Computational Analysis of Active Phytochemicals Came from GC-MS Chromatogram of *Rhynchanthus longiflorus* Hook.f. against Thymidine Phosphorylase Enzyme

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Abstract:

Background: A previous study reported fifteen identified compounds that were obtained from an essential oil extract from *Rhynchanthus longiflorus* Hook.f., carried out by using the GC-MS technique, which revealed possible potential candidates for further drug development in cancer chemotherapy.

Objective: This study aimed to determine and predict the most effective compound obtained from the GC-MS chromatogram of the essential oil extract from *Rhynchanthus longiflorus* Hook.f., active against the human thymidine phosphorylase enzyme, again carried out using computational analysis.

Materials and methods: All compounds identified by GC-MS analysis were three-dimensionally optimized and docked with a well-prepared crystal structure of the human thymidine phosphorylase enzyme. Additionally, the *in silico* pharmacokinetic properties, bioactive activities, and toxicity profiles prediction were determined.

Results: Amongst these identified compounds, beta-eudesmol showed the highest binding affinity against thymidine phosphorylase, with a binding energy of -7.44 kcal/mol, showing better values than that of the reference compound (5-iodouracil). The pharmacokinetic properties, bioactive activities, and toxicity profiles of all compounds met the acceptance criteria.

Conclusion: This study suggests that an active phytochemical, revealed by the GC-MS chromatogram, may be a most promising candidate drug, acting on the thymidine phosphorylase enzyme, and so should be studied further.

Keywords: In silico analysis, *Rhynchanthus longiflorus* Hook.f., Thymidine phosphorylase

Introduction

Thymidine phosphorylase (TP) is the nucleoside metabolism enzyme that mainly catalyzes thymidine to thymine and 2-deoxy-alpha-D-ribose-1-phosphate (dRib-1-P).[1] TP is an attractive target for the treatment of cancer, due to evidence from many studies, indicating that TP is correlated to malignant phenotypes of cancer cells.[2] The mechanism

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postulated is that a dephosphorylated product of dRib-1-P, called 2-deoxy-alpha-D-ribose (dRib), promotes angiogenesis and anti-apoptotic activity in cancer cells.[3] In an attempt to develop new substances for cancer chemotherapy, TP inhibition is an interesting concept for the inhibition of angiogenesis in many types of cancer.[1]

The plant studied has long been used to alleviate illness or diseases. Many plants from the family Zingiberaceae have been used in traditional for many purposes.[4] *Rhynchanthus longiflorus* Hook.f. (RL), one of the species in this family, is a rare and endangered epiphytic, perennial rhizomatous plant mainly found in Myanmar and India.[5] The decoction of the plant's rhizomes is being used for the treatment of hypertension and convulsions as folk remedies in some Akha villages.[6] In a previous study by our group, the chemical constituents of the RL rhizome was determined, using the GC-MS technique. This found twelve monoterpenes, two sesquiterpenes, and a phenylpropene identified from the essential oil.[7] Unfortunately, scientific data about the biological activities of RL is limited because of consideration of conservation of the scant plant material. However, the GC-MS chromatogram is valuable, not only for understanding of the chemical constituents of this plant, but also as a guide for computational screening of various drug targets for the treatment of many diseases. Thus, it is necessary to evaluate the TP inhibition of all of the phytochemicals, identified by GC-MS analysis, from the essential oil extract of R. longiflorus (RLO), by using computational analysis, to predict the role of these terpenoids and phenylpropanoid compounds.

Methodology

1. Preparation of phytochemical structures obtained from chromatogram of RLO

A detailed explanation of the RLO preparation and GC-MS analysis of active phytochemical constituents has already been reported in our previous paper.[7] All known structures that have been identified, from the main EI MS library of NIST/EPA/NIH Mass Spectral Library 2005 (NIST05), were constructed and the energy of freely rotatable bonds of all active constituents was determined, by using Chem3D Professional 10.0 (CambridgeSoft Inc., Cambridge, USA).

2. Structure preparation of human thymidine phosphorylase

A Crystal structure of human thymidine phosphorylase and another of 5-iodouracil (PDB ID: 2WK6),[8] were prepared, by removing all water molecules, any solvent, and the ligand (5-iodouracil as reference molecule), using the ViewerLite program (Accelrys, San Diego, USA).

3. Computational prediction of pharmacokinetic properties, bioactive activities, and toxicity profiles of compounds obtained from RLO

Canonical SMILES format of all known structures that have been identified from GC-MS chromatograms were retrieved from the PubChem compound database[9] and then entered into SwissADME (http://www.swissadme.ch/index.php), to compute their physicochemical and pharmacokinetic properties.[10] *In silico* bioactivity prediction of these compounds in SMILES format was evaluated using PASSonline software, which selected potential activity as a phosphorylase inhibitor, angiogenesis stimulant, as well as antineoplastic activity.[11] Computational toxicity analysis of all phytochemical compounds from RLO in SMILES format was performed by using a ProTox-II web-based tool.[12]

4. Molecular docking of compounds obtained from GC-MS of RLO against TP

The predicted binding free energy of compounds from RLO following docking

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was analyzed by using AutoDock 4.2.6 software.[13] Each energy-minimized compound from RLO was mated to the well-prepared targets with default parameters of docking procedures. The molecular docking protocol was obtained from the active site of the human TP, with a molecular grid set at 0.375 Å grid spacing. The location of the grid was located at x = 15.497, y = 17.393, and z = 47.496 Å. Docking results of all compounds were evaluated using the best predicted binding free energy (BE, kcal/mol), along with the inhibitory constant from all clusters of each conformational structure. Virtual analysis of the best results was then viewed and analyzed using UCSF Chimera.[14]

Results and discussion.

The challenge of drug discovery and development for antineoplastic agents is to reduce development times and effectively screen out unfavorable compounds.[15] Computational analysis is one of the approaches that can be used to search for more effective drug candidates with potentially fewer side effects.[15] Free accessible computer aided web-based tools for drug screening are versatile, user-friendly, and fairly efficient, being based on bioinformatics, cheminformatics, chemical biology, and molecular modeling.[10-12]

In our study, we selected SwissADME, PASSonline, and ProTox-II online tools to predict and estimate the potential pharmacokinetic properties, bioactive activities, and toxicity profiles, respectively. All compounds from RLO fell within Lipinski's rule of five, with no effect on any pharmacokinetic parameters. The logP parameter (octanol/water partition coefficient) is a physiochemical descriptor for lipophilicity which affects drug absorption, distribution, drug-receptor interactions, and drug metabolism.[16] The predicted logP values of known phytochemical compounds from RLO were within the acceptable range (range of 2.58 to 4.37), and so were expected to display good bioavailability (Table 1).

Table 1 Physiochemical properties and molecular docking analysis of compounds identified by GC-MS analysis from RLO toward TP

Compound number	Phytochemical name	Predicted binding energy (kcal/mol)[a]	Predicted inhibitory constant (μM)[a]	MW[b]	HBA[c]	HBD[c]	cLogP[c]
1	α-pinene	-5.44	102.31	136.23	0	0	3.44
2	(+)-sabinene	-5.29	132.63	136.23	0	0	3.25
3	(-)-β-pinene	-5.45	101.68	136.23	0	0	3.42
4	β-myrcene	-4.88	265.31	136.23	0	0	3.43
5	(+)-3-carene	-5.34	121.47	136.23	0	0	3.42
6	Eucalyptol	-5.41	109.16	154.25	1	0	2.67
7	*trans*-β-ocimene	-5.15	168.35	136.23	0	0	3.4
8	γ-terpinene	-4.87	269.91	136.23	0	0	3.35
9	Linalool	-5.21	150.49	154.25	1	1	2.66
10	Unknown	-	-	-	-	-	-
11	4-carromenthenol	-5.92	45.75	154.25	1	1	2.6
12	(-)-α-terpineol	-5.71	65.56	154.25	1	1	2.58

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Compound number	Phytochemical name	Predicted binding energy (kcal/mol)[a]	Predicted inhibitory constant (μM)[a]	MW[b]	HBA[c]	HBD[c]	cLogP[c]
13	2-carene	-5.37	116.16	136.23	0	0	3.12
14	Methyl eugenol	-5.78	57.76	178.23	2	0	2.58
15	(+)-9-aristolene	-6.32	23.23	204.35	0	0	4.37
16	β-eudesmol	-7.44	3.51	222.37	1	1	3.61
Ref[d]	5-iodouracil	-5.95	43.20	237.98	2	2	0.59

[a] Results were obtained from AutoDock 4.2.6 software.
[b] Calculated using ChemBioDraw Ultra16.0. MW: molecular weight
[c] Calculated using SwissADME. HBA: number of hydrogen acceptors; HBD: number of hydrogen donors; RB: number of rotatable bonds; tPSA: total polar surface area; cLog P: log octanol/water partition coefficient.
[d] Reference compound: 5-iodouracil

The prediction of potential bioactive activities of identified compounds were then analyzed, using the PASS Online web-based tool. The output result of potential activity, both as a phosphorylase inhibitor and antineoplastic agent, is given in Table 2, where "Pa" represents the probability to be active in the sub-class of bioactivities.[11] In this study all compounds from RLO showed antineoplastic activity in a variety of cancers. Compound 2, 7-12, and 16 displayed phosphorylase inhibition, which indicated their potential use as an inhibitor of the TP.

Table 2 In silico bioactivity prediction of compounds identified by GC-MS from RLO

Compound	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16
Phosphorylase inhibitor	0	0.15	0	0	0	0	0.17	0.13	0.17	0.14	0.14	0	0	0	0.14
Angiogenesis stimulant	0	0	0	0.24	0	0	0.51	0.19	0.31	0	0	0	0.26	0	0
Antineoplastic (bladder CA)	0	0.17	0.16	0	0.17	0	0	0.15	0	0	0	0.19	0.16	0	0
Antineoplastic (bone CA)	0	0.23	0	0	0	0	0	0	0	0	0	0	0	0	0.19
Antineoplastic (brain CA)	0	0	0	0.30	0.26	0	0.30	0	0.26	0	0	0.28	0.22	0	0
Antineoplastic (breast CA)	0	0.15	0.16	0.89	0	0.16	0.76	0	0.26	0	0	0	0.40	0	0.17
Antineoplastic (carcinoma)	0	0	0.12	0.56	0.11	0	0.46	0	0.23	0	0	0	0.16	0	0.18
Antineoplastic (cervical CA)	0	0	0	0.14	0	0.178	0.24	0	0	0	0	0	0.32	0	0
Antineoplastic (colon CA)	0	0	0	0.18	0	0.75	0.22	0	0	0	0	0	0.20	0	0
Antineoplastic (colorectal CA)	0	0	0	0.18	0	0.76	0.28	0	0	0	0	0	0.21	0	0
Antineoplastic (endocrine CA)	0	0.19	0.20	0	0	0	0	0	0	0	0	0	0.17	0	0.29
Antineoplastic (gastric CA)	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0
Antineoplastic (glioma)	0	0	0	0.22	0	0	0.3	0	0	0	0	0	0	0	0
Antineoplastic (liver CA)	0	0.38	0.35	0.35	0	0	0.30	0.15	0.26	0	0	0	0.24	0	0.30
Antineoplastic (lung CA)	0	0.41	0.49	0.68	0.19	0.78	0.64	0	0.37	0	0	0.16	0.24	0	0.41
Antineoplastic (lymphoma)	0	0	0	0	0	0	0	0	0.15	0	0	0	0.18	0	0
Antineoplastic (melanoma)	0	0.30	0.38	0.36	0.16	0	0.48	0	0	0	0	0	0.21	0	0.19
Antineoplastic (MM)	0	0	0	0	0	0	0	0.37	0	0	0	0	0	0	0
Antineoplastic (NHL)	0	0	0	0	0	0.34	0	0	0	0	0	0.3	0	0	0
Antineoplastic (NSCLC)	0	0	0	0	0	0	0.17	0	0	0	0	0	0.30	0	0
Antineoplastic (ovarian CA)	0.12	0.13	0.20	0.30	0.11	0.36	0.35	0	0	0.15	0	0	0.18	0	0.15
Antineoplastic (pancreatic CA)	0.22	0.49	0.44	0.25	0.25	0	0.28	0.25	0.29	0.33	0.34	0	0.26	0	0.40
Antineoplastic (renal CA)	0	0.19	0.19	0.50	0.14	0	0.50	0	0.17	0.18	0	0.18	0.19	0	0.14
Antineoplastic (SCLC)	0	0	0	0	0	0	0	0	0	0.21	0	0	0.26	0	0
Antineoplastic (solid tumors)	0	0	0	0	0	0	0.26	0.25	0	0	0	0	0.28	0	0
Antineoplastic (SCC)	0	0.13	0.16	0.29	0.16	0	0.35	0	0.10	0	0.09	0.09	0.16	0	0.21
Antineoplastic (thyroid CA)	0.18	0.24	0.26	0.15	0.19	0	0.17	0	0	0	0	0.20	0.19	0.21	0.30
Antineoplastic (uterine CA)	0	0	0	0	0	0	0	0	0	0	0	0	0.13	0	0

[a] Pa value is probability "to be active", by using PASSonline software; CA: cancer, SCLC: Small cell lung cancer; SCC: Squamous cell carcinoma; NSCLC: Non-small cell lung cancer; NHL: Non-Hodgkin lymphoma; MM: Multiple myeloma;

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In addition, the toxicity prediction of 15 compounds identified by GC-MS analysis from RLO were also evaluated using ProTox-II. Almost compounds showed prediction for low toxicity except for β-myrcene (4), methyl eugenol (14), and (+)-9-aristolene (15). Beta-myrcene (4) has classified toxicity *via* nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE) and heat shock factor response element (HSE) mechanisms. Methyl eugenol (14) was predicted to be carcinogenic and have anti-aromatase activity. (+)-9-aristolene (15) was predicted to exhibit immunotoxicity. For predicted toxicity class and toxic dose (LD50) values of compounds from RLO, almost compounds fell under toxicity class III except *trans*-β-ocimene (7) which fell in toxicity class III with the lowest LD50 as displayed in Table 3.

Table 3 Toxicity profiles of compounds identified by GC–MS from RLO

Compound number	Predicted LD50 (mg/kg)	Predicted toxicity class	Classification
1	3,700	5	Inactive
2	5,000	5	Inactive
3	4,700	5	Inactive
4	5,000	5	Nuclear factor (erythroid-derived 2)-like 2, Heat shock factor response element (HSE)
5	4,800	5	Inactive
6	2,480	5	Inactive
7	113	3	Inactive
8	2,500	5	Inactive
9	2,200	5	Inactive
10	-	-	-
11	1,016	4	Inactive
12	2,830	5	Inactive
13	4,800	5	Inactive
14	810	4	Carcinogenicity, Aromatase
15	4,800	5	Immunotoxicity
16	2,000	4	Inactive

[a] LD50: The median lethal dose, is the dose of a compound that is caused death for 50% of the animals in a dose group.

All compounds from RLO were docked with the human TP in the region of the receptor-protein binding interface. The binding energy of the reference compound, 5-iodouracil was -5.95 kcal/mol, with an estimated inhibition constant (Ki) of 43.20 μM. The compounds that exerted binding free energy greater than that of the reference compounds towards TP were (+)-9-aristolene (15) and β-eudesmol (16) which provided

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binding energies of -6.32 and -7.44 kcal/mol, respectively (Table 1).

This finding indicated that there are promising compounds exerting thymidine phosphorylase inhibition. In this study, we selected only β-eudesmol (16) for binding mode analysis. The interaction of β-eudesmol against TP is by H-bonding interaction with Ser117 residues, with bond distances of 2.98 Å, and also by hydrophobic interaction with the hydrocarbon chain of His116, Thr118, Gly119, Gly120, Asp123, Gly145, Arg146, Leu148, Thr151, Gly152, Gly153, Thr154, Tyr199, Ser217, Ile218, and Lys221 (Figure 1). This compound binds with an enzyme in a good conformational position, with proximity to the catalytic site of TP, including Arg202, Ser217, and Lys221.[17]

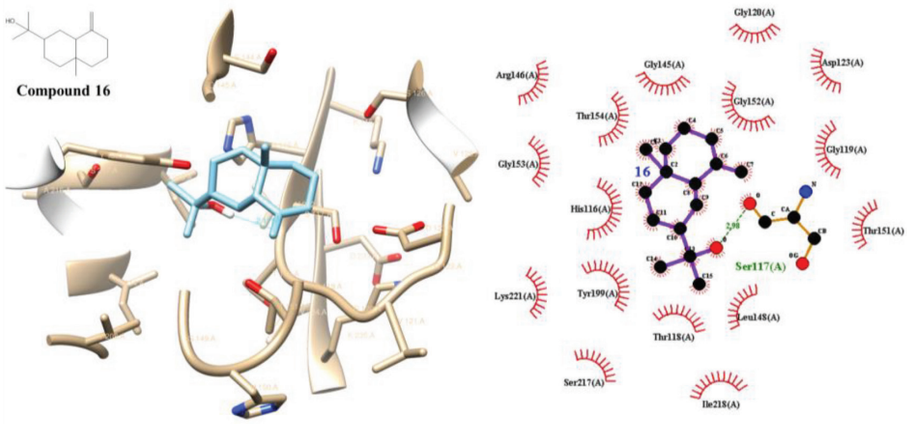

Figure 1 Binding mode of compound 16 toward human TP (2WK6)

The essential oils, which contain the most terpenoid compounds may play a role in pharmaceutical, food, and cosmetic applications. The biological effects of certain terpenes have been reported in a broad range of therapeutic areas, including anticancer chemotherapy, anti-inflammatory, antimicrobial chemotherapy, antioxidant, and anti-parasitic activities. This has led to widespread drug research and development.[18] Beta-eudesmol, a sesquiterpene from RLO. seems to be a promising compound, that binds effectively to the TP enzyme. This enzyme might have a possible role in the pathogenesis of cancer. There have been several previous reports about the beneficial roles of β-eudesmol in anticancer chemotherapy. For example, it had *in vitro* and in *vivo* activities against a wide range of cancers, including lung cancer, colon cancer, liver cancer, bile duct cancer/cholangiocarcinoma (CCA), gastric cancer, melanoma, and leukemia.[19] The mechanism of action of β-eudesmol against cancer cells was reported in cholangiocarcinoma cell lines *via* inhibition of STAT1/3 phosphorylation, heme oxygenase (HO)-1 activation, and NF-kB expression.[20] Therefore, the mechanisms of action of β-eudesmol *via* TP inhibition require further exploration.

In this particular study, even though the plant RL is rare and endangered, all compounds identified by GC-MS analysis from this plant are commercially available as pure chemical reagents which can be obtained without invasion of this plant's resources. This study paves the way for further drug development from active compounds of RLO, as a new class of cancer chemotherapy. It demonstrates that this rationale of *in silico* prediction is an approach that can be used to screen and design drug candidates, which is less time-consuming and provides essential information to prioritize drug discovery and ongoing development processes.

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Conclusion

This computational analysis revealed that β-eudesmol, selected from all of the compounds identified by GC-MS analysis from RLO, showed the best binding affinity towards human TP, so could be the start point of further cancer chemotherapeutic development.

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Conflicts of Interest

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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