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RESEARCH ARTICLE

In-silico Molecular Docking ADME Study and Synthesis of Eugenol Derivatives as Antiproliferative

Farah Haidar Abdulredha *

Department of Pharmacy, Faculty of Pharmacy, the University of Mashreq, Baghdad, Iraq

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***Corresponding Author:**

farah.haidar@uom.edu.iq

ABSTRACT

The use of semisynthetic alterations to natural materials has provided us with several anticancer medications. The current study involves the synthetic modification of eugenol, a natural substance, to create novel anticancer drugs. The ultimate compounds were verified for their structure using NMR, IR, and mass methods. Based on the cytotoxicity data, compound 17 containing morpholine was identified as the most potent cytotoxic agent, exhibiting IC50 values of 1.71 µM (MCF-7), 1.84 µM (SKOV3), and 1.1 µM (PC-3). Additionally, it demonstrated inhibitory activity against thymidylate synthase (TS) with an IC50 value of 0.81 µM. Subsequent cellular investigations demonstrated that compound 17 has the ability to trigger apoptosis and halt the cell cycle specifically during the S phase in PC-3 cancer. Based on the docking investigation, compound 17 is very likely to be a transition state inhibitor, as it exhibited a comparable interaction to 5-fluorouracil. The results obtained from docking and biological assessment are supported by in silico pharmacokinetics and DFT computational investigations, which indicate a favorable pharmacokinetic profile for the medicine to be administered orally. Compound 17 shown potential as a promising inhibitor of thymidylate synthase (TS), effectively inhibiting DNA synthesis and thus reducing DNA damage in prostate cancer cells.

INTRODUCTION

The development of cancer treatment has benefited greatly from natural products and the semisynthetic compounds they include[1,2] . The derivatives of semi-synthesized natural chemicals have proven advantageous due to their altered pharmacokinetic profile, which reduces toxicity and enhances their antiproliferative activity. [3,4] A naturally occurring aromatic light yellow phenol, eugenol (4-allyl-2-methoxyphenol) is completely soluble in organic solvents but only moderately soluble in water[5,6] .It has significant antiviral, antitumor, anticancer, and antioxidant properties [7,8]. This non-cancerous and non-mutagenic molecule has anticancer properties by means of downregulating β-catenin/E2F1/surviving, inhibiting DNA synthesis, increasing the formation of reactive oxygen species, reducing mitochondrial membrane potential, and so on.using cell cycle arrests to initiate apoptosis , Cell cycle arrests caused by cancer are the second most common cause of death [9,10] Lung cancer leads the list of cancer-related fatalities worldwide and is on the rise, with breast and colorectal cancers following closely after.[11] While tailored therapies are now gaining a lot of interest, chemotherapy, radiation, and surgery remain the most frequently used treatments to combat the illness. Oncology medicinal chemists are interested in thymidylate synthase (TS), a folate-dependent enzyme necessary for DNA replication, as one of the targets. While targeted treatments are the focus of a lot of interest right now. Oncology medicinal chemists are interested in thymidylate synthase (TS), a folate-dependent enzyme necessary for DNA replication, as one of the targets.This

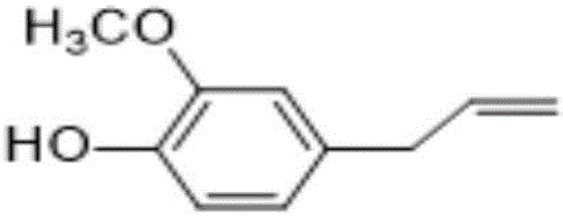
enzyme catalyzes methylation of deoxyuridine monophosphate (dUMP) to thymine monophosphate (dTMP) using CH_2THF cofactor, which after phosphorylation results in thymidine triphosphate (dTTP) formation, a precursor for DNA synthesis[12] .When TS is inhibited, thymine is depleted, which ultimately leads to dTTP depletion and eventual cell death. induction of apoptosis, and antiproliferation[13] .Additionally, the TS enzyme controls a number of proteins related to the process of apoptosis [14,15] New chemotherapeutic drugs with improved effectiveness and safety are a developing field in cancer treatment due to resistance, insensitivity, and toxicity of the current TS inhibitors [16, 17].Because of its broad mechanism of action, the creation of 1,3,4-oxadiazole based derivatives as anticancer medicines has grown significantly in medicinal chemistry during the last several decades [18] . For example, compound **I** showed a telomerase inhibitory activity with IC_{50} 2.30 μM, compound **II** as HDAC1 inhibitor with IC_{50} = 0.017 μM, [19] compound **III** was discovered to be 8-fold (IC_{50} 2.1 μM) greater efficacy as an inhibitor of FAK than Cisplatin (IC_{50} 8.6 μM), [20] while compound **IV** was 15-fold more potent TS inhibitor with IC_{50} 0.62 μM than pemetrexed [21].

Moreover, conjugation of oxadiazole scaffold with bioactive natural products also represents a promising approach. [22] Oxadiazole fused thymol (**V**), [23] furanolabdane, [24] and isosteviol derivatives [25] have shown significant inhibiting actions with IC_{50} 1.95 μM, GI_{50} 0.08–0.34 μM, and

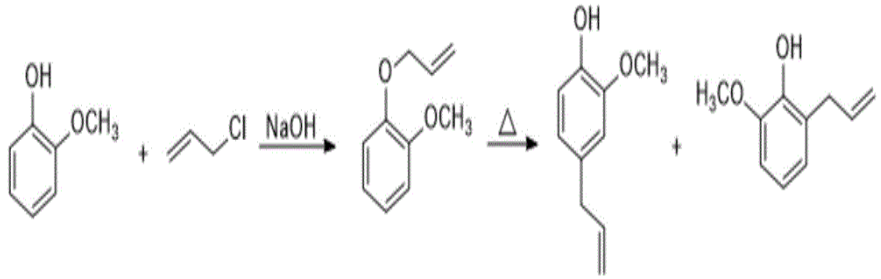
IC_{50} 0.95–3.36 μM, respectively, in the direction of tested cancer cells. These previously cited accounts of eugenol and 1,3,4-oxadiazole encouraging us to combine natural product with wide biological targets for a preventative measure eugenol with 1,3,4 oxadiazole scaffold. The present work reports the synthesis of eugenol-based 1,3,4-oxadiazole-hybrids (**5–17**) as well as their cytotoxicity, docking, and computational studies as TS inhibitors.

Synthesis method of eugenol

eugenol, also known as 4-allylguaiacol, has a strong clove scent, is colorless or light yellow, and is insoluble in water [26] .



Natural eugenol made by distilling and extracting dried flower buds of the Myrtaceae plant Eugenia caryophyllata. In order to produce eugenol, guaiacol is essentially used as a raw material for chemical reaction. A popular technique for producing eugenol from guaiacol is the claisen rearrangement process, whose chemical equation is as follows:



ortho-position is simpler to react, however even with short steps, the approach yields less than 50% of eugenol, a para-position product., and the boiling point of ortho-position isomer by-products is very similar to that of eugenol, which is challenging to remove, which has a significant impact on the quality of eugenol products. CN105294409 describes a synthesis method of eugenol in which

compounds like copper and cobalt salts are calcined into solid catalysts (TH L D) to catalyze the claisen rearrangement reaction. However, this method still produces ortho-position isomer by-products, and the complicated catalyst preparation process raises production costs. The use of cobalt and copper salts also contributes to pollution. Giguere et al. have discovered that by using microwaves to perform the claisen rearrangement reaction, eugenol selectivity can be increased to 87%. heating (Tetrahedron L et, vol.27, 4945-48) .

Synthetic Eugenol Derivatives

A natural product, eugenol **1**, was used as the starting material for the synthesis target compounds(**5–17**). All the intermediates **2–4** were created, and their melting points were verified by literature comparison.[27] The reaction between Compound 1 and ethyl bromo acetate when there is potassium carbonate and anhydrous acetone to yield compound **2**, which was treated with hydrazine hydrate in methanol to yield compound **3**. Next, after the inclusion of carbon disulfide drip by drop in the fundamental alcoholic concoction of compound **3** at 0–5 °C, the mixture was refluxed for 12 h, and acidification using HCl yielded the key intermediate **4** in 86% yield, which was utilized for the obtainment of final compounds **5–17**. Treatment of **4** with freshly prepared different N-substituted chloroacetamides in dry propanone afforded final hybrids **5–14** (68–90% yield) and with formaldehyde and different aliphatic amines in ethanol gave compounds **15–17** (70–85% yield).

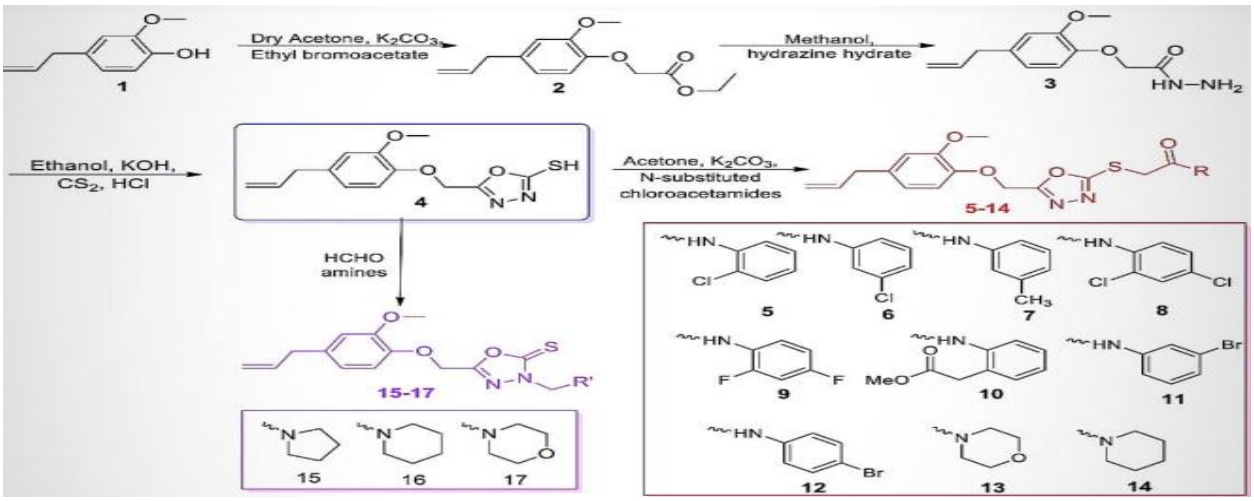


Figure1. Synthetic Pathway for the Eugenol Derivatives (5–17)

Mode of actions of Eugenol as anticancer agents

ultimate eugenol derivatives (**5–17**) were examined for their ability to inhibit the growth of three human adenocarcinomas. These includedbreast (MCF-7), prostate (PC-3), and ovarian (SKOV3) using the MTT technique as previously outlined.Of all the completed compounds, 1,3,4-oxadiazole-Mannich base bearing morpholine heterocycle (**17**) Exhibited the highest level of cytotoxic activity among all tested agents. IC_{50} 1.71, 1.84, and 1.1 μM, while doxorubicin exhibited IC_{50} 1.74, 2.88, and 2.61 μM, against MCF-7, SKOV3, and PC-3, respectively. Also, compound **9** having fluoro-substituted thioacetamide group demonstrated significant cytotoxicity against IC_{50} in the range 2.09–3.36 μM toward the tested cell lines. Against the breast cancer cells, compounds **8**, **12**, and **15** displayed significant cytotoxicity with IC_{50} < 10 μM, compounds **5**, **6**, and **11** were moderately cytotoxic with IC_{50} less than 25 μM and other compounds were found to be mild and inactive. Against ovarian and prostate cancer cells, compound **10** with the COOMe group displayed good sensitivity with IC_{50} 8.74 and 7.07 μM, respectively; Nevertheless, it exhibited a moderate level of toxicity against breast cancer cells. In addition, compound 15, which has a pyrrolidine ring in its Mannich base structure, exhibited

significant antiproliferative activity against prostate cancer. IC_{50} 10.01 μM. Compounds **5**, **11**, **12**, **15**, and **16** with IC_{50} in the range 14.09–26.33 μM and compounds **5**, **6**, **7**, **8**, **12**, **13**, and **16** with IC_{50} in the range 11.18–29.96 μM They exhibited a modest level of efficacy in eradicating ovarian and prostate cancer cells, respectively [28]. The remaining compounds were either mild (IC_{50} > 50) or less active (IC_{50} > 100). it is clear that most of the eugenol derivatives (except **14**) have the ability to hinder cancer cell proliferation.

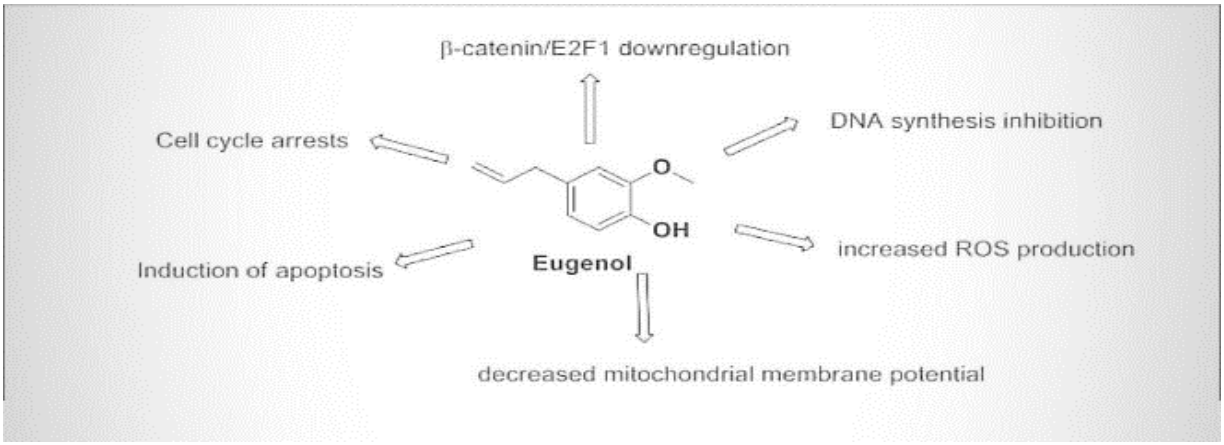


Figure2: Eugenol as anticancer agents.

Effect of Compound 17 Arrest Cell Cycle at S Phase

Disruption of the cell cycle is a significant factor in the rapid growth of cancer cells. Consequently, inhibiting the cell cycle is a very effective approach to impede cell proliferation [30]. Compound 17 is the most powerful substance that kills PC-3 cells. The compound that had the most inhibitory efficacy against TS was chosen to investigate its biological mechanism for suppressing cancer cell growth. PC-3 cells were exposed to compound 17 and a control. Both groups were stained with propidium iodide at their pre-calculated IC50 concentration for 48 hours.

The effects of the compounds on the cell cycle profile and apoptosis were then assessed using flow cytometry,Also **Compound 17 Induced Cancer Cell Apoptosis in PC-3 Cells .**In order to confirm the apoptotic potential of compound 17, a flow cytometric experiment was conducted using Annexin V-propidium iodide dual labeling. This assay allows for the distinction between living cells, cells in early and late stages of apoptosis, and necrotic cells [31].

In Silico Physicochemical and Pharmacokinetics Studies

The phrase 'in silico' refers to computer-based testing and is often used in relation to the biological words 'in vivo' and 'in vitro'[32].The process of developing new pharmaceuticals has been an expensive, hazardous, and demanding endeavor, characterized by a poor rate of success. The majority of molecules, according Unfavorable limitations and limited effectiveness in clinical studies prevent the product from being released to the market. Hence, use computational methods to anticipate the pharmacokinetics and toxicity of a medicine in the early phases of research offers insights into the therapeutic efficacy and likelihood of success of the molecule. Computational studies have facilitated the optimization of pharmacokinetic and toxicity characteristics, hence enhancing the efficiency of drug development.

The physicochemical and pharmacokinetic features of the recently created hybrids (5–17) have been assessed using Swiss ADME software [33] . The destiny of the produced molecules for a successful medicine is determined by certain criteria, such as Lipinski and Veber guidelines. The Lipinski guidelines are a fundamental guideline in drug development and research. They specify that a molecule should have a molecular weight (MW) of less than 500, a lipophilicity (i log Po/w) value of

less than 5, a hydrogen bond acceptor (HBA) count below 10, and a hydrogen bond donor (HBD) count below 5. These standards also encompassed the criteria of molecular flexibility (nROTB) and polar surface area (PSA) being below 10 and 140 Å2, respectively. The produced compounds have favorable oral absorption and permeability, since they do not exhibit any Lipinski violations with regards to molecular weight, HBA, HBD and lipophilicity.Furthermore, it was observed that all the compounds, with the exception of 10, exhibited flexibility, indicating the absence of any bioavailability issues 70.43–79.76%, except compound **10** that was shown low gastrointestinal absorption of 61.36%. All the compounds were discovered to be impermeable to the brain.

The results indicated that the synthesized compounds possess satisfactory physicochemical and pharmacokinetic characteristics, which are necessary for a molecule to be administered orally [33]. All the compounds were determined to be non-mutagenic, noncarcinogenic, and safe, with an LD50 within the acceptable range of 2.46–2.82 mol/kg. The chronic toxicity was shown to be within the safe range, and no skin sensitization impact was seen. Nevertheless, it was discovered that these chemicals exhibit hepatotoxicity.

Molecular Docking Study

Interactions occurring between a tiny molecule and a protein at the atomic level enable us to characterize the objective is to study the behavior of tiny molecules within the binding site of target proteins and to clarify essential biochemical processes. The docking method consists of two fundamental steps: the anticipation of the ligand's conformation, as well as its position and orientation inside the binding sites (often known as pose), and the evaluation of the binding affinity. The first phase pertains to sample procedures, while the second step concerns scoring schemes. These topics will be further explored in the theoretical section.

The eugenol derivatives that have been artificially produced (**5–17**) were docked against TS protein (PDB 6QXG) to corroborate our biological findings. According to reports, 5-fluorouracil (5-FU) interacts with the active site through GLY222, SER216, ASN226, ARG50, HIS256, ARG175, CYS195, GLY217, ASP218, ARG215, and ARG176 residues. The induce-fit-docking was applied to produce final postures via induce-fit-docking. The ultimate position was chosen based on the combination of the lowest binding free energy (ΔG) and RMSD.

The inhibition-constant (Ki) was performed for all the compounds **5–17**, which must be in the range 0.1–1.0 μM, and inversely proportional to binding energy efficiency. The results of our docking analysis showed that compounds 5–17 can interact with the important amino acids through several mechanisms, including hydrogen bonding, arene cations, and arene–arene interactions.(35) With the exception of compound 16, all the other compounds have almost same binding energy (ΔG in the range −7.20 to −7.89 kcal/mol), but lower than reference drug, 5-FU (ΔG −8.10) compounds **9** and **17** strongly support their in vitro TS inhibitory activity with IC_{50} 1.01 and 0.81 μM, respectively.

Based on the aforementioned outcomes, kcal/mol), and exhibited interactions with the active site that were akin to 5FU, The hypothesis is that the produced compounds engaged with TS protein equivalents in interactions. 5FU as TS inhibitor. The most active compounds **9** and **17** were found to bind with the TS pocket via H-bond with Arg50 amino acid, And compound 9 also formed other H-bond with Arg176 and π–π interaction with Trp109, whereas C═S of compound 17 formed strong H-bond with Arg50, Arg175, and π–π interaction with Arg215. Other compounds were stabilized in binding pocket of the vital TS backbone through binding with ASN226, CYS195, ASP218, ARG50, ARG175, ARG215, SER216, and ARG176. The docking interaction of compound **17** emerged as a promising lead for a TS inhibitor.[35]

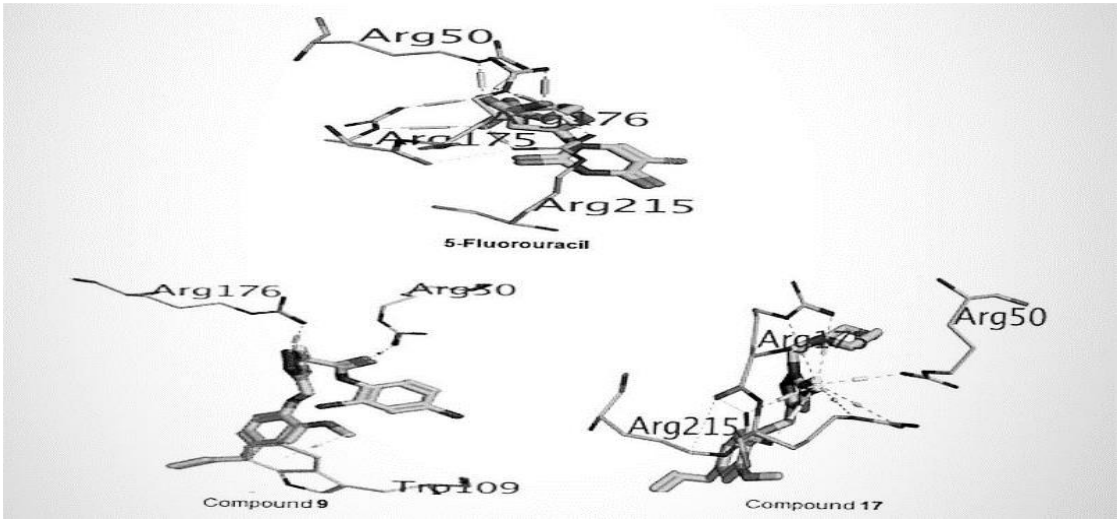


Figure.3: Docking poses of compounds 9, 17, and 5-FU.

CONCLUSIONS

Eugenol-based new 1,3,4-oxadiazole incorporated N-substituted acetamide and Mannich bases Anticancer drugs were synthesized with a satisfactory yield. In silico physicochemical and The toxicity investigations of the compounds revealed that the majority of them had drug-like characteristics and have been shown to be non-mutagenic, noncarcinogenic, and safe LD_{50} in the acceptable range of 2.46–2.82 mol/kg. Cytotoxicity results concluded that compound 17 bearing a morpholine ring was found to be the most active which blocks proliferation of breast, ovarian, and prostate cancer cells with IC_{50} 1.71, 1.84, and 1.1 μM, respectively, and TS inhibition effectively with IC_{50} of 0.81 μM. The activation of the S phase checkpoint in PC3 cells leads to S phase arrest, whereas the induction of irreversible DNA damage prompts apoptosis.

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