Charles Darwin University

Final Examination

Family Name						
Given Name/s						
Student Number						
Teaching Period	Semester 2, 2017					

PHA214 – Biotechnology and Pharmacogenomics	DURATION	
	Reading Time:	10 minutes
	Writing Time:	120 minutes

INSTRUCTIONS TO CANDIDATES

Answer all questions in Section A on the MCQ answer sheet proved.

Answer all questions in Section B in the booklet provided.

EXAM CONDITIONS

You may begin writing from the commencement of the examination session. The reading time indicated above is provided as a guide only.

This is a CLOSED BOOK examination

Any non-programmable calculator is permitted

No handwritten notes are permitted

No dictionaries are permitted

ADDITIONAL AUTHORISED MATERIALS	EXAMINATION MATERIALS TO BE SUPPLIED
No additional printed material is permitted	1 x 16 Page Book 1 x 5-Multiple Choice Answer Sheet 1 x Scrap Paper

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Section A
Multiple Choice Questions
Total No of Marks for this Section: 50

This section should be answered on the Answer Sheet provided. Please ensure that your name and student number have been written on the Answer sheet and placed in the completed Answer Booklet.

Marks for each question are indicated. Suggested time allocation for Section A: 50 mins

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Section B
Short Answer Questions: 11
Total No of Marks for this Section: 75

This section should be answered in the Answer Book provided.

Marks for each question are indicated. Suggested time allocation for Section B: 70 mins

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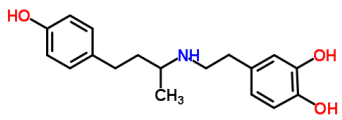
Question 1

(a) Dobutamine ($C_{18}H_{23}NO_3$) is a cardiotonic drug with specificity for Beta-1 adrenergic receptors.



Redraw the structure in your answer paper and label all the possible binding interactions that can take place in the binding site between dobutamine molecules and the amino acid residues of the beta-adrenergic receptor protein involved.

(Marks: 3)

(b) What is meant by *de novo* drug design?

(Marks: 1)

(c) The *log*P value of dobutamine is 1.94. Calculate the other properties of dobutamine relating to 'Lipinski-Rule of Five'. State whether the molecule is drugable and explain your answer. [Atomic weight of H=1.01g/mol, C=12.01g/mol, N=14.01g/mol, O=16g/mol]

(Marks: 4)

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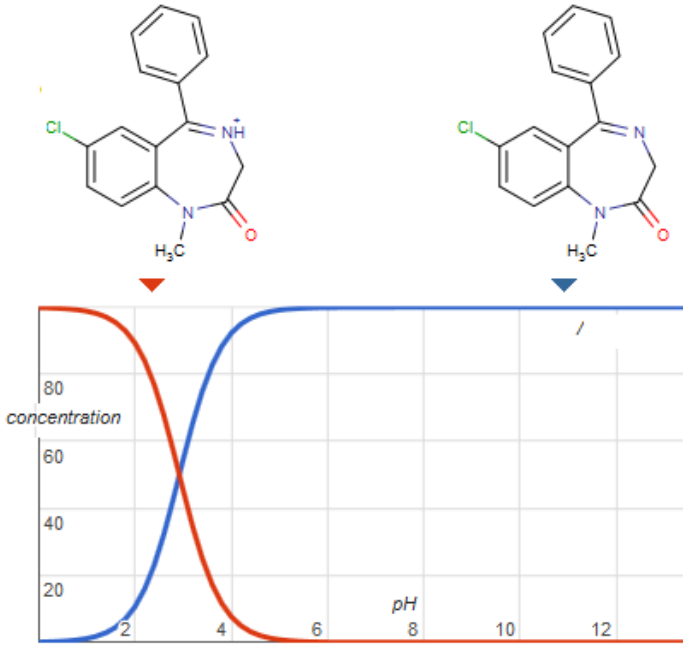
Question 2

(a) The graph below represents a plot of %concentration versus pH of diazepam. Diazepam's pKa=2.92 and *log*P=3.08.



(i) What does the logP value indicate about the nature of the molecule?

(ii) Discuss the nature of the major species at pH 7.4.

(iii) Calulate the logD value fo the molecule at pH 5.5.

$$\text{Log D}_{(\text{pH})} = \log \text{P} - \log[1+10^{(\text{pH-pKa})}]$$ -- for acids

$$\text{Log D}_{(\text{pH})} = \log \text{P} - \log[1+10^{(\text{pKa-pH})}]$$ -- for bases

(Marks: 4)

(b) During homology modelling, state the two important criteria you would apply to choose a template protein.

(Marks: 2)

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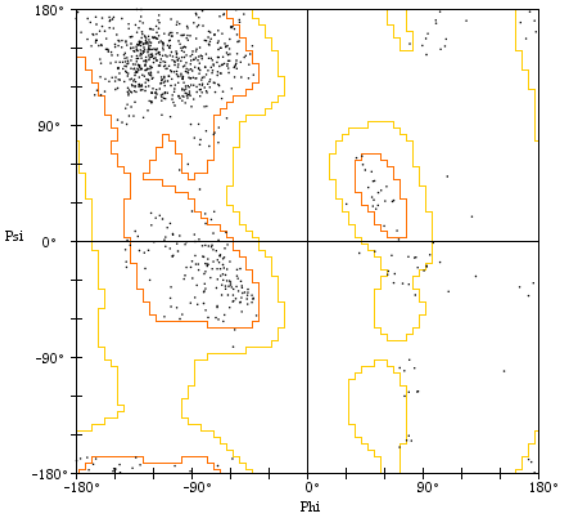
(c) In reference to the figure below, discuss the significance of Ramachandran plot in homology modelling of proteins.



(Marks: 2)

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Question 3

*Log*P of benzoic acid is 1.89. Using the data provided in the table below, for substituents of benzoic acid answer the questions below.

Substituent	π	σ_m	σ_p
H	0.00	0.00	0.00
Br	0.86	0.39	0.23
Cl	0.71	0.37	0.23
F	0.14	0.34	0.06
I	1.12	0.35	0.18
NO_2	-0.28	0.71	0.78
OH	-0.67	0.12	-0.37
NH_2	-1.23	-0.16	-0.66
SH	0.39	0.25	0.15
SO_2NH_2	-1.82	0.46	0.57

(a) Calulcate the logP value for:
- Nitrobenzoic acid
- Aminobenzoic acid
- Chlorobenzoic acid

(Marks: 3)

(b) Which of the above three (3) compounds will be most hydrophobic? Explain your answer.

(Marks: 2)

(c) What position of the benzoic acid would you add the amino group to, to make the acid stronger? Sketch the structures and explain your answer.

(Marks: 3)

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Question 4

What is meant by the term non-coding DNA?

(Marks: 2)

Question 5

Explain the role of UDP-glucuronosyltransferases play in bladder carcinogenesis.

(Marks: 4)

Question 6

Explain the role of Phase I, Phase II and Phase III reactions in drug metabolism.

(Marks: 3)

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Question 7

Laura is 14 and a forward on her school soccer team and leads the league scoring. For the last four months she has been really tired, but nothing seemed really wrong until her legs became covered in bruises. Just pressing her fingers on her skin was enough to make a bruise!

Beth is 13 and has never been athletic, she prefers reading and theatre. But she has been missing a lot of school because of one virus after another, lots of fevers and night sweats and a severe rash.

Both Laura and Beth have been suffering from anaemia, fevers, bleeding and are pale and thin. They have been diagnosed with Acute Lymphocytic Leukaemia. Their doctor, Dr. Ryder has decided to keep them in the hospital while treating them with a 'thiopurine' drug called 6-Mercaptopurine, which is known to be highly effective in treating leukaemia. Dr Ryder gives both the girls the same dosage of drug before he leaves the hospital for the night.

While making his rounds in the next few days, Dr Ryder sees Laura's vital signs plummet. Her anaemia has worsened; she has a fever and is having difficulty breathing. In contrast, Beth's anaemia has decreased; she is free of fever and is actually showing signs of improved health.

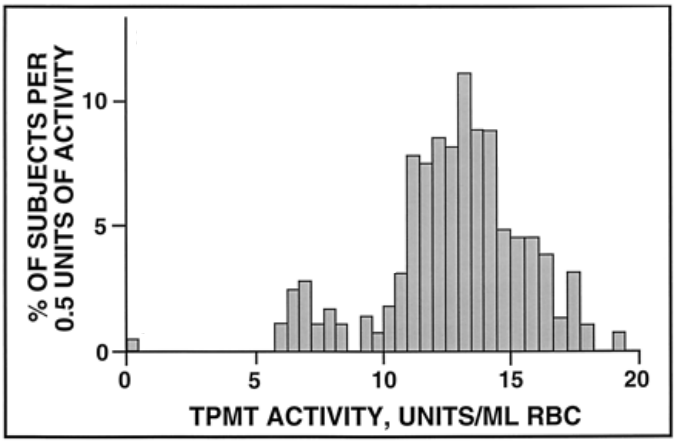
(a) Suggest why the drug might have affected the two girls differently.

(Marks: 1)

(b) What type of tests could Dr Ryder perform in order to determine how the two girls are reacting to the drug? Explain what each test would be determining.

(Marks: 1)

Dr. Ryder reviews the literature published about thiopurine methyltransferase (TPMT) and finds an interesting graph (below).



(c) What does this graph suggest to you about the genetics of TPMT? Explain your answer and include the likely genotypes for each.

(Marks: 3)

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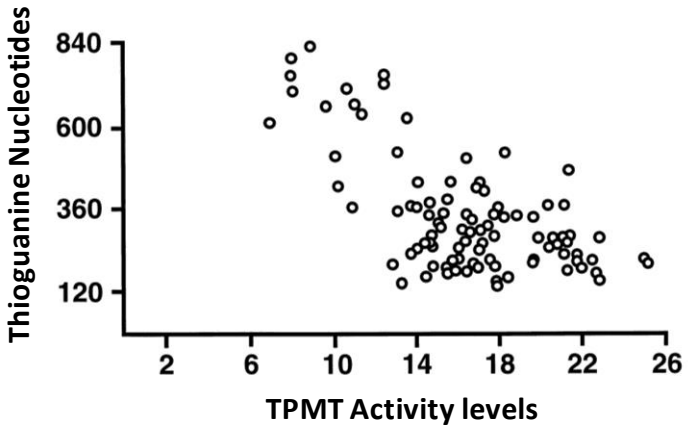
Dr Ryder compares the level of Thioguanine nucleotides (TGN) and TPMT enzyme levels in patients, and finds the following graph (below).



(d) Describe the relationship between TPMT activity, and TGN levels and why they are important.

(Marks: 2)

(e) Briefly summarise the importance of TPMT in this case study.

(Marks: 1)

Question 8

(a) What is a transgenic animal?

(Marks: 1)

(b) When requesting approval to construct a transgenic animal, the work needs to align to one of six categories. List the six categories and provide an example of an animal and how it is beneficial.

(Marks: 9)

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Question 9

A group of 20 recurrence breast cancer patients (taking tamoxifen) were selected for a study, regarding tamoxifen treatment. 19 aged matched women who had already completed 5 years of tamoxifen treatment with no recurrence, were used as controls. (Table 1). Genomic DNA was collected and amplified by PCR to determine if SNPs could influence the efficiency of tamoxifen treatment (Table 2).

Characteristic	Case group (n = 20)	Control group (n = 19)
Median age (years)	45.8	46.47
Menopausal status		
Premenopausal	16 (80%)	15 (78.9%)
Postmenopausal	4 (20%)	4 (21.1%)
Tumour size (cm)		
<2	4 (20%)	3 (15.8%)
2-5	15 (25%)	15 (78.9%)
>5	1 (5%)	1 (5.3%)
Number of positive nodes		
0	10 (50%)	9 (47.4%)
1-3	5 (25%)	5 (26.3%)
>4	5 (25%)	5 (26.3%)
ER status		
Positive	20 (100%)	19 (100%)
Negative	0	0

Table 1: Patient characteristics

SNP	Allele Frequencies	
	Case group (n = 20)	Control group (n = 19)
*CYP2D6*10* (C100T; C- wild type)		
T/T	11 (55%)	1 (5.3%)
C/T	4 (20%)	8 (42.1%)
C/C	5 (25%)	10 (52.6%)
*CYP2D6*4* (G1846A; G – wild type)		
A/A	0	0
G/A	1 (5%)	0
G/G	19 (95%)	19 (100%)

Table 2: Patient genotypes and frequencies

(a) Using the above data, which of the following SNPs most likely plays a role in tamoxifen treatment?

(Marks: 2)

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(b) Using your understanding of drug metabolism, explain why the patients displaying the variant genotype had a shorter disease free survival than the matched controls.

(Marks: 6)

Question 10

DNA fragments for two genes (Bt resistance and GFP) were generated through PCR with the addition of restriction enzymes (*Hind*III) at the 5'- and 3'-ends. The DNA fragments were inserted into a plasmid and transformed into plants so that they were resistant to certain insects (Bt gene) as well as fluoresce (GFP gene).

(a) List the reagents that are necessary for a reaction to occur and explain the role of each reagent.

(Marks: 4)

(b) State the reaction steps and conditions for a general PCR reaction and what is occurring in each step.

(Marks: 3)

(c) List three (3) advantages of PCR.

(Marks: 3)

(d) Would it have been possible for the plant to be transformed with the Bt gene and not the GFP gene? Explain your answer.

(Marks: 2)

Question 10

Jane is a 47-year-old female who was stabilised on warfarin (a substrate for CYP2C9), with her prothrombin time being optimised by substantially increasing the normal dosage of anticoagulant required, despite her blood levels being within normal limits. Ten (10) days later Jane stops taking rifampicin and she suffers a mild haemorrhage. Explain what might have happened to Jane.

(Marks: 4)

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