Investigating the potential inhibitory effect of the megaphone (molecule) on nasopharyngeal cancer growth factor receptors

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Abstract: **Nasopharyngeal cancer (NPC)** is a type of cancer that originates in the nasopharynx, which is the upper part of the throat behind the nasal cavity. Like other cancers, the growth and progression of nasopharyngeal cancer are influenced by various proteins involved in cell signaling, growth regulation, and tumor development such as Epidermal Growth Factor Receptor (**EGFR**), Vascular Endothelial Growth Factor (**VEGF**), Fibroblast growth factor receptor (**FGFR**) and Cyclin D1 (**CD1**). Megaphone ((1′R,5′R,7R,8S)-7-Hydroxy-3,4,5,5′-methoxy-5′,6′-dihydro-2′H-8,1′-neolign-8′-en-2′-one, **MG**) is the main component of the alcohol extract of the ground root of *Aniba megaphylla*, which *in vitro* inhibits the growth of cells derived from human nasopharyngeal carcinoma. Due to the lack of literature data, the main goal of this study was to examine the first step of the mechanisms of anti-carcinogenic activity by examining the inhibitory potential of **MG** against the above-mentioned cancer cell growth factor receptors.

Keywords: Megaphone, Nasopharyngeal cancer, EGFR, VGFR, FGFR

1. Introduction

The most common form of nasopharynx cancer, also known as **nasopharyngeal carcinoma (NPC)**, is most frequently detected in the postero-lateral nasopharynx or pharyngeal recess (fossa of Rosenmüller). It is essential to differentiate nasopharyngeal cancer from other throat-related malignancies, such as laryngeal and esophageal cancer. In terms of cancer incidence worldwide, nasopharyngeal cancer rates are 22nd among all cancer types. It is the **18th most prevalent cancer in men** and the **22nd most prevalent cancer in women**. There were over **133,000 newly reported cases** of nasopharyngeal cancer in 2020 alone [1].

In the 1960s, research revealed that an alcoholic extract of the ground root of *Aniba megaphylla* inhibited the proliferation of human nasopharyngeal carcinoma cells. In 1978, scientists isolated the active components of the extract using silica gel chromatography, identified them, and named the main compound Megaphone ((1′R,5′R,7R,8S)-7-

Hydroxy-3,4,5,5′-methoxy-5′,6′-dihydro-2′H-8,1′-neolign-8′-en-2′-one, $C_{22}H_{30}O_6$, **MG**, solid)). **MG** is a neolignane with considerable cytotoxic properties [2]. Surprisingly, despite its potential, no additional research has been conducted on the mechanisms of anticancer activity of this compound. Determining the inhibitory effects of **MG** on proteins essential for the growth of nasopharyngeal carcinoma cells, namely Epidermal Growth Factor Receptor (**EGFR**), Vascular Endothelial Growth Factor (**VEGF**), Fibroblast growth factor receptor (**FGFR**), and Cyclin D1 (**CD1**), is the purpose of this study (Figure 1).

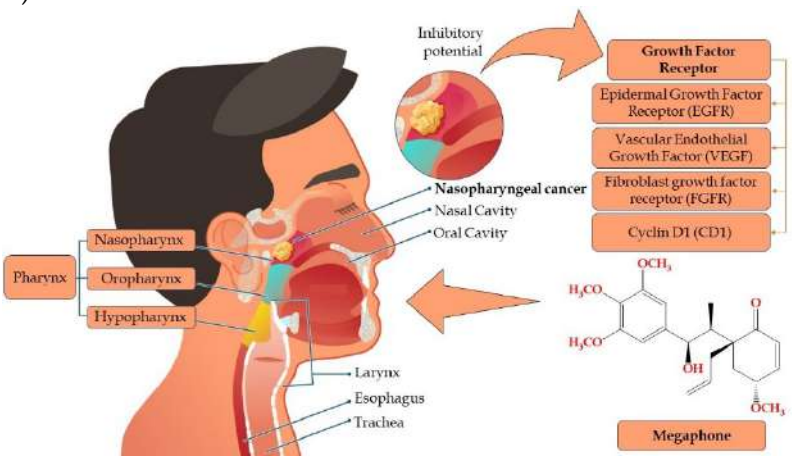

Figure 1. The location of nasopharyngeal carcinoma and the potential mechanism of the anticancer effect of megaphone (a compound isolated from the plant *Aniba megaphylla*) on the receptors for the growth and proliferation of cancer cells.

2. Methodology

In order to study the interactions between the **MG** and EGFR, VEGF, FGFR and CD1 receptor, molecular docking simulations were carried out using the AutoDock 4.2 program package. The three-dimensional crystal structure of the investigated receptors was retrieved from RCSB Protein Data Bank with PDB code: EGFR (1M17) [3], VEGF (4ASD) [4], FGFR (4V05) [5] and CD1 (2W96) [6]. In order to prepare the receptors, the non-protein components (co-crystallized water molecules) were removed, and missing amino acid residues were added. Before docking simulations, the structure of the investigated compound was optimized in *Gaussian 16* at the B3LYP-D3J/6-311++G (d,p) level of theory. The active site definition had been aligned to taken crystal structure co-crystallized with an erlotinib (**EGFR**, x=22.014, y=0.253, z=52.724 Å), sorafenib (**VGFR**, x=-24.262, y=-0.388, z=-10.926 Å), AZD4547 (**FGFR**, x=85.700, y=1.144, z=9.265 Å) and GOL1266 (**CD1**, x=17.135, y=9.905, z=59.265 Å). The other docking parameters have been selected based on standard protocols that have been expanded upon in previous studies. The calculation was performed with the Lamarckian Genetic Algorithm (LGA) technique.

3. Results and discussion

The thermodynamic parameters obtained from the molecular docking simulations are presented in Table 1. Based on the free binding energy (ΔG_{bind}) and the inhibition constant (K_i), the inhibitory activity according to the growth processes of cancerous cells decreases in the following order: **EGFR-MG** < **VGFR-MG** < **FGFR-MG** < **CD1-MG**. This indicates that **MG** has the greatest inhibitory effect on the transmembrane **EGFR** receptor. By blocking the **EGFR** binding site on the extracellular domain of the receptor, interruption of **EGFR** signaling can prevent the growth of EGFR-expressing tumors and improve the patient's condition. This represents one of the potential mechanisms of anticancer activity of **MG** against nasopharyngeal carcinoma.

Table 1. Thermodynamic parameters (ΔG_{bind} free energy binding, K_i constant of inhibition, ΔG_{total} final total internal energy, ΔG_{tor} torsional free energy, ΔG_{unb} unbound system's energy, ΔG_{elec} electrostatic energy and $\Delta G_{vdw+hbond+desolv}$ is the sum of dispersion and repulsion (ΔG_{vdw}), hydrogen bond (ΔG_{hbond}), and desolvation (ΔG_{desolv}) energy, kcal mol^{-1}) for the most stable conformations of MG in the different receptors obtained after molecular docking simulations.

Complexes	ΔG_{bind}	K_i (μM)	ΔG_{inter}	$\Delta G_{vdw+hbond+desolv}$	ΔG_{elec}	ΔG_{total}	ΔG_{tor}	ΔG_{unb}
EGFR-MG	-7.59	2.73	-10.57	-10.34	-0.23	-1.33	2.98	-1.33
VEGF-MG	-7.54	2.96	-10.52	-10.42	-0.10	-1.95	2.98	-1.95
FGFR-MG	-6.86	9.32	-9.85	-9.65	-0.20	-1.31	2.98	-1.31
CD1-MG	-7.01	7.26	-9.99	-9.34	-0.65	-2.42	2.98	-2.42

Figure 2 presents the amino acid environment of **MG** in the active site of the investigated receptors.

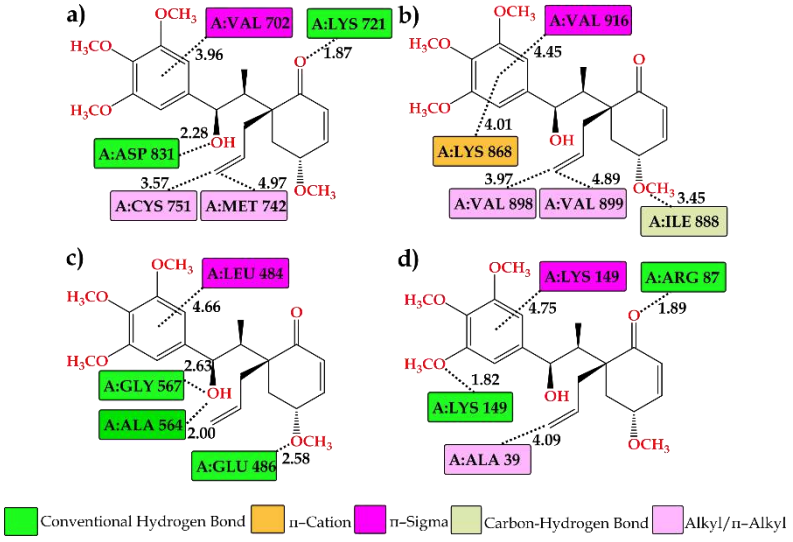

Figure 2. 2D representation of interactions between **MG** and amino acid residues: a) **EGFR-MG**, b) **VEGF-MG**, c) **FGFR-MG**, d) **CD1-MG** complexes with interatomic distance (Å) obtained after molecular docking simulations.

Through polar functional groups, **MG** establishes the most important type of interactions, conventional hydrogen bonds, with different amino acid residues: LYS, ASP, GLY, ALA, and ARG of investigated receptors. A characteristic π-cation interaction is established between the partially positive $-NH_3^+$ group of the A:LYS 868 (VGFR receptor) and the aromatic ring of **MG** (4.01Å). **MG** with amino acid residues of different receptors: VAL, CYS, MET, LEU, establish hydrophobic contacts: π-sigma, π-alkyl, alkyl, which contribute to the stabilization of the protein-ligand complex.

3. Conclusions

Based on the presented results, it can be concluded that the **MG** shows significant inhibitory activity toward nasopharyngeal cancer growth factor receptors: **EGFR**, **VGFR**, **FGFR**, **CD1**. According to the study's findings, **MG** has the strongest **EGFR** receptor-inhibitory action. The obtained results and a thorough analysis of the typical intramolecular interactions between **MG** and growth factor receptors serve as a crucial foundation for a further investigation of the mechanisms of **MG**'s anticancer activity.

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