

## SINTESIS, UJI SITOTOKSISITAS DAN UJI IMUNOSITOKIMIA TURUNAN N-FENILPIRAZOLINA DARI 4-KLOROASETOFENON SEBAGAI SENYAWA ANTIKANKER

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### INTISARI

Penelitian ini bertujuan untuk menemukan senyawa turunan N-fenilpirazolina yang potensial sebagai kandidat obat kemoterapi penyakit kanker, terutama untuk kanker payudara, leher rahim dan kolon. Penelitian ini dilakukan melalui empat tahapan, yaitu: 1) sintesis kalkon sebagai bahan dasar, 2) sintesis senyawa N-fenilpirazolina, 3) uji sitotoksitas, dan 4) uji imunositokimia.

Reaksi kondensasi *Claisen-Schmidt* dilakukan melalui metode konvensional dan metode sonokimia antara 4-kloroasetofenon dengan 5 jenis benzaldehida, yaitu benzaldehida, 4-anisalaldehida, veratraldehida, vanilin dan 4-dimetilaminobenzaldehida sehingga menghasilkan senyawa (*E*)-1-(4-klorofenil)-3-fenil-2-propen-1-on (kalkon 1), (*E*)-1-(4-klorofenil)-3-(4-metoksifenil)-2-propen-1-on (kalkon 2), (*E*)-1-(4-klorofenil)-3-(3,4-dimetoksifenil)-2-propen-1-on (kalkon 3), (*E*)-1-(4-klorofenil)-3-(4-hidroksi-3-metoksifenil)-2-propen-1-on (kalkon 4), dan (*E*)-1-(4-klorofenil)-3-(4-(dimetilamino)fenil)-2-propen-1-on (kalkon 5). Pada metode konvensional dibutuhkan waktu selama 1,75-26,5 jam untuk menghasilkan kalkon 1-5 dengan *yield* 53,47-97,06%, sedangkan metode sonokimia membutuhkan 0,9-4,2 jam untuk menghasilkan kalkon 1-5 dengan *yield* 47,22-96,69%. Reaksi siklokondensasi antara kalkon 1-5 dan fenilhidrazina dilakukan melalui metode konvensional dengan sistem refluks selama 4-7 jam sehingga diperoleh senyawa 3-(4-klorofenil)-1,5-difenil-2-pirazolina (pirazolina 1) dengan *yield* 47,54%, senyawa 3-(4-klorofenil)-5-(4-metoksifenil)-1-fenil-2-pirazolina (pirazolina 2) dengan *yield* 44,19%, senyawa 3-(4-klorofenil)-5-(3,4-dimetoksifenil)-1-fenil-2-pirazolina (pirazolina 3) dengan *yield* 50,24%, senyawa 3-(4-klorofenil)-5-(4-hidroksi-3-metoksifenil)-1-fenil-2-pirazolina (pirazolina 4) dengan *yield* 11,03%, dan senyawa 3-(4-klorofenil)-5-(4-(dimetilamino)fenil)-1-fenil-2-pirazolina (pirazolina 5) dengan *yield* 75,53%. Elusidasi struktur seluruh senyawa hasil sintesis dilakukan menggunakan FT-IR, GC-MS, <sup>1</sup>H- dan <sup>13</sup>C-NMR.

Uji sitotoksitas senyawa kalkon 1-5 dan pirazolina 1-5 dilakukan secara *in vitro* terhadap sel kanker payudara (MCF-7 dan T47D), leher rahim (HeLa), kolon (WiDr) dan sel normal (Vero) dengan metode MTT. Senyawa pirazolina 1 diketahui memiliki potensi sebagai agen antikanker terbaik dengan nilai IC<sub>50</sub> 0,27 µg/mL terhadap sel kanker HeLa dan selektivitas tinggi terhadap sel normal. Uji imunositokimia dilakukan dengan metode tidak langsung untuk mengetahui mekanisme molekuler antikanker. Hasil uji menunjukkan bahwa pirazolina 1 meningkatkan ekspresi protein p53, Bax, Caspase-9, Caspase-3, IL-10 dan menurunkan ekspresi Bcl-2, COX-2, H-Ras. Dengan demikian, pirazolina 1 dapat menghambat sel kanker HeLa melalui beberapa jalur, yaitu meningkatkan apoptosis, menekan proliferasi dan menekan inflamasi pada sel HeLa.

Kata kunci: kalkon, pirazolina, sonokimia, antikanker, imunositokimia

## SYNTHESIS, CYTOTOXICITY AND IMMUNOCYTOCHEMISTRY EVALUATION OF N-PHENYLPYRAZOLINE DERIVATIVES FROM 4-CHLOROACETOPHENONE AS ANTICANCER COMPOUND

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### ABSTRACT

The aims of this study are to discover potent *N*-phenylpyrazoline derivatives as chemotherapeutic drug candidate on cancer disease, especially for breast, cervical and colorectal cancer. This research was carried out through four steps, i.e.: 1) synthesis of chalcones as precursor, 2) synthesis of *N*-phenylpyrazolines, 3) cytotoxicity evaluation, and 4) immunocytochemistry evaluation.

*Claisen-Schmidt* condensation reaction was performed via conventional and sonochemical methods between 4-chloroacetophenone with 5 different benzaldehydes, which were benzaldehydes, 4-anisaldehyde, veratraldehyde, vanillin and 4-dimethylaminobenzaldehyde to produce (*E*)-1-(4-chlorophenyl)-3-phenyl-2-propen-1-one (chalcone **1**), (*E*)-1-(4-chlorophenyl)-3-(4-methoxyphenyl)-2-propen-1-one (chalcone **2**), (*E*)-1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-2-propen-1-one (chalcone **3**), (*E*)-1-(4-chlorophenyl)-3-(4-hydroxy-3-methoxyphenyl)-2-propen-1-one (chalcone **4**), and (*E*)-1-(4-chlorophenyl)-3-(4-(dimethylamino)phenyl)-2-propen-1-one (chalcone **5**). The conventional method took 1.75-26.5 h for producing chalcone **1-5** in 53,47-97,06% yield, while the sonochemical method took 0.9-4.2 h for producing chalcone **1-5** in 47,22-96,69% yield. Cyclocondensation reaction between chalcone **1-5** and phenylhydrazine was done via conventional method with reflux for 4-7 h to produce 3-(4-chlorophenyl)-1,5-diphenyl-2-pyrazoline (pyrazoline **1**) in 47,54% yield, 3-(4-chlorophenyl)-5-(4-methoxyphenyl)-1-phenyl-2-pyrazoline (pyrazoline **2**) in 44,19% yield, 3-(4-chlorophenyl)-5-(3,4-dimethoxyphenyl)-1-phenyl-2-pyrazoline (pyrazoline **3**) in 50,24% yield, 3-(4-chlorophenyl)-5-(4-hydroxy-3-methoxyphenyl)-1-phenyl-2-pyrazoline (pyrazoline **4**) in 11,03% yield, and 3-(4-chlorophenyl)-5-(4-(dimethylamino)phenyl)-1-phenyl-2-pyrazoline (pyrazoline **5**) in 75,53% yield. Structure elucidation of all synthesized compounds was confirmed by FT-IR, GC-MS, <sup>1</sup>H- dan <sup>13</sup>C-NMR.

*In vitro* cytotoxicity evaluation of chalcones **1-5** and pyrazolines **1-5** was evaluated against breast cancer cell lines (MCF-7 and T47D), cervical cancer cell line (HeLa), colorectal cancer cell line (WiDr) and normal cell line (Vero) via MTT assay. Pyrazoline **1** was found to be the most potent anticancer agent with IC<sub>50</sub> value of 0,27 µg/mL against HeLa cancer cell line and possess high selectivity toward normal cell. Immunocytochemistry evaluation was performed via indirect method to study the anticancer molecular mechanism. The results showed that pyrazoline **1** increased the expression of p53, Bax, Caspase-9, Caspase-3, IL-10 proteins and decreased the expression of Bcl-2, COX-2, H-Ras proteins. Thus, pyrazoline **1** inhibits HeLa cancer cell line through multiple pathways, which are inducing apoptosis, suppress proliferation and inflammation of HeLa cell.

Keywords: chalcone, pyrazoline, sonochemical, anticancer, immunocytochemistry