Original Research Article

**Vepris nobilis** plant: a potential source of anticancer agents

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**ABSTRACT**

**Background:** Cancer is one of the major causes of death worldwide. Current cancer therapy is costly, it has poor therapeutic outcomes and many side effects. Therefore, new medications are needed. Plants have been used as sources of anticancer drugs. *Vepris* species have anticancer properties. The purpose of this study is to assess *Vepris nobilis*, a plant found in Kenya as a potential source of anticancer drugs.

**Methods:** The dichloromethane/methanol (CH2Cl2/M ethanol) 1:1 extract of the stem bark of *Vepris nobilis* led to the isolation of an alkaloid named, 4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinolone. SwissADME online tool was used to assess the compound’s pharmacokinetic parameters. Pass online tool identified potential targets while protox server described the toxicity of the compound. Chimera and Avogadro softwares were used for molecular docking studies.

**Results:** In-silico pharmacokinetic studies, showed that the isolated compound complied with Lipinski rule of five, it showed high gastrointestinal activity, and it also inhibits cytochrome P450 (CYP) isoforms 1A2, 2C9 and 2C19. In toxicity studies the compound was relatively safe with a predicted median lethal dose (LD50) of 1600 mg/kg, apart from potential immunotoxicity and mutagenicity. Molecular docking studies demonstrated that, the compound has potential anticancer activity, it interacted with deoxyribonucleic acid (DNA) topoisomerase I in an almost similar manner to camptothecin though it had less binding potential.

**Conclusions:** 4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinolone derived from *Vepris nobilis* is a potential drug for the management of cancer which can be administered orally.

**Keywords:** Cancer, In-silico, Molecular docking, Pharmacokinetic, *Vepris nobilis*

**INTRODUCTION**

Cancer is a group of diseases due to abnormal growth of cells, which comprise a complex environment of different types of malignant cells which support its growth and development.1,2 It is among the leading cause of death worldwide. Globally, 9.8 million deaths were reported in 2018.3-5 The number of cancer deaths are expected to rise to 13 million by 2030.6

In Kenya, cancer is a major health problem, it is a third leading cause of mortality, responsible for 7% of the annual deaths.7 The current treatment methods are facing many challenges; they are only effective at early stages, very expensive and resistance to the chemotherapeutic drugs has occurred in some cases.8-13 In addition, the severe side effects are not effectively mitigated. Hence there is an urgent need for continuous research to find alternative agents to fight against cancer.

Plants have been used as sources of anticancer drugs. For instance, camptothecin, an alkaloid extracted from *Camptotheca acuminata* is a drug used in cancer treatment by Thomas et al. Other anticancer drugs derived from plants include but not limited to vincristine, vinblastine from *Catharanthus roseus* and taxol from *Taxus*.
brevifolia.\textsuperscript{16} The genus Vepris is a rich source of furoquinoline and acridone alkaloids, which have been reported to have anticancer antiplasmodial, antimicrobial and antioxidant activities.\textsuperscript{17-20} Therefore, it was worthwhile to isolate the secondary metabolites of this genus.

Drug discovery is very expensive and requires a lot of time. However, \textit{in silico} drug discovery is cheaper and not as time-consuming as conventional methods of drug discovery like \textit{in vitro} and \textit{in vivo} studies. \textit{In silico} drug discovery involves use of softwares and databases to assist in discovery and development of drugs. It facilitates predictions of how ligands and drugs may interact with various targets or receptors.

**METHODS**

**General**

The $^1$H (200, 600 MHz) and $^{13}$C (50, 150 MHz) were acquired using Varian-Mercury and Bruker instrument using residual solvent signals as reference. Column chromatography was on normal silica gel 60G (Merck, 70-230 mesh) and Sephadex LH-20. Analytical thin layer chromatography (TLC) using silica gel 60 F254 (Merck) pre-coated plates were used to monitor the separation of compounds. For qualitative work, the TLC plates were visualized under ultraviolet (254 and 366 nm) light, exposure to iodine (I$_2$) vapor or spraying with Dragendorff reagent.

**Plant material**

The stem bark of Vepris nobilis was collected from Kakamega forest, Kenya, in July 2010. The plant was identified at the University Herbarium, School of Biological Sciences, University of Nairobi.

**Extraction and isolation**

The dried and ground stem bark (3.2 kg) of Vepris nobilis was extracted thrice using dichloromethane/methanol (CH$_2$Cl$_2$/MeOH) 1:1 by cold percolation. The crude extract (80 g) was subjected to column chromatography on silica gel (600 g). Gradient elution with n-hexane containing increasing amount of ethyl acetate and finally washed with MeOH afforded twenty major fractions (labeled A-T). Fraction M (eluted with 55% CH$_2$Cl$_2$ in n-hexane) was used to obtain compound 1 (24 mg) as colourless solids, after further purification on a silica gel (50 g) column with n-hexane containing increasing amount of CH$_2$Cl$_2$ (1 to 99% v/v).

**In-silico pharmacokinetic analysis**

SwissADME online tool (http://www.swissadme.ch/) was used to predict the pharmacokinetic profile of 4,6-dimethoxy-7-(3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinoline.\textsuperscript{23} Canonical SMILES of the compound were uploaded to the SwissADME tool which predicts and evaluates medicinal chemistry likeness, drug-likeness and pharmacokinetic properties.

**In-silico toxicity prediction**

Canonical SMILES of 4,6-dimethoxy-7-(3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinoline were uploaded to the ProTox server (http://tox.charite.de/protox_H/) which was used to predict the toxicity profile including hepatotoxicity, cytotoxicity, mutagenicity, immunotoxicity, carcinogenicity, toxicological pathways and toxicity targets.\textsuperscript{24}

**Determination of potential targets**

Using the pass online website, potential targets for 4,6-dimethoxy-7-(3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinoline were identified.\textsuperscript{25}

**Molecular docking**

4,6-dimethoxy-7-(3-methylbuta-1,3-dien-1-yl)oxy)furo [2,3-b]quinoline was drawn using pubchem sketcher. A moffile of the compound was downloaded and converted to 3-D using Avogadro software.\textsuperscript{26} Optimisation of the chemical structure to the most stable conformation was done using the Avogadro software. Based on the pass online results, the compound had antineoplasic activity and targeted beta-glucuronidase. Anticancer that are also affected by beta glucuronidase are the topoisomerase inhibitors. Therefore, DNA topoisomerase (PDB ID:1A36) was downloaded from the protein databank. Residues were removed from the DNA topoisomerase enzyme using the chimera software.\textsuperscript{27} Molecular docking between DNA topoisomerase enzyme and 4,6-dimethoxy-7-(3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinoline was done using autodock vina feature in chimera software. The binding energies were compared with the binding between campothecin and DNA topoisomerase. Ligand-receptor interactions were observed using Discovery studio software.

**RESULTS**

Compound 1 was isolated as yellow oil with retention factor (Rf) value of 0.5 (1% MeOH in CH$_2$Cl$_2$). The spot turned orange when sprayed with Dragendorff reagent which is an indication of an alkaloid. The $^1$H NMR spectrum revealed the presence of a pair of AB doublets corresponding to the two furan protons (H-2 and H-3) of furoquinoline alkaloids along with a downfield shifted methoxyl which is also characteristic of a methoxyl group at C-4 for furoquinoline alkaloids.\textsuperscript{28} The $^1$H NMR spectrum further showed a second methoxyl group resonating at 6H 4.02, with corresponding carbon resonating at 6C 56.1.

The presence of two singlet aromatic protons at 7.52 (H-5, $\delta$C 104.6) and 7.32 (H-8, $\delta$C 101.2) is consistent with C-6 and C-7 substituted c ring. One of these substituents being
methoxyl ($\delta$H 4.02, $\delta$C 56.1) groups and was placed at C-6 based on Nodiff experiment. Irradiation of methoxyl at C-4 showed interaction with H-3 and H-5, and irradiation of the methoxyl group resonating at $\delta$H 4.02 ppm (6-OMe) showed interaction with H-5.

The substituent at C-7 is 3-methylbuta-1,3-dienyloxy, as evidenced by the presence in the $^1$H NMR spectrum of a pair of doublets resonating at $\delta$H 6.85 (J=12.2 Hz, for H-1'), and 6.32 (J=12.2 Hz, H-2'), terminal methylene protons resonating at $\delta$H 4.94 ($^1$H, Br S) and 4.89 ($^1$H, Br S), and a methyl group at $\delta$H 1.91 for Me-5. The corresponding carbon atoms of this group appeared at $\delta$C 142.3 (C-1'), 115.3 (C-2'), 114.5 (C-3'), 118.9 (C-4') and 18.9 (C-5'). Therefore this compound was characterized as 7-(3-methylbuta-1,3-dienyloxy)-4, 6-dimethoxyfuro [2,3-b]quinolone.

Pharmacokinetics

Based on SwissADME, 4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinolone has high gastrointestinal activity, it crosses the blood brain barrier and inhibits cytochrome P450 (CYP) isoforms 1A2, 2C9 and 2C19. In terms of Pan-assay interference compounds (PAINS) alert, it had no alert. The compound has a molecular weight of 311.33 g/mol, partition coefficient (log P) of 3.76, 0 hydrogen bond donors and 5 hydrogen bond acceptors. The bioavailability score was 0.55.

Toxicity

The predicted median lethal dose (LD50) for 4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinolone was 1600 mg/kg which indicates that the compound is in toxicity class 4 (LD50 between 300 and 2000). Assessment of organ toxicity, toxicity end points, toxicological pathways and toxicity targets indicated that the compound can cause immunotoxicity and mutagenicity.

Potential targets

Based on Pass online website, the major potential actions of 4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinolone were antineoplastic activity, beta glucuronidase inhibitor and gluconate-2-dehydrogenase inhibitor.

Molecular docking

4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo [2,3-b]quinolone bound to DNA topoisomerase I as shown in Figure 1. However, the binding energies were less optimal compared to the binding energies of camptothecin and DNA topoisomerase I. The compound intercalated with the DNA of the topoisomerase enzyme.

The compound interacted with DNA topoisomerase via pi-cation interactions with lysine at position 493, hydrogen bond with threonine at position 501, alkyl interactions with alanine at position 499, arginine at position 364, lysine at position 493 and position 532. More interactions are shown in Figure 2.

DISCUSSION

4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo [2,3-b]quinolone lacks any PAINS alert and thus is a very good lead compound to be developed to a drug.\textsuperscript{29,30} This
compound complies with rules of drug likeness proposed by Lipinski which recommended that a potentially orally active drug has a molecular weight of less than or equal to 500, a log P of less than or equal to 5, less than or equal to 10 hydrogen acceptors and less than or equal to 5 hydrogen bonds.\textsuperscript{31} It also complied to Veber’s rules on drug likeness which recommended less than or equal to 10 rotatable bonds and less than or equal to 140 angstroms in terms of polar surface area.\textsuperscript{32} The compound also has high gastrointestinal activity. This indicates that the compound is a potential drug that can be administered orally.

The compound inhibits cytochrome P450 isofrom IA2 and thus may affect metabolism of caffeine, clozapine, oflanzapine, lidocaine, ropivacaine, melatonin, tacrine, tizanidine, triamterene, zolmitriptan and frovatriptan.\textsuperscript{33} It also inhibits CYP2C9 which is critical in metabolism of warfarin, phenytoin, tolbutamide, some non-steroidal anti-inflammatory drugs, losartan, candesartan, cyclophosphamide, zafirlukast and other drugs.\textsuperscript{34} It also inhibits CYP2C19 and thus affects metabolism of carisoprodol, omeprazole, pantoprazole, lansoprazole, moclobemide, diazepam, mephenytoin, mephobarbital and hexobarbital.\textsuperscript{35} Therefore, this compound has many drug-drug interactions and cost versus benefit analysis should be done for patients with several comorbidities.

This compound is generally safe since it does not affect toxicological pathways, toxicity targets and hepatotoxicity. However, it causes immunotoxicity and mutagenicity. A number of anticancer drugs like doxorubicin, cyclophosphamide, busulphan and mercaptopurine are also mutagenic.\textsuperscript{36} This still indicates the potential of this compound as an anticancer agent.

4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinolone intercalates the DNA in a similar manner to camptothecin. It also interacts with arginine amino acid at position 364 similar to camptothecin. However, it also inhibits CYP2C19 and thus affects metabolism of carisoprodol, omeprazole, pantoprazole, lansoprazole, moclobemide, diazepam, mephenytoin, mephobarbital and hexobarbital.\textsuperscript{37,38} Therefore, this compound has many drug-drug interactions and cost versus benefit analysis should be done for patients with several comorbidities.

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\textbf{CONCLUSION}

4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b]quinolone derived from \textit{Vepris nobilis} is a potential drug for the management of cancer which can be administered orally. However, it has many drug-drug interactions.

\textbf{Recommendations}

\textit{In vitro} and \textit{in vivo} studies are needed to test 4,6-dimethoxy-7-((3-methylbuta-1,3-dien-1-yl)oxy)furo[2,3-b] quinolone for management of cancer.

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