and student number and departmental address given. Errors in formatting. Word count 2000 ± 200.	Title does not indicate the subject matter of the paper. Name and student number and departmental address given. Errors in formatting. Word count > 2200.	Title, author's name and/or address not given. Formatting requirements not followed. Word count >2200 or<1800.
Introduction _____________ **x 2**	Concise and clear account of the scientific background and the rationale of the experiment. Final sentence summarises the broad conclusions of the paper.	Clear account of the scientific background and the rationale of the experiment. Minor omissions or errors. Final sentence summarises the broad conclusions of the paper.	A good introduction of the scientific background and the rationale of the experiment. A few factual error or omissions. Final sentence summarises the broad conclusions of the paper.	Some introduction to the scientific background and the rationale of the experiment. More detail needed. Improved summary of the major finding needed.	Lacking detail of the rationale of the experiment and scientific background. Summary of the major finding not given.
Methods _____________ **x 1**	Appropriate detail and referencing of methods used.	Sufficient detail and referencing of methods used. Minor details missing.	Insufficient detail and referencing of methods used. Minor errors.	Methods given but not referenced. Lacks details and has errors.	Methods not written in paragraph style.
Results _____________ **x 1**	Excellent description of the experimental results. No conclusions or interpretation of results presented. Data analysis was performed correctly.	Good description of the experimental results. No conclusions or interpretation of results presented. Minor errors in data analysis.	Good description of the experimental results. Lacks some required detail. No conclusions or interpretation of results presented. A few errors or omissions in data analysis.	Description of the experimental results lacks required detail. Some conclusions or interpretation of results presented. Some errors or omissions in data analysis .	No description of results. Results not written in paragraph style. Errors in data analysis. Some data analysis not presented.
Tables, Figures & Legends _____________ **x 1**	Graph axes labelled and units of measurement given. Legends explain the figures in sufficient detail that they can be understood without reference to the text. Tables self-explanatory, with necessary descriptions provided in footnotes underneath the table	Graph axes labelled and units of measurement given. Legends explain the figures in sufficient detail that they can be understood without reference to the text. Tables self-explanatory, with footnotes underneath the table. A few minor errors in data presentation	Graph axes labelled and units of measurement given. Not all legends explain the figures in sufficient detail that they can be understood without reference to the text. Most tables self-explanatory, with footnotes underneath the table. Some minor errors in data presentation.	Most graph axes labelled and units of measurement given. Not all legends explain the figures in sufficient detail that they can be understood without reference to the text. Most tables are self-explanatory. Some significant errors in data presentation	Results poorly presented or missing. Graph axes not labelled and units of measurement absent. Legends do not explain the figures in sufficient detail that they can be understood without reference to the text. Tables are not self-explanatory.
Discussion & Conclusion ___________ **x 2.5**	Discussion is clear and succinct. Extensive interpretation of the results with reference to previous scientific studies. No re-statement of the results. Main conclusions conveyed in a final paragraph.	Good interpretation of the results, greater reference to previous scientific studies needed. Some re-statement of the results. Main conclusions conveyed in a final paragraph.	Some interpretation of the results, greater reference to previous scientific studies needed. Minor errors in interpretation of the results Some re-statement of the results. Conclusions conveyed in a final paragraph.	Some interpretation of the results, greater reference to previous scientific studies needed. Errors in interpretation of the results Some re-statement of the results. Some conclusions conveyed in a final paragraph.	No interpretation of the results with reference to previous scientific studies given. Results presented. Main conclusions not conveyed in a final paragraph.
Referencing _____________ **x 1**	In-text citations and reference list follow BJP conventions. Relevant information selected. A wide range of references used.	In-text citations and reference list follow BJP conventions. Relevant information selected. A wider range of references needed.	In-text citations and reference list follow BJP conventions, with minor errors. Relevant information selected. A wider range of references needed.	In-text citations and/or reference do not follow BJP conventions. Relevant information selected. A wider range of references needed.	BJP conventions not followed. Non-peer reviewed sources used. Information in introduction and discussion not referenced. Wider range of references needed.

Writing Conventions ______________ **x 1**	Excellent sentence structure, correct grammar and word usage. Sentences and paragraphs well connected. Appropriate written expression- using discipline specific vocabulary and formal not oral language. Has been proof read.	Good sentence structure, correct grammar and word usage. Sentences and paragraphs well connected. Appropriate written expression- using discipline specific vocabulary and formal not oral language. Proof reading needed to eliminate minor errors.	Good sentence structure, correct grammar and word usage. Sentences and paragraphs not always well connected. Appropriate written expression- better use of discipline specific vocabulary and formal not oral language needed. Proof reading needed to eliminate minor errors.	Poor sentence structure, grammar and word usage. Sentences and paragraphs not well connected. Appropriate written expression- better use of discipline specific vocabulary and formal not oral language needed. Proof reading needed to eliminate errors.	Use of paragraphs and improved sentence structure needed. The report is difficult to read due to poor grammar and word usage. No evidence of proof reading.
TOTAL /100					

Preclinical Toxicology: Ames test

INTRODUCTION

The Ames Test is a pre-clinical toxicity test aiming to establish the genotoxicity of a drug - the potential to damage genetic material. More precisely, the Ames Test detects mutagens - drugs which mutate DNA or RNA. A drug which is a mutagen is a potential carcinogen (cancer-causing compound). The Ames Test detects mutations known as point-mutations, alterations in base-pairs or frame-shifts. The Ames Test is one of the most basic and widely-performed toxicity tests, not just for potential pharmaceutical compounds but for any commercial, industrial or environmental chemical, even samples of industrial waste, effluent or foodstuffs.

The Ames Test is named after its' inventor, Professor Bruce N. Ames. It relies on the reverse-mutation of histidine-dependent bacterial strains. These are strains of bacteria unable to synthesize an essential amino acid. As a result, the bacteria are unable to multiply and grow in a medium deficient in the amino acid. Mutagens cause the bacteria to 'reverse-mutate' so their ability to synthesize the amino-acid is restored and the bacteria grow and form colonies in the amino-deficient medium. These are 'revertant' colonies. Mutagenic potential of a test drug is assessed based on the number of revertant colonies formed.

This practical will utilise a histidine-dependent strain of *Salmonella typhimurium* designated TA100. The bacterial strain will be cultured together with several concentrations of a test chemical or negative or positive control.

The practical is conducted over two weeks, as it takes 3 days for the revertant (reverse-mutated) strains to grow and form a colony large enough to be observed with the naked eye. In the first week, the bacterial plates incorporating the test chemical will be prepared. In the second week, the plates will be scored (revertant colonies counted) and results analysed.

A couple of points:

The *S. typhimurium* strains are not pathogenic, nevertheless use caution when pipetting bacteria or pouring plates:

- **wear gloves**
- **dispose of contaminated waste (tubes and tips) in autoclave bag**

In addition**, the materials (plates, pipettes, tubes, chemicals) have been sterilized**, as we only want to grow the bacteria added to the plate - not environmental bacteria. It is important to try and maintain sterility as much as possible:

- **do not touch the surface of agar plates, or the interior of lids**
- **use a fresh tip for every pipetting step**
- **use a fresh tube for every plate poured**

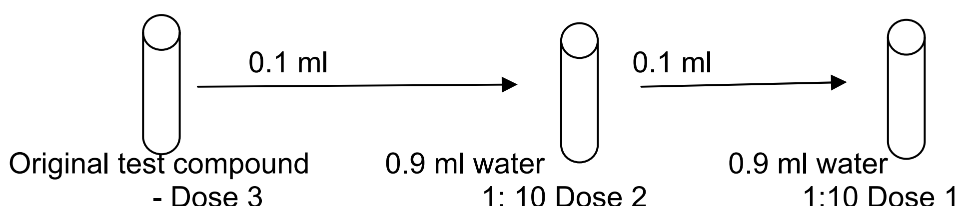
MATERIALS AND METHODS

WEEK ONE

1. Prepare test-substance dilutions.

   You have a 'test drug' at your work-station - each group has a different one, which will be revealed prior to week 2. In addition you have a 'negative control' - purified water - and a positive control, for this particular *S. typhimurium* strain, TA100, the positive control is sodium azide (NaN_3). Sodium azide is not especially dangerous, indeed it is commonly used in scientific laboratories. However, it is toxic and care should be exercised in it's use - wear gloves, don't mess around.

   Prepare serial 1:10 dilutions of your test-compound. Take two tubes and add 0.9 water. Take the test compound and add 0.1 ml to 0.9 ml of water in a fresh tube. Mix thoroughly. From this 1:10 dilution take 0.1 ml and add to 0.9 ml of water. This final dilution will be 'dose 1' of the compound, the first dilution 'dose 2' and the original, undiluted test compound 'dose 3'.



2. Label Plates

   You should have 15 glucose-agar plates at your workstation. These plates contain a base-coating of agar, a naturally-occurring jelly, incorporating the essential nutrients for all bacteria to grow - glucose, water and some basic salts.

   The practical is designed for you to prepare triplicate (three) plates of five treatments, making fifteen plates in total. The first step is to label the plates with the marker pen. The following information must be present:

   - your group name (or number)
   - the treatment (five in total; negative control, dose 1, 2 or 3 of the test drug, positive control)
   - the no. of plate (in the triplicate group; 1, 2 or 3).

   So, for example the first plate might say:

   G5, Neg Con 1
   (Group 5, negative control, plate 1).

   It is best to write your label on the side of the bottom part of the dish - writing on the bottom will interfere with counting, writing on the lid may be a problem if lids are dropped or mixed-up.

3. Prepare pour-tube and pour plate.

It is important to work quickly when preparing the pour tube and pouring plates. Otherwise the agar will set in the tubes and the sample will be wasted. For this reason, it is best to prepare one tube at a time.

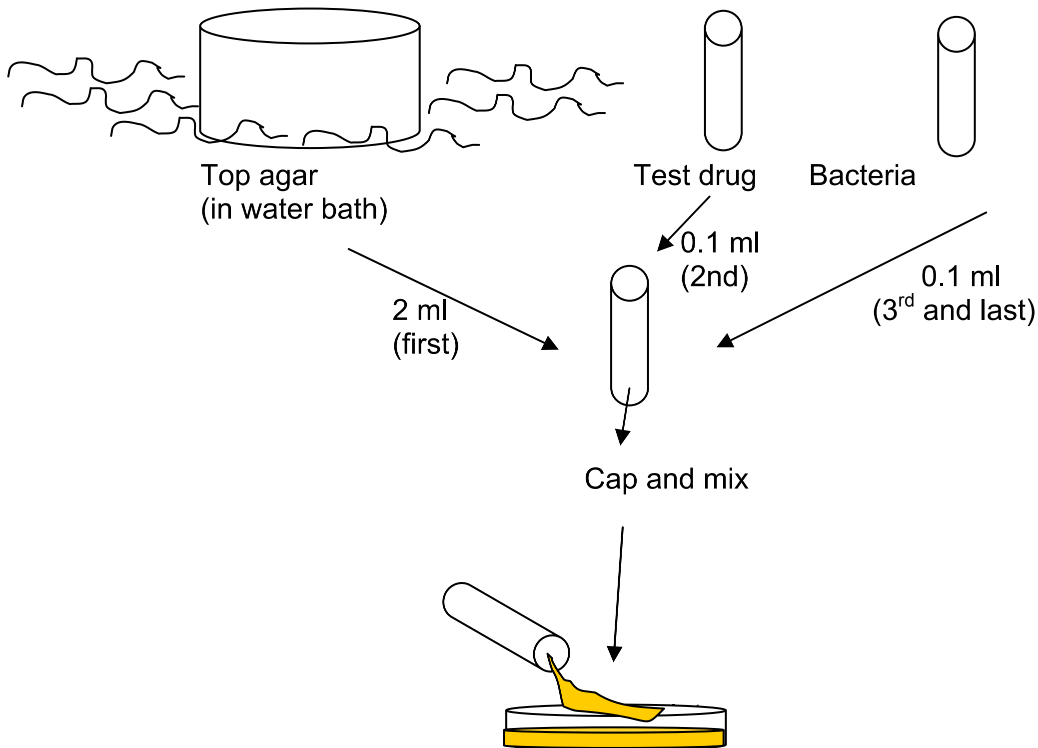
Take a labeled glucose-agar plate and remove the lid. Take one of the empty tubes at your workstation. Add 2 ml of 'top agar', the molten agar in your water-bath, to one of the tubes.

IN QUICK SUCCESSION add 0.1 ml (100 μl) of the test drug (or water, for negative controls) followed by 0.1 ml of bacteria culture. Cap the tube and mix briefly by inversion.
WITHOUT DELAY remove the cap and pour the entire contents of the tube onto the appropriate, labelled glucose-agar plate. Tilt the plate about a bit to ensure an even coverage of the poured agar mix - if the agar collects against one side of the plate, the colonies will 'bunch up' and make counting difficult. Replace the lid and leave the poured plate lying flat on the bench to set.

Discard the used tips and tube in the autoclave bag.

Move onto the next plate.

When you have prepared all 15 plates, stack them in groups of 3 according to the treatment-group. A demonstrator will collect them later and place them in the incubator at 37°C, for 3 days (72 hours). At the end of this period, the plates will be removed from the incubator and placed in the fridge.



WEEK TWO

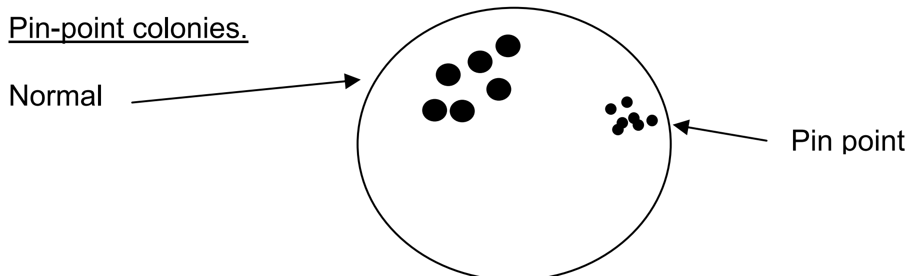
1. Score plates

The demonstrator will place your groups' plates at your work-station. Colonies of revertant *S. typhimurium* will be present (hopefully) as small, pale-yellow spots. Count the number of colonies on each plate and record the number in the table on page 6.

There are several methods of counting - the best way is to invert the plate (lid on) and count from the bottom, marking each colony with a marker on the plastic plate-bottom as you go. You may wish to remove the lid and count the plate from the top, although be very careful not to touch the plate as you'll destroy the colonies.

Also look for the following:

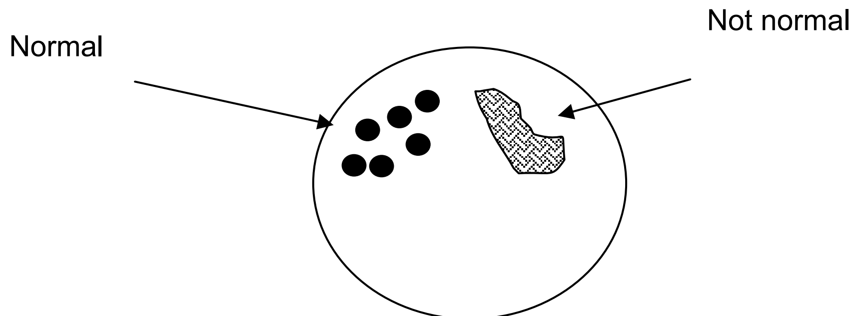
<u>Pin-point colonies.</u>



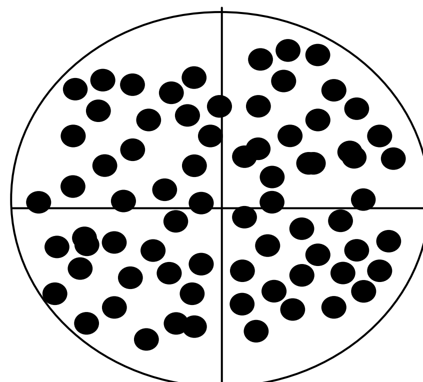
These colonies are smaller than normal revertant colonies, and there are likely to be a great deal of them, tightly bunched. They are indicative of toxicity (general, rather than genotoxicity) of the test drug towards the bacteria.

<u>Aberrant colonies</u>

If your plate contains colonies which are unusually large, a different shape or a different colour to the *S. typhimuriurm* colonies, then the plate may have been contaminated. Consult a demonstrator if you are unsure.



Some plates may have many colonies. In this situation, assuming the spread or coverage of colonies is even, it is acceptable to count the number of colonies in a quarter-plate and multiply the figure by 4 for the total.



TREATMENT OF RESULTS

Ames Test results
Group:
Test Chemical:

Treatment	Plate 1	Plate 2	Plate 3	Mean	SEM
Negative control (water)					
Test Chem – Dose 1					
Test Chem – Dose 2					
Test Chem – Dose 3					
Positive Control (Sodium Azide)					

2. Analyze results

Believe it or not, there is no agreement amongst the various testing authorities as to what constitutes a positive result in an Ames test. Various formulae are used:
- t-test v negative control
- ratiometric increase (1.5-fold or 2-fold above control)
- concentration-dependent increase above control

Try analyzing your data using each of these methods (t-test may not be possible, unless you have a calculator). Do the findings conflict?

QUESTIONS

1. Does one (or more) of your plates have no colonies present? What might this mean?

2. Some drugs are not mutagens but may be metabolized to mutagenic compounds in the body. Is there any way an Ames test can assess this potential?

3. The Ames Test takes 3-4 days to determine a result. How may this process be improved from a time point of view?

REFERENCE

Mortelmans, K. & Zeiger, E. (2000). The Ames *Salmonella* / microsome assay. Mutation Research 455, 29-60.

Examples of Past Exam Questions:

Short answer questions
Allow 10 minutes to answer each of these questions. Typically, a choice of 8 questions is given, with students required to answer 6 questions. If more than 6 questions are answered, only the first 6 are marked.

1. You have discovered a drug that shows *in vitro* activity, and you now wish to conduct whole animal experiments using several routes of administration that may require surgical intervention.
What are the **THREE** guiding principles underpinning the use of animals for scientific purposes that you need to consider when preparing your submission to the institutional ethics committee? Give examples of how you would achieve these.

2. List **FIVE** limitations to drug action. Using examples, describe a drug delivery strategy to overcome **ONE** of these.

3. High-throughput screening techniques used in the generation of 'hit' and 'lead' compounds can be loosely divided into two categories; biochemical assays and cell-based assays. Give an example of each category, and briefly discuss the relative advantages and disadvantages of each category.

4. Choose **TWO** of the techniques listed below and for each:
(a) Briefly describe the technique and
(b) Describe how the technique could be used for Target Selection, Lead Discovery or Lead Development.
a. Linkage Analysis
b. Virtual Screening
c. Homology Modelling
d. Common Module Profiling
e. Common Pathway Scanning

5. Discuss the **THREE** clinical rationales for using enzyme inhibition as a therapeutic approach. Include in your answer examples of drugs and the disease/disorder treated.

6. You have developed a novel series of DNA-binding cancer drugs that are dual poisons of the enzymes topoisomerase I and topoisomerase II. Several drugs that individually target these enzymes are currently used clinically.
Describe the process by which you would secure a full patent on your invention, pointing out the situations and events that would limit or disqualify your application.

7. Clinical trials are an important phase of the drug design and development process.
a. List the different phases of clinical trial and for each phase describe:
(i) the objectives of the phase
(ii) the type of subjects enrolled and
(iii) sample size used
b. List the regulatory authorities that monitor the conduct of clinical trials and approval/ documentation that must be obtained before a trial can start in Australia.

8. With regard to the oral bioavailability of a drug:
a. List the **FIVE** possible physico-chemical and structural properties of a drug that can be manipulated to enhance bioavailability.
b. For **TWO** of these properties briefly describe;
(i) a definition
(ii) how it affects bioavailability
(iii) its method of calculation or measurement.

Long answer questions
Allow 30 minutes to answer each of these questions. Typically, a choice of 6 questions is given, with students required to answer 4 questions. If more than 4 questions are answered, only the first 4 are marked.

1. Three principal tests are used to determine the genotoxicity of a pharmaceutical, the Ames test, the chromosome aberration test, and the micronucleus test. For **EACH ONE** of these tests, describe:
a. the specific purpose of the test, i.e. what it is designed to demonstrate
b. the basic method of the test, including whether the test is conducted *in vitro* or *in vivo*, the types of cells involved, what the data consist of, and how a positive or negative result is determined.

2. DNA and soluble enzymes make drug targets that lend themselves to the development of lead compounds by the application of structure-based approaches. Discuss the methods used to determine the 3-dimensional structure of such targets, their strengths and weaknesses, and indicate how the structural information may be used for rational drug design. Illustrate your answer with practical examples discussed in your lectures.

3. Discuss combinatorial chemistry approaches to lead discovery, focusing on its basic principles. What are the main benefits and drawbacks of the approach, and how can its use be exploited in a cost-effective manner? In what situations may this be the only practical way of proceeding?

4. Give an overview of the drug discovery process, from target selection through to the filing of a clinical trial exemption scheme application. In your essay include; the challenges faced and techniques/technology used to facilitate the progression of the drug discovery process through each stage.

5. Your company currently has an α_1-adrenergic receptor antagonist in clinical development for the treatment of hypertension. The compound is called Bepelowosin. The pre-clinical and Phase I-II clinical studies have not indicated any adverse reactions. In Phase II studies it was found that 20 μg twice a day was more effective than placebo in lowering blood pressure. You now wish to design a Phase III trial to compare Bepelowosin to Losartan, an angiotensin II receptor antagonist that is currently used in the treatment of hypertension.
Prepare a report (in essay form) for your project team outlining the design of the trial. Include your recommendations for the: location, objectives, subject population, inclusion/exclusion criteria, controls, blinding, design, randomisation, sample size and end-points of the trial.

6. PAR is a newly identified nuclear receptor. It has been shown to play a role in pancreatic cancer. You have chosen PAR as your target for the rational design of a drug to treat pancreatic cancer. No crystal structures exist for PAR. Searches of various databases have generated the following information:
PAR shares homology with the oestrogen receptor (ER), peroxisome proliferator-activated receptor γ(PPARγ), and the progesterone receptor (PR). PAR has 14 % sequence similarity with the ER, 35 % sequence similarity with the PPARγ, and 28 % sequence similarity with the PR.

Answer the following EIGHT parts:

(i) Describe the difference between homology and sequence similarity

(ii) Which of the above receptors would you choose to base your homology model of PAR on? What are your reasons for making this choice?

(iii) Describe the steps involved in building an homology model

(iv) You have carried out a virtual screen by docking a library of compounds onto your homology model of the PAR receptor. You are searching for drug-like molecules that you will subsequently screen *in vitro*. The Table below shows the "hits" from your virtual screen.
Which of the "hits" from this screen meet "Lipinski's rule of five" for drug-like molecules?

Compound	Log P	Molecular Weight	Hydrogen bond donor	Hydrogen bond acceptor
1	5.2	367	5	10
2	6	285	3	7
3	4.8	333	4	8
4	2.6	510	2	5
5	3.4	580	4	3
6	5.2	287	6	5
7	2	495	3	6
8	1.8	158	6	2
9	1.1	275	3	4
10	4.9	380	2	11

The "hits" from your docking study have been tested *in vitro* for affinity and potency. These data were then used to generate a Quantitative Structure Activity Relationship (QSAR) according to the Hansch equation:

$$\log(1/C) = x\log P + y\sigma + zMR$$

(i) **C, log P, σ** and **MR**, the constants in the Hansch equation, are measures of what properties of the drug?

(ii) What is the relationship between log P and the *in vivo* activity of a compound?

(iii) Of the drug-like molecules from your virtual screen, which one is less likely to have CNS side effects? Explain the rationale behind your choice.

(iv) What characteristics of your lead compound can be optimised by the use of QSAR?