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Appendix A. AUCC approval letters

100 1908 - 2008

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Ref: H010-09

31 March 2009

ANIMAL USE AND CARE COMMITTEE
Private Bag X04
Onderstepoort
0110

Tel: 012-529 8434
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Prof CE Medlen
Department of Pharmacology
Faculty of Health Sciences
(connie.medlen@up.ac.za)

Dear Prof Medlen

H010-09: The toxicity and anti-cancer activity of novel drugs developed by CANSA and BioPAD sponsored "Anti cancer drug development consortium"

The application for ethical approval, dated 13 March 2009 was approved by the Animal Use and Care Committee at its meeting held on 30 March 2009.

Best regards

EMostert

Elmarie Mostert
AUCC Coordinator

Copy Dr R Anar

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Ref: H010-09 (Amendment 1)

03 June 2010

Prof CE Medlen / Dr D Cromarty
Department of Pharmacology
Faculty of Health Sciences
(connie.medlen@up.ac.za / duncan.cromarty@up.ac.za)

Dear Prof Medlen

The title addition, "Efficacy evaluation of Riminocelles ™ against MDR, HCT-15 colon adenocarcinoma xenografts in Balb/C nude mice use" to the original application **H010-09** "The toxicity and anticancer activity of novel drugs developed by the CANSA and BioPAD sponsored "Anti cancer drug development consortium" was approved by the AUCC

Best regards

E Mostert

Elmarie Mostert

AUCC Coordinator

Copy: D Koot

Appendix B. Review of Riminophenazine QSAR

Table 1. Increased sensitivity of various Riminophenazine derivatives using non-toxic doses of Vinblastine and Doxorubicin against two Pgp expressing cell lines. Adapted from: Medlen *et al.* (US Patent). [166]

At non-toxic [ ]	Increased sensitivity (fold reduction in IC_{50} value of Riminophenazine)			
	Vinblastine		Doxorubicin	
Compound	K562/MMB	H69/LX4	K562/MMB	H69/LX4
B4100	3.53	6.38	1.2	1.37
B4119	1.46	5.69	1.88	1.03
B4121	97.83	7.57	3.12	2.34
B4128	6.49	2.82	1.78	1.57
B4163	10.63	3.46	1.5	1.15
B4169	24.66	7.36	2.95	1.45
B4103	0.69	5.21	1.3	1.37
B4126	4.45	2.69	1.28	1.54
B4127	6.07	2.91	2.53	1.01
B4178	6.03	1.97	1.1	1.5
B3786	not tested	3.6	not tested	1.02
B3962	4.07	2.15	1.79	1.32
B4019	not tested	2.11	not tested	1.34
B4070	2.94	2.63	0.82	1.68
B4090	7.3	5.64	1.42	1.49
B4112	19.5	21.38	2.36	3.54
B4123	6.8	4.69	1.84	1.15
B4125	11.96	1.97	1.65	1.5
B4158	4.69	3.29	1.75	1.19
B4159	7.02	5.79	1.77	1.42
B4174	1.78	0.86	1.5	0.79
B4177	not tested	10.71	not tested	0.7
B663	5.92	6.72	1.67	1.9

Table 2. Mean IC_{50} value (µg/ml) for various Riminophenazines against various neoplastic and normal cell cultures

Cell culture	B663	B4112	B4121	B4125	B3962	B4090	B4100	B4123	B4128	B4169	B3786	B4331	B4103	B4119	B4126	Reference
Neoplastic																
FaDu (human pharynx squamous carcinoma)	2															26
T24(human bladder carcinoma)	1															26
PLL/PRF/5 (PLC), (human Hepatocellular carcinoma)**	1.65	0.705	0.81	0.77	0.49		0.72		1.25				0.63	0.97	0.55	26, 27, 36, 163
WIL (human non-small cell lung cancer)**	4.8															31
HepG2 (human Hepatocellular carcinoma**	1.3	0.5	0.8	0.6	0.3		0.6		0.8				0.4	0.4	0.5	27, 36, 163
HeLa (human cervical)	0.8	0.4	0.8	0.7	0.5		0.4		0.8				0.7	0.6	0.4	26, 27, 36
Mahlavu (human Hepatocellular carcinoma)**	1.5															27
CaCO2 (human colorectal)**	1.9	1.0	0.7	0.2	0.3		0.3		0.8				1.3	0.3	0.4	27, 36, 163
K562/MMB (human erythroleukaemia)*	0.23	0.18	2.25		0.29	0.20	0.34	0.33	0.38	2.86						163, 164
COLO320DM (human colorectal carcinoma)*	2.1	0.7	0.9	0.4	0.4		0.5		1.0				0.5	0.7	0.6	36, 163
HT-29 (human colorectal carcinoma)*	1.4	0.4	0.7	0.1	0.3		0.5		0.9				0.4	0.4	0.4	36, 163
WHO 3 (human oesophageal)	1.1	0.3	0.6	0.3	0.3		0.5		0.7				0.3	0.6	0.4	36, 163
Du145 (human prostate)		0.7														163
H69/LX4 (human small cell lung cancer)*	2.9	0.4	0.8		0.6		0.8				0.6	0.7				38
H69/P (human small cell lung cancer)	1.6	0.8	0.5	0.8	0.4		0.7		1.2				0.9	1.2	0.6	38
Novikoff (rat hepatocellular)**	2.3	0.8	0.9	0.8										0.8		165
Jurkat (human acute T cell leukaemia)	1.2	0.3	0.4	0.3										0.2		165
MCF7(human breast)	1.6	0.3	0.4	0.6										0.3		165
Mean	1.7	0.5	0.8	0.5	0.4	0.2	0.5	0.3	0.9	2.9	0.6	0.7	0.6	0.6	0.5	
Normal																
Vervet kidney cells	>4															26
Human Fibroblasts	5.0	1.1	1.0	1.7										0.6		26, 165
Fibro (MRC5)	>2.5	0.8	0.8	1.0	0.6		0.8		0.9				1.0	0.8	0.4	36
Resting human lymphocytes	1.8	0.7	0.9	0.8										0.5		165
Stimulated (PHA) lymphocytes	0.62	0.49	0.88	0.69										0.33		165
Mean	2.5	0.8	0.9	1.1	0.6		0.8		0.9				1.0	0.5	0.4	

* Pgp expressing (classical resistance)
** Intrinsic non-classical resistance

Appendix C. Pharmacokinetic and tissue distribution study schedule

Day 8 (Pharmacokinetic and tissue distribution study)

55 female mice are required for this experiment.

Groups and caging

1. PTX [10 mg/kg] Riminocelles™ to 25 mice (5 cages of 5), i.e. cage A1-A5.
2. PTX [10 mg/kg] Taxol® to 25 mice (5 cages of 5), i.e. cage B1-B5.
3. Administer saline (negative control) to 5 mice (1 cage of 5), i.e. cage C1.

Experiment schedule

Before IV dosing through the tail vein, each animal was weighed and an appropriate dose calculated. Precise timing of when the dose was given and when the animal was euthanised was documented in study monitoring sheets.

07h00	Administer [10 mg/kg] PTX-Riminocelles to A1 (6 hr group).
07h25	Administer [10 mg/kg] PTX-Taxol to B1 (6 hr group).
07h50	Administer [10 mg/kg] PTX-Riminocelles to A2 (3 hr group).
08h15	Administer [10 mg/kg] PTX-Taxol to B2 (3 hr group).
08h40	Administer [10 mg/kg] PTX-Riminocelles to A3 (30 min group).
09h10	Euthanise, collect terminal blood[a] and organ[b] samples from A3.
09h35	Administer [10 mg/kg] PTX-Taxol to B3 (30 min group).
10h05	Euthanise Sacrifice, collect terminal blood and organ samples from B3.
10h50	Euthanise, collect terminal blood and organ samples from A2.
11h15	Euthanise, collect terminal blood and organ samples from B2.
11h40	Administer [10 mg/kg] PTX-Riminocelles to A4 (24 hr group).
12h05	Administer [10 mg/kg] PTX-Taxol to B4 (24 hr group).
12h30	Administer negative control (saline) to C1 (24 hr group).
13h00	Euthanise, collect terminal blood and organ samples from A1.
13h25	Euthanise, collect terminal blood and organ samples from B1.
14h00	Administer [10 mg/kg] PTX-Riminocelles to A5 (1 hr group).
14h25	Administer [10 mg/kg] PTX-Taxol to B5 (1 hr group).
15h00	Euthanise, collect terminal blood and organ samples from A5.
15h25	Euthanise, collect terminal blood and organ samples from B5.

Next day

11h40	Euthanise, collect terminal blood and organ samples from A4.
12h05	Euthanise, collect terminal blood and organ samples from B4.
12h30	Euthanise, collect terminal blood and organ samples from C1

[a]Heparinised blood samples are to be drawn via cardiac puncture from 5 (isoflurane anaesthetized) mice at 30 min, 1 hr, 3, 6 for drug level quantitation. At 24 h two blood samples are to be drawn for both toxicity marker profiling and drug quantitation.

[b]Organs (Liver, spleen, kidney, lungs and adipose tissue) are to be collected and weighed before being analysed for drug content via LC-MS/MS.