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ASX Announcement

BioDiem's BDM-I presented at international conference

Melbourne, 16 September 2013: Australian infectious disease therapy and vaccine development company BioDiem Ltd (ASX: BDM) has announced that antifungal study results relating to its novel antimicrobial BDM-I have been presented in a poster at the annual Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), run by the American Society of Microbiology, in Denver, Colorado.

The results were generated via the U.S. National Institute of Allergy and Infectious Diseases' (NIAID's) In Vitro Assessment for Antimicrobial Activity Service[1*]. Under NIAID's preclinical services program, internationally recognised researchers, Professors Melanie Cushion[3] and Thomas Patterson[4,5] tested BDM-I's activity against a range of fungi which can cause serious human infection. This was expanded testing following on from an earlier assessment. The poster titled "Antifungal and antipneumocystis activity of the investigational antimicrobial BDM-I" is attached to this announcement.

Nearly 70 different strains of opportunistic or hospital-acquired fungi have been assessed for sensitivity to BDM-I. These micro-organisms can all cause illnesses which are difficult to treat. Examples include life-threatening bloodstream infections and pneumonia.

In these bench-top studies, BioDiem's BDM-I demonstrated activity against several of the Cryptococcus species as well as endemic fungi such as *Blastomyces dermatitidis*, *Coccidioides immitis/posadasii* and *Histoplasma capsulatum*. Marked activity was also demonstrated by BDM-I against *Pneumocystis carinii* and *murina*. It was noted by the researchers Professors Cushion and Patterson that "further studies are warranted to determine the potential of this broad-spectrum antifungal agent".

Based on these encouraging results, BioDiem previously announced new studies to assess both optimal dose and overall effectiveness of BDM-I as a novel treatment in a mouse model of the fungal disease Pneumocystis. These studies are again supported by NIAID's preclinical services program[2**].

"The presentation of the data at this international conference and the progression of evaluation of BDM-I's antifungal activity to studies in an animal model of pneumocystis infection is a significant step forward for BDM-I's development program towards use in difficult-to-treat infections. This is where new treatments are urgently needed. BioDiem greatly appreciates NIAID support enabling this progress to occur" said BioDiem Chief Executive Julie Phillips.

Pneumocystis is a yeast-like fungus that many healthy people carry without symptoms. However in patients with a suppressed or compromised immune system such as cancer patients and particularly HIV patients, it is very problematic causing pneumonia which is a major cause of death in such patients who do not have preventative treatment.

According to the U.S. Centers for Disease Control and Prevention (CDC), Atlanta, the incidence of pneumocystis pneumonia (PCP) in the U.S. is estimated to be 9% among hospitalised HIV/AIDS patients and 1% among solid organ transplant recipients. In immunocompromised patients, the mortality rate ranges from 5% to 40% in those who receive treatment. The mortality rate approaches 100% without therapy.

In August 2012, BioDiem renewed its Non-Clinical Evaluation Agreement with NIAID, part of the U.S. National Institutes of Health (NIH), under which the research has progressed.

NIAID Contract Number * HHSN272201100018I ** HHSN272201000029I

1. (http://www.niaid.nih.gov/LabsAndResources/resources/dmid/invitro/Pages/invitro.aspx)
2. (http://www.niaid.nih.gov/labsandresources/resources/dmid/animalmodels/Pages/default.aspx)

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About BioDiem Ltd

BioDiem (ASX: BDM) is an ASX-listed biopharmaceutical company developing vaccines and antimicrobials targeting treatment and prevention of infectious diseases and related cancers. The lead technology is the LAIV (Live Attenuated Influenza Virus) vaccine used for seasonal and pandemic influenza vaccines and is given intranasally. BDM-L, a therapeutic hepatitis vaccine project targeting hepatitis D and B is underway at the University of Canberra. BioDiem's antimicrobial, BDM-I, is in preclinical development for fungal and bacterial diseases, also schistosomiasis. The SAVINE (scrambled antigen) technology is in development for tuberculosis and also EBV-related disease including nasopharyngeal cancer. BioDiem's retinal product BDM-E, being developed for retinitis pigmentosa, is available for outlicence. BioDiem's research is ongoing in partnerships with internationally recognised laboratories and commercial groups.

About BDM-I

BDM-I is a synthetic compound targeting the treatment of serious human infections. BDM-I is in the preclinical stage with outlicensing as the intended outcome. BDM-I is active against a range of pathogenic micro-organisms including gram-positive and gram-negative bacteria, fungi and protozoa. Key patents have been granted in Europe, Japan and the US around BDM-I's antimicrobial activity, including activity against *Plasmodium falciparum*, responsible for causing the most commonly severe form of malaria, and *Trichomonas vaginalis*, the protozoan responsible for causing trichomoniasis.

About National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH)

NIAID, part of NIH, conducts and supports research throughout the United States and worldwide to study the causes of infectious and immune-mediated diseases, and to develop better means of preventing, diagnosing, and treating these illnesses. News releases, fact sheets and other NIAID-related materials are available on the NIAID Web site at www.niaid.nih.gov.

NIH, the nation's medical research agency, includes 27 Institutes and Centers and is a component of the U.S. Department of Health and Human Services. NIH is the primary federal agency conducting and supporting basic, clinical, and translational medical research, and is investigating the causes, treatments, and cures for both common and rare diseases. For more information about NIH and its programs, visit www.nih.gov.

[3]About University of Cincinnati College of Medicine

The University of Cincinnati College of Medicine is one of 14 colleges of the University of Cincinnati—a top-ranked public research university with more than 42,000 students. In September 2012, UC was labeled "among the top tier of the best national universities" by U.S. News and World Report. The UC College of Medicine, established in 1819 as the Medical College of Ohio by pioneering physician Daniel Drake, became a part of the University of Cincinnati in 1896. The college is considered the oldest medical college in operation west of the Allegheny Mountains and is the second-oldest public college of medicine in the U.S. The College of Medicine has an exceptional list of alumni and current and past faculty who have made considerable contributions to medicine and to the medical sciences, including Albert Sabin, MD, who developed the live-virus oral polio vaccine.

[4]About The University of Texas Health Science Center at San Antonio

The University of Texas Health Science Center at San Antonio, one of the country's leading health sciences universities, ranks in the top 3 percent of all institutions worldwide receiving National Institutes of Health funding. The university's schools of medicine, nursing, dentistry, health professions and graduate biomedical sciences have produced more than 29,000 graduates. The $736 million operating budget supports eight campuses in San Antonio, Laredo, Harlingen and Edinburg. For more information on the many ways *"We make lives better®,"* visit www.uthscsa.edu.

[5]About South Texas Veterans Health Care System

South Texas Veterans Health Care System (STVHCS) serves one of the largest primary service areas in the nation providing health care services for about 80,000 Veterans in the San Antonio area.

For additional information, please visit www.biodiem.com

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Antifungal and Antipneumocystis Activity of the Investigational Antimicrobial BDM-I

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ABSTRACT

Background: There is a critical need for the development of new antimicrobials with broad-spectrum activity. In an initial screen, the novel antimicrobial agent BDM-I demonstrated broad-spectrum activity against both fungi and bacteria. Our objective was to further evaluate the in vitro antifungal and antipneumocystis activity of BDM-I against select fungi, including causative agents of opportunistic and endemic mycoses.
Methods: Clinical isolates, including *Cryptococcus* species, *Candida glabrata*, *Blastomyces dermatitidis*, *Coccidioides* species, and *Histoplasma capsulatum*, were evaluated. MICs were determined using CLSI reference methods for yeasts (M27-A3) and filamentous fungi (M38-A2). Antipneumocystis activity was measured using a well-described ATP luciferin-luciferase reaction assay, and the IC50 of BDM-I was determined against *P. carinii* and *P. murina*. Mammalian cell toxicity was also measured using the ATP assay in the human lung carcinoma cells (A549) and the rat lung fibroblasts (L2).
Results: BDM-I demonstrated potent activity against endemic fungi, including *B. dermatitidis*, *Coccidioides* species, and *H. capsulatum* (MIC90 range 0.25 – 0.5 µg/ml at 100% growth inhibition). Similarly, activity was also observed against *C. neoformans* and *C. gattii* (MIC90 2 µg/ml at 100% growth inhibition) as well as *C. glabrata* (MIC90 2 µg/ml). BDM-I also had marked activity against *P. carinii* and *P. murina* (IC50 on day 3 of exposure <0.1 and 0.174 µg/ml, respectively). While toxicity was observed against the L2 cell line (IC50 <0.1 µg/ml), the antipneumocystis activity was 30 times lower than that observed against the A549 cell line (IC50 0.174 vs. 5.26 µg/ml, respectively).
Conclusions: The investigational agent BDM-I has both antifungal and antipneumocystis activity in vitro. Further studies are warranted to determine the potential of this broad-spectrum agent.

Fungal Species	*Cryptococcus neoformans* (N= 13)	*Cryptococcus gattii* (N = 10)	*Candida glabrata* (N = 10)	*Blastomyces dermatitidis* (N = 10)	*Coccidioides species* (N = 10)	*Histoplasma capsulatum* (N = 10)
50% Inhibition Endpoint (µg/ml)						
MIC Range	0.25 – 2	1 – 2	0.5 – 1	0.06 – 0.125	0.125 – 0.25	< 0.03 – 0.25
MIC50	1	2	1	0.125	0.25	0.06
MIC90	2	2	1	0.125	0.25	0.25
GM MIC	1.00	1.74	0.81	0.09	0.22	0.05
100% Inhibition Endpoint (µg/ml)						
MIC Range	2	2	1 – 2	0.125 – 0.25	0.25	0.03 – 0.5
MIC50	2	2	1	0.25	0.25	0.125
MIC90	2	2	2	0.25	0.25	0.5
GM MIC	2.00	2.00	1.15	0.20	0.25	0.9

BACKGROUND

- The development of novel antimicrobial agents is of critical importance due to evolving antimicrobial resistance.
- BDM-I (**Figure 1**) is a novel antimicrobial agent currently under development by BioDiem Ltd., who has a Non-Clinical Evaluation Agreement with the NIH/NIAID for the pre-clinical evaluation of this investigational agent.
- In initial screens, BDM-I demonstrated broad-spectrum activity against both fungi and bacteria.

OBJECTIVE

Our objective was to further evaluate the in vitro antifungal and antipneumocystis activity of BDM-I against select fungi. This included causative agents of opportunistic and endemic mycoses and *Pneumocystis* species. In addition, the potential for mammalian cell toxicity was also evaluated.

MATERIALS AND METHODS

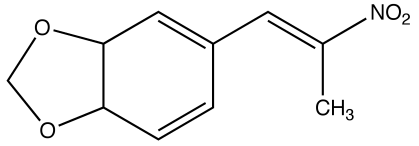
Isolates
- Clinical isolates of *Cryptococcus neoformans* and *Cryptococcus gattii*, *Candida glabrata*, *Blastomyces dermatitidis*, *Coccidioides* species, and *Histoplasma capsulatum*, were obtained from the Fungus Testing Laboratory at the UT Health Science Center at San Antonio.
- Isolates of *Pneumocystis carinii* and *Pneumocystis murina* were maintained at the Cincinnati Foundation for Biomedical Research and Education.

Antifungal Activity
- In vitro antifungal activity was measured according to the CLSI M27-A3 and M38-A2 guidelines. These assays are performed in the Fungus Testing Laboratory at the UT Health Science Center at San Antonio.
- After the appropriate period of incubation (24 to 48 hours for *Candida glabrata*, 72 hours for *Cryptococcus* species, and 72 – 96 hours for *Blastomyces dermatitidis*, *Coccidioides* species, and *Histoplasma capsulatum*) the MIC values were determined.
- Two MIC values were used: 1) the concentration resulting in a prominent reduction in growth (50% of the growth control), and 2) the concentration resulting in complete inhibition of growth (optically clear well).
- The MICs that inhibited 50% and 90% of the fungi (MIC50 and MIC90, respectively), and the geometric mean (GM) MICs were determined.

Antipneumocystis Activity
- Antineumocystis activity was measured against *P. carinii* and *P. murina*. This work was performed at the Cincinnati Foundation for Biomedical Research and Education.
- For each study, a set of controls was included: 1) growth control (untreated *Pneumocystis*); 2) pentamidine at 0.3 or 1 µg/ml; 3) ampicillin at 10 µg/ml; 4) media control or vehicle control (at the highest concentration used). Plates are incubated at 37°C with 5% CO_2 in a water-jacketed incubator.
- At 24, 48, and 72 hours, 50 µl samples were removed for ATP analysis.
- Antipneumocystis activity was measured using an ATP assay (ATPlite luminescence ATP Detection Assay, Perkin Elmer), which is based on the release of bioluminescence driven by ATP in the luciferin-luciferase reaction.
- Activity was classified by the IC50 value as highly active (<0.010 µg/ml), very marked (0.011 - 0.099 µg/ml), marked (0.10 – 0.99 µg/ml), moderate (1.0 – 9.99 µg/ml), slight (10.0 – 49.9 µg/ml), or inactive (≥ 50 µg/ml).

Mammalian Cell Toxicity
- The ATP assay described above was used to evaluate the viability of cell monolayers in order to assess for potential toxicity to mammalian cells.
- Confluent monolayers consisted of the human lung cell carcinoma cell line A549 (ATCC CCL-185) and the rat lung fibroblast line L2 (ATCC CCL149).

Figure 1. Chemical structure of the novel antimicrobial agent BDM-I.



CONCLUSIONS

The novel antimicrobial agent BDM-I demonstrated in vitro activity against *Cryptococcus neoformans* and *Cryptococcus gattii* as well as the endemic fungi *Blastomyces dermatitidis, Coccidioides immitis/posadasii*, and *Histoplasma capsulatum*. This investigational agent also demonstrated marked activity against both *Pneumocystis carinii* and *Pneumocystis murina*. Although toxicity was observed against the rat lung fibroblast line L2, the antipneumocystis activity was 30 times lower than that observed against the human lung cell carcinoma cell line A549. Further studies are warranted to determine the potential of this broad-spectrum antimicrobial agent.

RESULTS (cont.)

Table 1. MIC ranges, MIC50, MIC90 and GM MIC values for BDM-I versus *Cryptococcus* species and endemic fungi.

Parameter	*Cryptococcus neoformans* (n = 13)	*Cryptococcus gattii* (n = 10)	*Blastomyces dermatitidis* (n = 10)	*Histoplasma capsulatum* (n = 10)	*Coccidioides* spp. (n = 10)
50% Inhibition Endpoint					
MIC Range	0.25 - 2	1 - 2	0.06 - 0.125	≤ 0.03 - 0.25	0.125 - 0.25
MIC50	1	2	0.125	0.06	0.25
MIC90	2	2	0.125	0.25	0.25
GM MIC	1.00	1.74	0.09	0.05	0.22
100% Inhibition Endpoint					
MIC Range	2	2	0.125 - 0.25	0.03 - 0.5	0.25
MIC50	2	2	0.25	0.125	0.25
MIC90	2	2	0.25	0.5	0.25
GM MIC	2.00	2.00	0.20	0.09	0.25

Table 2. Antipenumocystis activity of BDM-I as measured by percent reduction in ATP.

Species	*Pneumocystis carinii*			*Pneumocystis murina*		
Time Point	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Media	0	2.04	0.84	0	0	12.18
Ampicillin 10µg/ml	0	0	0	0	0	12.73
Pent. 1 µg/ml	75.69	96.03	83.08	78.99	78.22	71.83
BDM-I 100 µg/ml	97.30	95.74	91.92	98.85	98.76	98.77
BDM-I 10 µg/ml	96.45	96.81	92.76	97.81	99.03	98.47
BDM-I 1 µg/ml	53.55	90.35	90.16	63.18	98.90	97.71
BDM-I 0.1 µg/ml	34.76	10.27	73.08	26.39	21.08	21.68
BDM-I IC50 (µg/ml)	0.047	0.411	< 0.1	0.441	0.172	0.174

Table 3. Toxicity to mammalian cells as measured by percent reduction in ATP.

	A549 Cell Line				L2 Cell Line			
Agent	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Antimycin A 75 µg/mL	26.79	79.34	72.56	94.85	76.37	49.59	61.84	83.05
BDM-I 100 µg/mL	99.36	99.72	99.71	99.66	99.30	99.41	99.20	99.70
BDM-I 10 µg/mL	11.50	31.28	52.95	39.47	99.42	99.26	99.62	99.66
BDM-I 1 µg/mL	11.17	10.48	11.77	19.85	99.15	97.19	99.59	97.08
BDM-I 0.1 µg/mL	8.06	7.87	7.46	7.07	80.37	62.54	40.30	47.16
IC50 (µg/ml)	13.70	8.45	5.26	6.09	< 0.1	< 0.1	< 0.1	< 0.1

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