CYTOTOXICITY, GENOTOXICITY AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) STUDIES OF SYNTHESIZED CARBONYL THIOUREA DERIVATIVES ON ACANTHAMOEBA SPP. AND HUMAN CORNEAL EPITHELIAL CELLS

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Cytotoxicity, genotoxicity and quatitative structure-activity relationship (QSAR) studies of stnthesized carbonyl thiourea derivatives on acanthamoeba SPP. and human corneal epitheli: cell / Maizatul Akma Ibrahim.

Lihat Sebelah

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Thesis submitted in fulfillment of the requirement for the Doctor of Philosophy in the School of Fundamental Sciences Universiti Malaysia Terengganu

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Dedicated to

My beloved parents and sisters

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfillment of the requirement for the degree of Doctor of Philosophy

CYTOTOXICITY, GENOTOXICITY AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) STUDIES OF SYNTHESIZED CARBONYL THIOUREA DERIVATIVES ON *ACANTHAMOEBA* SPP. AND HUMAN CORNEAL EPITHELIAL CELLS

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OCTOBER 2013

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Forty-four synthesized carbonyl thiourea derivatives were evaluated for their potential as anti-amoebic agent, aiming for a new discovery in *Acanthamoeba* keratitis treatment. The compounds were tested on four isolates of *Acanthamoeba* namely *A. castellanii* (CCAP 1501/2A), *A. polyphaga* (CCAP 1501/3A), *Acanthamoeba* sp. (Hospital Kuala Lumpur isolate) and *Acanthamoeba* sp. (Setiu Wetland isolate). Experiments conducted consisting of cytotoxicity and genotoxicity assays which were; IC_{50} determination by eosin dye method, morphological observation by light microscopy, evaluation of membrane integrity by acridine orange propidium iodide (AO/PI) staining, mode of cell death determination by DNA fragmentation test and assessment of DNA damage by alkaline comet assay. The IC_{50} values obtained were 6.26-30.46 μM for *A. castellanii*, 11.21-31.91 μM for *A. polyphaga*, 8.63-26.81 μM for *Acanthamoeba* sp. (HKL isolate) and 6.91-21.64 μM for *Acanthamoeba* sp. (SW isolate), indicating that these compounds are cytotoxic against *A. castellanii*, *Acanthamoeba* sp. of HKL and *Acanthamoeba* sp. of SW, but moderately cytotoxic on *A. polyphaga*. Two representative compounds, 2-(3-benzoylthioureido)-3-mercaptopropanoic acid and 2-(3-benzoylthioureido)-4-(methylthio)butanoic acid, which were labeled as M1 and M2 respectively, contained the best anti-amoebic activity and further tested. These compounds shortened acanthapodia structures, transformed the amoeba cells to become rounded, and exhibited no distinct vacuoles and nucleus. These show that the cells became encysted and inactive with the thiourea treatment. The compounds also disrupted the integrity of *Acanthamoeba* cells' membrane, making them non-intact. These thiourea promoted apoptosis in amoeba but did not significantly affected their DNA by giving DNA damage at score 0, 1 and 2. Positive control, chlorhexidine, gave better anti-amoebic activity but promoted necrosis and caused greater DNA damage. These two derivatives were later investigated on non-target human corneal epithelial cells (HCEC) through IC_{50} determination by using MTT assay, compound classification

by Selectivity Index (SI), cells proliferation by MTT test, morphological observation by light microscopy, membrane integrity and mode of cell death's examination by AO/PI staining, and DNA damage assessment by alkaline comet assay. Results showed moderate cytotoxicity of the compounds toward HCEC with IC_{50} at 132.69 μM for M1 and 98.20 μM for M2 compound. SI described M1 and M2 derivatives as moderate in their selectivity toward the amoeba cells. Cells' proliferation showed HCEC's reaction to the thiourea treatment in time-dependent manner, but maintaining the cells' high viability after 72 hours. The compounds did not greatly alter corneal cells' cellular morphology by only affecting the cells' differentiation. These derivatives were also found to disrupt HCEC's membrane integrity and promoted apoptosis but non-genotoxic on HCEC's DNA with damage score at 0 and 1. Positive control, hydrogen peroxide, was comparable to the thiourea derivatives in its cytotoxicity but displayed higher genotoxicity effect on HCEC. QSAR modeling from the data set of 44 thiourea analogs with anti-amoebic activity (pIC_{50}) for pathogenic *Acanthamoeba* sp. (HKL isolate) was carried out by applying stepwise-multiple linear regression (stepwise-MLR), genetic algorithm-multiple linear regression (GA-MLR), and genetic algorithm-partial least square (GA-PLS) methods. Stepwise-MLR selected four variables of fairly high squared correlation coefficient, r^2=0.732, and cross-validation, r^2_{cv}=0.522, with low root mean square error of calibration, *RMSEC*=0.058, and cross-validation values, *RMSECV*=0.08, while squared correlation coefficient of test set, and root mean square error of prediction obtained are r^2_{test}=0.739, and *RMSEP*=0.062. The model proved to have high predictive ability with residual in test set less than +0.88. *Y*-randomization gave r^2=0.052-0.248, which concludes that the model was not from random chance correlation. The model from GA-MLR contains five descriptors with r^2=0.848, r^2_{cv}=0.767, *RMSEC*=0.044, and *RMSECV*=0.055. Its validation gave r^2_{test}=0.777 and *RMSEP*=0.057 which proved that the constructed model is acceptable. Its prediction of test set produced residual less than 0.55. *Y*-randomization gave r^2 in the range of 0.004 to 0.300, confirming that the chance correlation was negligible. Meanwhile, GA-PLS came out with a statistically robust model of five variables with r^2=0.827, r^2_{cv}=0.682, *RMSEC*=0.047, *RMSECV*=0.064, r^2_{test}=0.790 and *RMSEP*=0.051. Small residual with values less than 0.25 from prediction in the test set explains robustness of the model. *Y*-randomization confirmed that the model did not occur from chances of correlation with r^2=0.015-0.372. The GA-PLS model is considered as the best model due to its high predictive power which is encoded by EEig09d, N-070, JGI6, MATS2m and MATS3m. These chosen set of descriptors provide the information about hydrophobicity and dispersive interaction which are involved in biological transport and distribution of the compounds' molecules through the cells' membrane. This study could assist in the prediction of new thiourea-based compounds with optimized properties to develop new anti-amoebic agent for *Acanthamoeba* keratitis.

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SITOTOKSITI, GENOTOKSITI DAN KAJIAN HUBUNGAN KUANTITATIF STRUKTUR-AKTIVITI (QSAR) SEBATIAN KARBONIL TIOUREA TERHADAP *ACANTHAMOEBA* SPP. DAN SEL KORNEA EPITELIA MANUSIA

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Empat puluh empat sebatian tiourea karbonil hasil disintesis telah diuji untuk mengenalpasti potensi sebagai agen anti-amebik bagi mencapai penemuan baru sebagai ubatan untuk penyakit *Acanthamoeba* keratitis. Sebatian-sebatian ini diuji ke atas empat isolat iaitu *A. castellanii* (CCAP 1501/2A), *A. polyphaga* (CCAP 1501/3A), *Acanthamoeba* sp. (isolat Hospital Kuala Lumpur) and *Acanthamoeba* sp. (isolat Laguna Setiu). Ujikaji yang dijalankan melibatkan ujian sitotoksik dan genotoksik iaitu; penentuan IC_{50} oleh kaedah pewarnaan eosin, pemerhatian morfologi menggunakan mikroskop cahaya, penilaian ketahanan membran melalui pewarnaan akridin jingga propidia iodin (AO/PI), penentuan mekanisma kematian sel melalui ujian fragmentasi DNA, dan juga penilaian terhadap kerosakan DNA dengan kaedah komet alkali. IC_{50} memberi nilai 6.26-30.46 μM bagi *A. castellanii*, 11.21-31.91 μM bagi *A. polyphaga*, 8.63-26.81 μM bagi *Acanthamoeba* sp. (isolat HKL) dan 6.91-21.64 μM bagi *Acanthamoeba* sp. (isolat SW), yang mana menunjukkan bahawa sebatian-sebatian ini sitotoksik terhadap *A. castellanii*, *Acanthamoeba* sp. isolat klinikal, dan *Acanthamoeba* sp. isolat persekitaran, tetapi separa sitotoksik ke atas *A. polyphaga*. Dua wakil sebatian, 2-(3-benzoiltioureido)-3-asid merkaptopropanoik dan 2-(3-benzoiltioureido)-4-(metiltio) asid butanoik, masing-masing dilabel sebagai M1 dan M2, menunjukkan aktiviti anti-amebik terbaik, dan diuji dengan ujian lanjutan. Sebatian-sebatian ini merencat struktur akantapodia dan mengubah bentuk sel ameba menjadi bulat, dan tidak mempunyai vakuol dan nukleus yang jelas. Ini menggambarkan sel ini mengalami proses perubahan pada sist dan tidak aktif dengan rawatan tiourea tersebut. Sebatian ini mengganggu ketahanan membran sel, menjadikan ia tidak tegar. Sebatian tersebut juga mengakibatkan apoptosis, tetapi tidak memberi impak yang tinggi pada DNA ameba dengan kerosakan DNA pada skor 0, 1 dan 2. Kawalan positif, klorheksidin, menujukkan aktiviti yang lebih baik tetapi mempamerkan kesan yang lebih tinggi dengan mengakibatkan nekrosis dan memberi kesan yang kuat ke atas DNA. Kedua-dua sebatian tiourea ini turut diuji ke atas sel bukan target iaitu sel epitelia kornea

manusia (SEKM) melalui ujian penentuan IC_{50} oleh ujian MTT, pengelasan sebatian menggunakan Indeks Selektif (SI), ujian sel proliferasi melalui kaedah MTT, pemerhatian morfologi menggunakan mikroskop cahaya, pemeriksaan ketahanan membran dan mekanisma kematian sel melalui pewarnaan AO/PI, serta penilaian kerosakan DNA menggunakan kaedah komet alkali. Hasil menunjukkan sebatian ini separa sitotoksik terhadap SEKM dengan nilai IC_{50} 132.69 μM bagi M1, dan 98.20 μM bagi sebatian M2. SI pula menunjukkan kedua-dua sebatian adalah separa selektif terhadap sel ameba. Sel proliferasi menunjukkan SEKM bertindak dengan pertambahan jangkamasa dan mengekalkan kadar sel hidup yang tinggi selepas 72 jam rawatan. Sebatian-sebatian ini didapati tidak merubah morfologi sel secara ketara. Rawatan menunjukkan sel tidak berbahagi secara sempurna. Sebatian ini juga dilihat mengganggu ketahanan membran SEKM dan mengakibatkan apoptosis, tetapi tidak bersifat genotoksik terhadap sel tersebut dengan memberikan skor komet pada tahap 0 dan 1. Kawalan positif, hidrogen peroksida, menunjukkan kesan yang sama dalam sifat sitotoksinya tetapi lebih genotoksik terhadap kornea. Pembinaan model QSAR melalui data 44 tiourea analog bersama aktiviti anti-amebik (pIC_{50}) ke atas *Acanthamoeba* sp. (isolat HKL) digunakan bagi analisa melalui kaedah stepwise-multiple linear regression (stepwise-MLR), genetic algorithm-multiple linear regression (GA-MLR), dan genetic algorithm-partial least square (GA-PLS). Model stepwise-MLR memberikan empat pembolehubah yang menghasilkan r^2=0.732, r^2_{cv}=0.522 berserta *RMSEC*=0.058, dan *RMSECV*=0.08. Hasil set uji, memberikan r^2_{test}=0.739, dan *RMSEP*=0.062. Model ini membuktikan ianya mempunyai kebolehan meramal yang tinggi dengan penghasilan nilai residu kurang dari +0.88. Ujian *y*-randomization pula yang menghasilkan r^2=0.052-0.248 merumuskan bahawa model ini tidak terhasil dari statistik rawak. Model dari GA-MLR pula menghasilkan lima pembolehubah dengan r^2=0.848, r^2_{cv}=0.767, *RMSEC*=0.044, *RMSECV*=0.055, r^2_{test}=0.777 dan *RMSEP*=0.057 membuktikan model ini dapat diterima, disokong oleh ujian ramalan yang memberikan residu kurang dari 0.55. Ujian *y*-randomization menghasilkan r^2 antara 0.004-0.300 membuktikan model tidak terhasil secara rawak. Manakala, GA-PLS memberikan hasil statistik yang bagus dengan menghasilkan model dengan lima pembolehubah berserta r^2=0.827, r^2_{cv}=0.682, *RMSEC*=0.047, *RMSECV*=0.064, r^2_{test}=0.790 dan *RMSEP*=0.051. Residu yang bernilaian kurang dari 0.25 hasil dari ramalan dari set uji membuktikan ketahanan model. *Y*-randomization pula mengesahkan ianya tidak terhasil dari statistik rawak dengan r^2 antara 0.015-0.372. Model GA-PLS adalah model terbaik kerana kebolehan meramalnya yang tinggi dan diwakili oleh EEig09d, N-070, JGI6, MATS2m dan MATS3m. Set pembolehubah ini memberi maklumat mengenai sifat hidrofobik dan serakan interaksi yang terlibat dalam pengangkutan dan pengagihan unsur sebatian melalui sel membran. Ujikasi ini dapat membantu meramal dalam penghasilan sebatian kimia dari tiourea dengan ciri optimum bagi menghasilkan agen anti-amebik untuk mengubati *Acanthamoeba* keratitis.