Desain dan Sintesis Turunan Kalkon dan Pirazolina Tersubstitusi Kloro sebagai Antikanker Payudara

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INTISARI

Tujuan penelitian ini mendesain, sintesis dan uji aktivitas antikanker payudara dari turunan kalkon dan pirazolina tersubstitusi kloro untuk mengidentifikasi senyawa yang potensial sebagai obat kemoterapi kanker payudara. Penelitian meliputi 6 tahap: 1) desain senyawa klorokalkon dengan aktivitas sitotoksik potensial menggunakan analisis QSAR (*Quantitative Structure Activity Relationship*), 2) sintesis senyawa klorokalkon berdasarkan rekomendasi QSAR, 3) sintesis senyawa kloropirazolina, 4) karakterisasi senyawa klorokalkon dan kloropirazolina menggunakan FT-IR, GC-MS, ^{1}H- dan ^{13}C-NMR, 5) uji sitotoksik senyawa klorokalkon dan kloropirazolina, 6) uji antiproliferasi, penghambatan siklus sel, induksi apoptosis dan analisis *molecular docking* senyawa klorokalkon dan kloropirazolina terpilih.

Analisis QSAR menghasilkan persamaan QSAR: pIC_{50} = 3,869 + (1,427 x qC1) + (4,027 x qC10) + (0,856 x qC15) - (35,900 x ELUMO) + (0,208 x Log P). Persamaan ini digunakan untuk mendesain 9 senyawa klorokalkon yang memiliki IC_{50} prediksi berkisar 2,06–8,29 µg/mL. Sintesis 9 senyawa klorokalkon melalui reaksi kondensasi *Claisen-Schmidt* dengan metode pengadukan selama 24 jam diperoleh rendemen yang sangat baik (92,9–98,8%). Reaksi siklokondensasi antara 7 senyawa klorokalkon dan fenilhidrazina dengan metode refluks selama 24 jam diperoleh 7 senyawa kloropirazolina dengan rendemen yang baik (71,0–88,4%).

Berdasarkan uji sitotoksik 9 senyawa klorokalkon dan 7 senyawa kloropirazolina terhadap sel kanker payudara MCF7 dan T47D *cell lines* dengan metode MTT diperoleh senyawa klorokalkon terbaik yaitu klorokalkon **C** ((E)-1,3-bis(4-chlorophenyl)prop-2-en-1-one) dengan nilai IC_{50} sebesar 13,64±1,73 dan 16,58±0,57 µg/mL sedangkan kloropirazolina terbaik yaitu kloropirazolina **B** (3-(2-chlorophenyl)-5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole) dengan nilai IC_{50} 25,34±0,35 dan 19,84±0,99 µg/mL yang keduanya memiliki nilai Indeks Selektivitas yang baik (IS>3). Uji antiproliferatif senyawa klorokalkon **C** dan kloropirazolina **B** pada MCF7 dan T47D *cell lines* menunjukkan *doubling time* yang lebih lama dibandingkan kontrol sel sehingga memperlambat proliferasi. Uji penghambatan siklus sel dengan metode *flow cytometry* menunjukkan senyawa klorokalkon **C** menginduksi fase sub-G1 pada MCF7 *cell lines* sedangkan pada T47D *cell lines* menginduksi fase sub-G1 dan menahan pembelahan sel pada fase G2/M. Kloropirazolina **B** menginduksi fase sub-G1 pada MCF7 maupun T47D *cell lines*. Uji induksi apoptosis dengan metode *flow cytometry* menunjukkan senyawa klorokalkon **C** dan kloropirazolina **B** menginduksi kematian MCF7 dan T47D *cell lines* melalui jalur apoptosis dan bukan nekrosis. Berdasarkan analisis *molecular docking*, mekanisme molekuler klorokalkon **C** dan kloropirazolina **B** melalui jalur aktivasi p53 *mutant*, inhibisi Bcl-2 dan COX-2.

Kata Kunci: QSAR, sintesis, antikanker payudara, klorokalkon, kloropirazolina

Design and Synthesis of Chloro-substituted Chalcone and Pyrazoline Derivatives as Breast Anticancer

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ABSTRACT

The aim of this study was to design, synthesize and test breast anticancer activity from chloro-substituted chalcone and pyrazoline derivatives to identify potential compounds as breast cancer chemotherapy drugs. The research involved 6 stages, including 1) the design of chlorochalcone derivatives with potential activity using QSAR analysis, 2) the synthesis of chlorochalcone derivatives based on the QSAR recommendations, 3) the synthesis of chloropyrazoline derivatives, 4) the characterization of chlorochalcone and chloropyrazoline derivatives using FT-IR, GC-MS, ^{1}H- and ^{13}C-NMR, 5) the cytotoxic assay of chlorochalcone and chloropyrazoline derivatives, and 6) the assesment of antiproliferation, cell cycle arrest, apoptosis induction assays and molecular docking analysis of selected chlorochalcone and chloropyrazoline derivatives.

The QSAR analysis yielded a QSAR equation: pIC_{50} = 3.869 + (1.427 x qC1) + (4.027 x qC10) + (0.856 x qC15) - (35.900 x ELUMO) + (0.208 x Log P). This equation was used to design 9 chlorochalcones, which were predicted to have IC_{50} values ranging from 2.06–8.29 µg/mL. The synthesis of 9 chlorochalcones was achieved through a Claisen-Schmidt condensation reaction with stirring for 24 hours, resulting in a high yield (92.9–98.8%). Chloropyrazoline was synthesized via cyclocondensation reaction between 7 chlorochalcone and phenylhydrazine, using the reflux method for 24 hours, and yielded 7 chloropyrazoline at good yields (71.0–88.4%).

Based on the cytotoxic assay of 9 chlorochalcone and 7 chloropyrazoline against breast cancer cells MCF7 and T47D cell lines using the MTT method, it was found that the most effective chlorochalcone was chlorochalcone **C** ((E)-1,3-bis(4-chlorophenyl)prop-2-en-1-one) with IC_{50} values of 13.64±1.73 and 16.58±0.57 µg/mL, while the most effective chloropyrazoline was chloropyrazoline **B** (3-(2-chlorophenyl)-5-(4-chlorophenyl)-1-phenyl-4,5-dihydro-1H-pyrazole) with IC_{50} values of 25.34±0.35 and 19.84±0.99 µg/mL, both of which had a good Selectivity Index (SI>3). The antiproliferative assay of chlorochalcone **C** and chloropyrazoline **B** on MCF7 and T47D cell lines showed a longer doubling time than control cells, which slowed down proliferation. The cell cycle inhibition assay showed that chlorochalcone **C** induced the sub-G1 phase (apoptosis) in MCF7 cell lines and induced the sub-G1 phase and cell cycle arrest in the G2/M phase in T47D cell lines.

Meanwhile, chloropyrazoline **B** induced the sub-G1 phase in both MCF7 and T47D cells. Based on the apoptosis induction assay using the flow cytometry method, it was determined that the compounds chlorochalcone **C** and chloropyrazoline **B** induced MCF7 and T47D cell lines death through apoptosis and not necrosis. Finally, molecular docking analysis revealed that the molecular mechanism of chlorochalcone **C** and chloropyrazoline **B** was through the activation of p53 mutant, inhibition Bcl-2 and COX-2.

Keywords: QSAR, synthesis, breast anticancer, chlorochalcone, chloropyrazoline