SINTESIS DAN EPOKSIDASI OLEAMIDA TERSUBSTITUSI *N*-ARIL (FENIL DAN *O*-TOLIL) DARI ASAM OLEAT MINYAK KELAPA SAWIT SEBAGAI KANDIDAT ANTIKANKER: STUDI *IN VITRO* DAN *IN SILICO*

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INTISARI

Tingginya prevalensi kanker secara global dan keterbatasan kemoterapi, meliputi resistensi dan toksisitas, menjadi tantangan signifikan dalam bidang kesehatan yang menekankan perlunya pendekatan terapeutik baru. Penelitian ini bertujuan untuk mensintesis dan mengevaluasi sitotoksisitas empat senyawa kandidat antikanker berbasis asam oleat dari minyak kelapa sawit: oleamida aromatik (**OA-1** dan **OA-2**) yang diperoleh melalui reaksi amidasi dengan anilin dan *o*-toluidin menggunakan $SOCl_2$ sebagai *coupling agent*, kemudian diepoksidasi menggunakan mCPBA untuk menghasilkan **E-1** dan **E-2**. Elusidasi struktur dilakukan dengan FTIR, GC-MS, ^{1}H-NMR, ^{13}C-NMR, dan LC-MS. Uji aktivitas antikanker dan uji selektivitas dilakukan secara *in vitro* terhadap sel kanker T47D, HeLa, dan WiDr, serta sel normal Vero menggunakan metode MTT. Prediksi sifat farmakokinetik dan uji penambatan molekuler terhadap enzim FASN domain tioesterase dilakukan secara *in silico*.

Reaksi transesterifikasi menghasilkan FAME dengan persen hasil 70,88% dengan kandungan metil oleat 48,48%; yang berhasil ditingkatkan menjadi 70,61% melalui metode UIC. Hidrolisis FAME oleat menghasilkan asam oleat dengan persen hasil sebesar 71,50%. Data elusidasi mengkonfirmasi bahwa **OA-1, OA-2, E-1, dan E-2** sesuai dengan struktur kimia senyawa target dengan persen hasil berturut-turut sebanyak 55,93%; 90,22%; 34,85%; dan 33,59%. Analisis lebih lanjut menunjukkan bahwa **OA-2** serta **E-1 dan E-2** memiliki aktivitas sitotoksik signifikan terhadap sel HeLa dengan nilai IC_{50} berturut-turut 12,84, 6,16, dan 11,23 µg/mL. Sementara itu, **E-1 dan E-2** menunjukkan aktivitas moderat (20 µg/mL < IC_{50} ≤ 200 µg/mL) terhadap sel T47D dan WiDr. Seluruh senyawa menunjukkan selektivitas baik terhadap sel kanker HeLa, T47D, dan WiDr (SI > 2). Studi *in silico* mendukung hasil studi *in vitro* bahwa keterbatasan sifat farmakokinetik, khususnya kelarutan dan absorpsi, berkontribusi terhadap rendahnya efektivitas sitotoksik senyawa oleamida. Selain itu, senyawa turunan epoksida menunjukkan aktivitas sitotoksik yang lebih baik, didukung oleh interaksi sinergis senyawa **E1** terhadap residu Glu2251 serta interaksi senyawa **E-2** dengan residu katalitik enzim FASN (Ser2308, Asp2338, His2481), dan afinitas ikatan yang lebih kuat dibandingkan senyawa oleamida (**E1** = –6,90 kkal/mol dan **E2** = –6,57 kkal/mol).

Kata kunci: minyak kelapa sawit, oleamida, epoksida, antikanker, farmakokinetik, penambatan molekuler

SYNTHESIS AND EPOXIDATION OF *N*-ARYL-SUBSTITUTED OLEAMIDES (PHENYL AND *O*-TOLYL) FROM PALM OIL OLEIC ACID AS ANTICANCER CANDIDATES: IN VITRO AND IN SILICO STUDY

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ABSTRACT

The increasing global prevalence of cancer, along with the limitations of current chemotherapeutic agents, especially resistance and systemic toxicity, emphasizes the urgent need for alternative treatment methods. This study presents the synthesis and biological evaluation of four oleic acid derivatives from palm oil: *N*-aril oleamides (**OA-1** and **OA-2**), produced through amidation with aniline and *o*-toluidine using $SOCl_2$ as a coupling agent. Subsequent epoxidation with mCPBA yielded the corresponding epoxides (**E-1** and **E-2**). The chemical structures of all compounds were verified by FTIR, GC-MS, ^{1}H-NMR, ^{13}C-NMR, and LC-MS. Cytotoxicity and selectivity were assessed in vitro against T47D, HeLa, and WiDr cancer cell lines, as well as Vero normal cell line, using the MTT assay. Additionally, in silico analyses were performed to predict pharmacokinetic properties and investigate molecular interactions with the thioesterase domain of FASN enzyme.

The transesterification process yielded 70.88% of FAME, with a methyl oleate content of 48.48% and was successfully increased to 70.61% via the UIC method. Hydrolysis of the oleate methyl ester produced oleic acid in 71.50% yield. Structural elucidation confirmed that **OA-1**, **OA-2**, **E-1**, and **E-2** corresponded to the target structures, with yields of 55.93%, 90.22%, 34.85%, and 33.59%, respectively. Biological evaluation showed that **OA-2**, **E-1**, and **E-2** had strong cytotoxic effects against HeLa cells, with IC_{50} values of 12.84, 6.16, and 11.23 µg/mL, respectively. Furthermore, **E-1** and **E-2** demonstrated moderate cytotoxicity (20 µg/mL < $IC_{50} \leq$ 200 µg/mL) against T47D and WiDr cell lines. All synthesized compounds exhibited good selectivity toward HeLa, T47D, and WiDr cell lines, with selectivity indices (SI) values greater than 2. In silico results supported the in vitro findings, suggesting that limited solubility and absorption may partly explain the moderate cytotoxicity of oleamide derivatives. Notably, the epoxide derivatives showed higher activity, supported by favorable binding interactions. **E-1** formed a synergistic interaction with Glu2251, while **E-2** interacted with key catalytic residues of the FASN enzyme (Ser2308, Asp2338, His2481), displaying stronger binding affinities (**E-1** = –6.90 kcal/mol; **E-2 =** –6.57 kcal/mol) compared to oleamide analogs.

Keywords: palm oil, oleamide, epoxide, anticancer, pharmacokinetic, molecular docking