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Design and Synthesis of Folic Acid-Phytochemical Conjugates as Anti-Cancer Agents

Cancer treatment continues to be a major challenge for the medical community mainly due to non-specificity, leading to serious toxic effects. The use of vitamins such as folic acid and biotin are presently being studied through the synthesis of vitamin-drug conjugates as a new approach to improve the affinity of the drug molecules with cancer cells. Current research emphasizes on the design and synthesis of novel phytochemical vitamin conjugates as anticancer agents through *in silico* studies. Conjugates of quercetin (QC), curcumin (CUR) and berberine (BER) with folic acid (FA) were evaluated through docking experiments using Autodock Vina. Quercetin-folic acid conjugate 1 (QC-FA 1) demonstrated the highest binding affinity (-12.0 kcal/mol) to the human folate receptor (PDB ID: 4LRH) indicating the possibility of rapid uptake leading to internalization within the cells, improving the specificity to cancer tissues. The conjugates were further examined for their pharmacokinetic properties using AdmetSAR and SMARTcyp tools, which implied the incapability of these conjugates to cross the blood brain barrier but with favorable pharmacokinetic and CYP450 potential indicating their ability to act as drug-like molecules. Further QC-FA 1 was synthesized and effectively characterized. This conjugate will be further tested for its efficacy in treatment of cancer.

Keywords: Vitamin-drug conjugate, Folic acid, Anticancer, Quercetin, Curcumin, Berberine, In silico study, Cancer treatment.

Introduction

One in every six deaths worldwide is caused by cancer, making it a key concern for global public health [1]. The WHO cancer estimates that about 10 million people died from the disease in 2020 [2]. Cancer is abnormal cell growth that can grow out of control and, in many cases, may spread to other body parts [3]. The most popular treatment for cancer is chemotherapy, but its disadvantage is that it is not selective, which leads to serious, often fatal side effects [4, 5]. The drawback of widely used cytotoxic medications like doxorubicin, cisplatin, paclitaxel, is that they are unable to distinguish between healthy and abnormal cells. This nonspecificity causes systemic toxicity, which in turn leads to a cascade of other negative side effects, including hair thinning, damage of the kidney, lung, and bone marrow [6].

Further, it is extremely difficult to deliver anticancer drugs to the tumor site. It is necessary to develop tailored drug delivery system so that pharmaceuticals can act exclusively on actively growing malignant cells with minimal side effects [7]. Such targeted medications, which are preferentially absorbed by tumor cells, are expected to significantly improve the effectiveness of cancer therapy. Recent research has focused on developing medications with different targeted ligands, including use of polysaccharides, peptides, and folate, to improve efficacy of anticancer drugs [8–12]. The best way to improve the safety and efficacy is to use a targeting ligand to deliver a therapeutic drug with high affinity for malignant tumours but low affinity for healthy cells [13].

Tumour targeting moiety and chemotherapeutic drug should be directly coupled through a linker so as to enable efficient tumor-specific drug delivery. Additionally, the targeted drugs can deliver therapeutic doses precisely into the cancer cell, blocking nonspecific absorption and associated damage of healthy cells [14, 15]. As a result, a conjugate acting "prodrug" can be developed that will quickly dissociate when incorporated into a malignant cell and restore the activity of the anticancer drug [16]. Due to the rapid growth, cancer cells have an enhanced demand for essential vitamins, which is a physiological trait shared by all living cells [17, 18]. Recent studies in the literature have demonstrated that receptors required for vitamin absorp-

tion are overexpressed in malignant cells. Essential vitamins such as folic acid, riboflavin, vitamin B12, and biotin are required for efficient tumor growth. Combining pharmaceuticals with vitamins that target tumor-associated antigens is one way to boost the sensitivity of malignant cells to ligand-targeted therapies while decreasing the susceptibility of normal cells to medications.

Recently, it was found that cancer cells have higher levels of overexpressed folate receptors than normal, healthy cells. Thus, it is recognized that folate receptors function as excellent targets [19–22]. Folate receptor alpha (FRA) protein is overexpressed on the exterior of several forms of tumours, including ovarian, breast, pancreatic, and liver malignancies. Thus folate and anticancer drugs can be combined. The optimal approach is to combine therapeutic probes with folic acid to prepare conjugates targeting FRA-positive tumour cells. Folate conjugates make it possible to administer cancer-specific medications with little or no harm to the healthy cells [23–25]. This conjugation scaffold can increase the bioavailability and efficacy of cytotoxic substances. Since such vitamin-anticancer medication conjugates can provide a large amount of the cytotoxic agents to the target malignant cell, the essential vitamins including biotin, folic acid, vitamin B12, and riboflavin may act as a promising approach to kill the malignant cells.

Folic acid-drug conjugates, biotin-drug conjugates, vitamin B12-drug conjugates, vitamin E-drug conjugates, and vitamin C-drug conjugates are now being extensively studied [26]. Over the past few decades, innovative lead compounds derived from natural sources have increased [27]. New research has also confirmed that the natural molecules are useful in making cancer cells more susceptible to chemotherapy, assisting to defeat multi drug resistance (MDR) [28]. Among the reported phytomolecules; quercetin (QC), curcumin (CUR), and berberine (BER) have gained large attention in the last decade due to their multifaceted therapeutic activities. The anti-proliferative, anti-metastatic, anti-migratory, and pro-apoptotic properties of these molecules have proven their potential in the treatment of a variety of malignancies [29-35]. However, since these molecules have very low bioavailability due to poor solubility, their use in cancer therapy is limited.

The present study attempted to design and evaluate folic acid (FA) conjugates with these identified phytomolecules with proven efficacy in various types of cancer through systematic in silico and synthetic studies. Conjugation of folic acid with these anticancer agents is hypothesized to provide targeted drug delivery of these phytomolecules with increased efficacy and fewer side effects.

Experimental

Materials: All chemicals used in this study were of analytical grade. Quercetin was obtained as a gift sample from Cayman Chemical Company, USA, whereas biotin and folic acid were procured from Sigma Aldrich, Merck KGaA, Darmstadt, Germany. DCC, dimethyl amino pyridine, N-hydroxy succinimide were obtained from SD Fine Chem. Mumbai, Maharashtra, India. All other laboratory solvents and regents used in the study were purchased from Loba Chemie Pvt Ltd, Mumbai, Maharashtra, India.

Instruments used: The docking studies were carried out by using Autodock Tools and Discovery Studio was applied for the visualization and interpretation of the docking results. Melting point apparatus (Veego) was used to find melting point. Spectral analysis of the synthesized compound was carried out using UV-Visible spectrophotometer 1800 and FTIR 8400S, Make-Shimadzu. ^{1}H-NMR spectra was recorded on a Bruker Avance 400 MHz spectrometer using DMSO-d_6 as a solvent with Tetra Methyl Silane (TMS) as internal standard. The reaction completion was monitored by TLC using readymade F254 silica plates.

In Silico study:

Protein preparation: Human folate receptor (PDB ID: 4LRH) was downloaded in PDB format from RCSB protein data bank with 2.80Å resolution. The protein structure was prepared, processed and verified for missing atoms, bonds and contacts using protein preparation wizard in AutoDock 1.5.6. Then hydrogen atoms were added to the protein structure. All water molecules were erased from the protein along with ligand molecules and heteroatoms. Kolman charges were added to remove non integral charges present on amino acid in the polypeptides, 7.0 pH range was set and charged fields were added using AutoDock. Finally, the structures were refined and saved as PDBQT files for further docking studies [36, 39].

Ligand Preparation: 3D structure of phytoconstituent-vitamin conjugates were drawn using ChemDraw 18.1. 3D ligand energy minimization was performed using the MM2 method of Chem3D 18.1, and the results were saved in a PDB file. PDB file for folic acid was converted to PDBQT format using the Open Babel GUI program. The energy-minimized ligand PDBQT file was used for further docking studies [37].

Receptor grid generation: Using H-bond interactions from the PDBsum, the active pocket was located, and grid settings were determined. Grid was created by grid box in Autodock. The targeted molecule's active

location was enclosed by a cubic box. The receptor grid was then created using the following coordinates: 5.69, 20.79, and 4.05 for X, Y, and Z, respectively (by calculation method). Grid points were separated by 0.375. Maximum 10 conformers were taken into account throughout the docking process [38, 39].

Docking studies: Human folate receptor alpha (PDB ID: 4LRH) was chosen as the target protein because this receptor is overexpressed on breast cancer cells. Folic acid was selected as a reference drug, and 6 phytochemical-vitamin conjugates were chosen as ligands. The reference drug selected for docking acts as a folate receptor inhibitor. Lamarckian genetic algorithm method was used for docking. The docking process was carried out using the Autodock Vina software, and the outcomes were visualized and analyzed using the Discovery Studio Visualizer. Analysis was done based on the binding energy, bond length, interactions bond length, and participation of amino acid residues.

Pharmacokinetic predictions:

ADME/T property: To estimate each ligand's pharmacokinetic features, such as human abdominal absorption, carcinogenicity, cytochrome P inhibitory promiscuity, etc., the ADME/T profile of each ligand has been examined using the ADME/T SAR server [40-42].

P450 site of metabolism prediction: SMARTcyp web server was utilized to predict the P450 site of metabolism for each ligand molecule. This prediction provides information on the metabolism of the cytochrome P450 family of enzymes, like CYP2C9, CYP3A4, CYP2D6 [43].

Synthesis of Quercetin- Folic Acid Conjugate 1:

Scheme for synthesis of QC-FA 1 conjugate is presented in Figure 1.

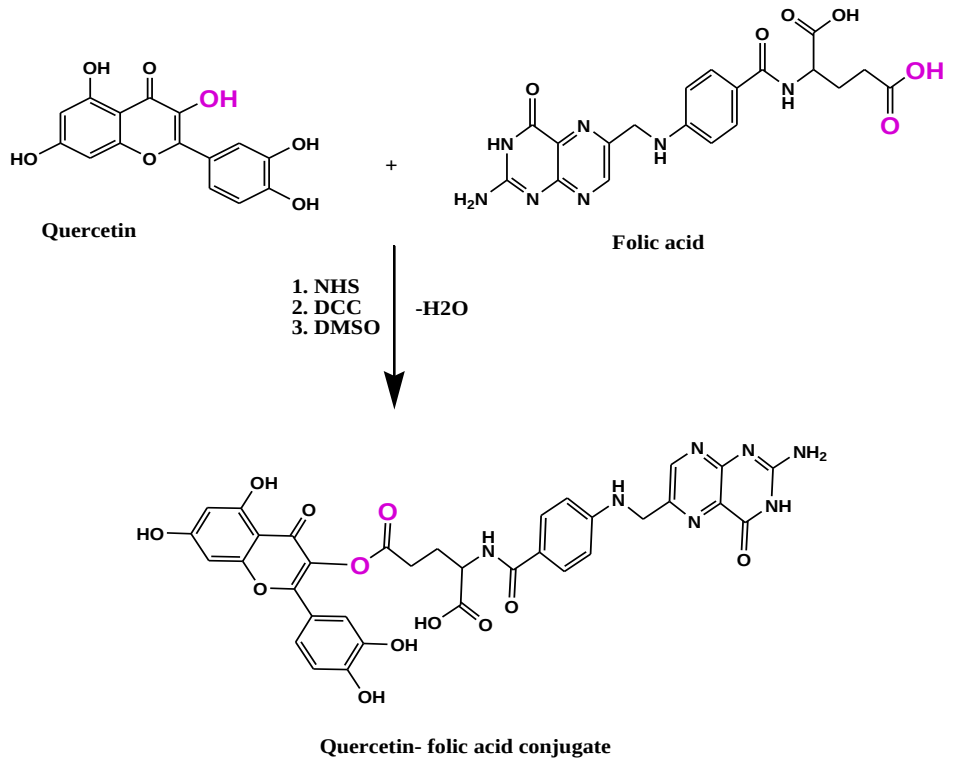

Figure 1. Reaction scheme for the synthesis of Quercetin-Folic Acid Conjugate 1

STEP 1: Activation of folic acid (FA): In a clean, dry 50 ml round bottom flask, 1.34 mmol folic acid was dissolved in 10 ml of dimethylsulphoxide (DMSO) with mild heating for about 10–15 min (time may vary as per dissolution of FA in DMSO). Then equivalent amount of N-Hydroxysuccinamide and Dicyclohexylcarbodiimide (DCC) was added in the above mixture. Reaction mixture was then stirred for 16 hours at room temperature. After which urea precipitated as dicyclohexyl urea (DCU) was filtered off to get activated folic acid.

STEP 2: Conjugation of QC and activated FA: In a round bottom flask, activated FA was added with solvent from Step 1 followed by the addition of equivalent amount of triethylamine and QC. This mixture

was then kept under continuous stirring overnight in the presence of molecular sieves, which ensure the entrapment of the water molecules generated during the synthesis process.

Physical and Spectral data of QC-FA Conjugate 1:

The physical characteristics of the synthesized conjugate, including its color, percentage yield, and melting point, were documented. Additionally, an absorption UV spectrum was obtained using a Shimadzu UV-Visible spectrophotometer-1800. The infrared (IR) spectra of the synthesized compounds were recorded using a Shimadzu 8400-S FT-IR spectrophotometer with potassium bromide. The synthesis of the conjugate was further validated through analysis using 1H-NMR and mass spectrometry techniques. The ^{1}H NMR spectra were recorded in DMSO using NMR spectrometer Bruker 400 MHz. The chemical shift values are given in parts per million, downfield from Tetra Methyl Silane (TMS) used as the internal standard. The mass spectra were recorded using BRUKER IMPACT mass analyser.

Results and Discussion

Physical and Spectral data of QC-FA Conjugate 1:

Folic acid binds to the folate receptor through the α-carboxylic acid of a glutamate residue, thus it should be free for its metabolism and function. The γ-carboxylic acid, located away from the binding site, can be modified. In order to leave the α-position unmodified, conjugation of pharmacophores to folic acid must be specific to the γ-carboxylic acid in order to maintain ligand binding affinity, thus we have selected terminal carboxyl group for modification [44]. To increase the acidic strength of folic acid as well as to ensure esterification at γ carboxyl group, we have activated folic acid as per the method described by Figliola, et al. [45]. The quercetin molecule offers five hydroxyl groups that can be modified through esterification reactions. Among these, the hydroxyl group at position 3 displays higher reactivity owing to its proximity to an adjacent carbonyl group. Existing literature supports the idea that modifying the enol hydroxyl group at position 3 enhances the stabilization of the pharmacophore, all while maintaining the antioxidant and anticancer properties of quercetin [46, 47]. Additionally, naturally occurring glycosides of quercetin utilize the 3-OH group to form glycosidic linkages [48, 49]. The above literature helps to support our hypothesis of enhanced reactivity of the 3-OH group in our study.

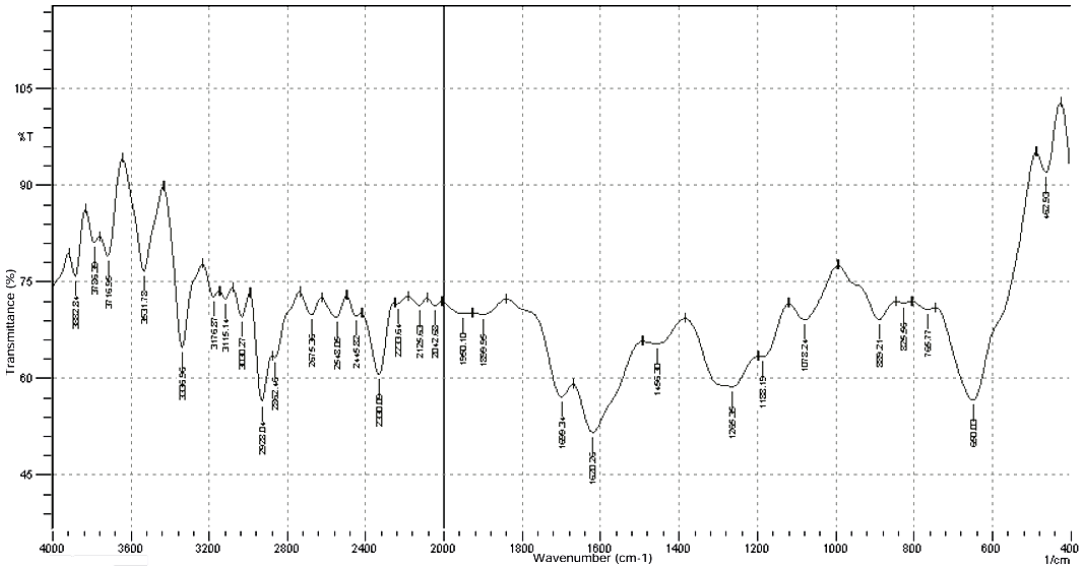

Figure 2. IR Spectrum of Quercetin-Folic Acid Conjugate 1

QC-FA Conjugate 1 was obtained as yellowish-brown powder with the yield of 67 % and melting point 213 °C. UV spectrum of the conjugate showed λ_{max} at 254.60 nm (0.669). IR (KBr, 4000–400 cm^{-1}): 3531.78 (Phenolic, O-H), 3336.96 (Amide, Asymmetrical N-H stretching), 3176.87 (Amide, Symmetrical N-H stretching), 3030.27 (Aromatic, C-H), 2938.04 (Aliphatic, C-H stretching), 1780 (Ester, C=O stretching), 1699.34 (Acid, C=O stretching), 1620.25 (Amide, C=O stretching), 1562.39 (N-H bending), 1496.30 (Aromatic, C=C), 1266 (Ester, C-O stretching), 1188.19 1078.24 (C-N stretching). The peak observed at 1780 cm^{-1} and 1266 cm^{-1} indicates the C=O ester stretching and C-O ester stretching respectively (Fig. 2). The peak of ester C=O stretching appears to be broadened due to the overlapping with the broad peak of the

acid C=O, due to the dimeric nature of carboxylic acid with intermolecular hydrogen bond formation. These two peaks give high assurance of formation of ester bond. Other functional groups are present in this spectrum confirm the formation of the QC-folic acid conjugate.

^{1}H NMR of synthesized conjugate displayed multiplet (m) from 6.2 to 7.9 ppm, representing 9 aromatic hydrogens (Ar-H) and 1 hydrogen on a pyrazine ring (pyrazine CH). A singlet (s) at 10.9 ppm, indicates a carboxylic hydroxyl group (carboxylic OH). Two singlets at 5.2 and 5.6 ppm correspond to 4 hydroxyl protons attached to the aromatic rings (Ar-OH). A multiplet from 2.08–2.24 ppm, representing 6 methylene hydrogens (Methylene CH). A triplet (t) at 4.87 ppm, revealing 1 methine hydrogen. A singlet (s) each at 4.00, 4.2 and 8.00 pm indicates the presence of the -NH_2, two -NH and one ring NH protons respectively. The presence of only one carboxylic hydroxyl group indicates the formation of linkage between quercetin O-H and carboxylic group of folic acid, confirming the formation of target compound.

MS: m/z (%) =725 (M+), 726 (M^++1).

Molecular docking: All chosen ligands docked well with the folate receptor, demonstrating the necessary binding affinities and good binding energies. Six ligand molecules, including the BER-FA conjugate, the QC-FA conjugates 1, 2 and the CUR-FA conjugates 1, 2, and 3 was used for docking studies (Fig. 3).

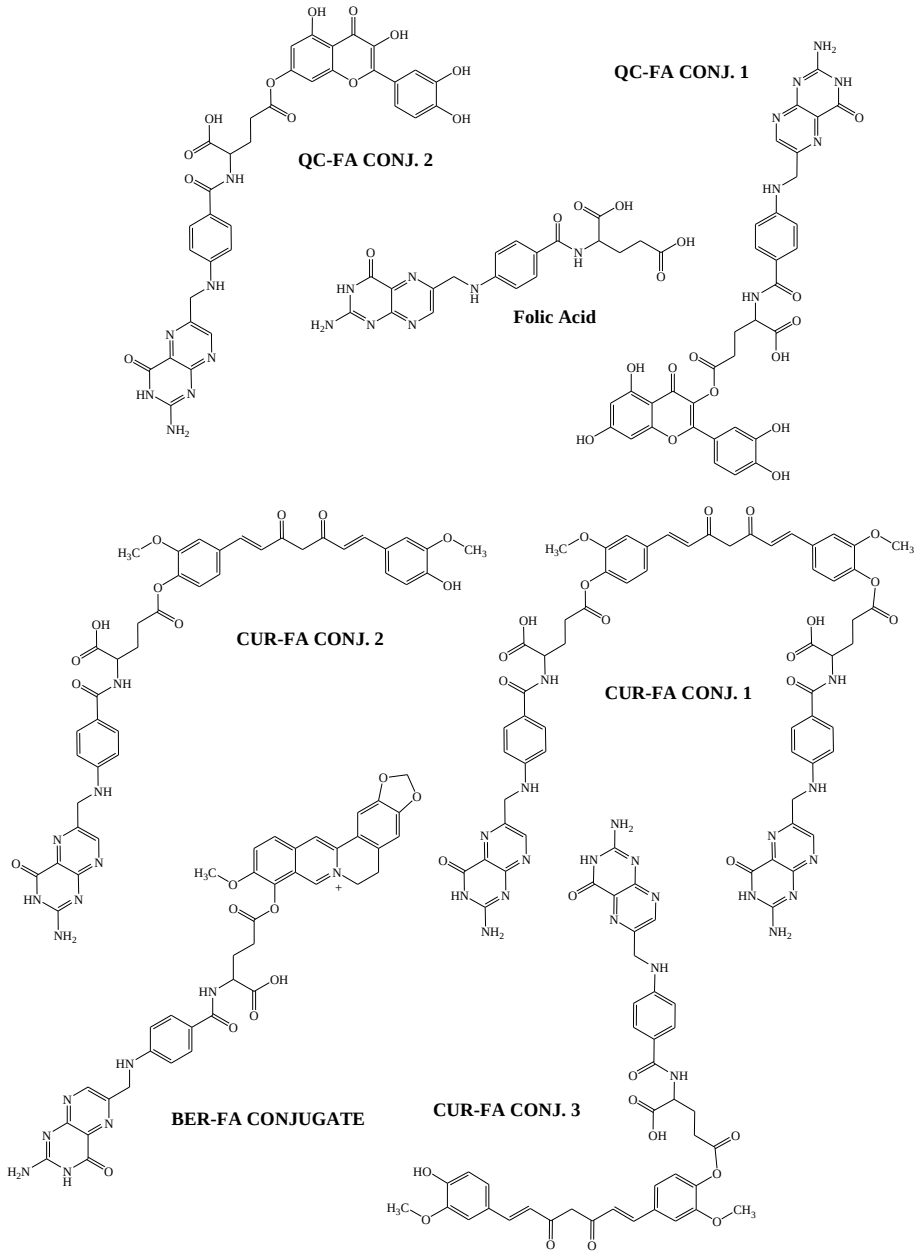

Figure 3. Structures of ligands

Reference drug FA docked in the binding site of the folate receptor with a binding energy of -10.6 kcal/mol by forming Pi-Pi bonding with amino acid Tyr60, Trp140 and hydrogen bonding with the amino acid residues Tyr60, Thr82, Arg103, Arg106, Ser 174. QC-FA conjugate 1 and BER-FA conjugate displayed highest binding energy of -12.0 and -11.2 kcal/mol respectively. QC-FA conjugate1 forms Pi-Pi bonding with Trp64, Tyr85, Trp140, Trp171 and hydrogen bonding with the amino acid residues Trp 102, Trp138 and Thr141. BER-FA conjugate displays Pi-Pi bonding with amino acid residues Tyr60, Arg61, Phe62, Trp102, Trp140, Trp171 and conventional hydrogen bonding with Arg61 and Trp102. Good binding energy through increased Pi-Pi bonding of QC-FA conjugate 1 and BER-FA conjugate indicates their affinity to the target. Other ligands showed binding energy lesser than FA, indicating lower affinity to the target. Binding energies of all ligands are summarized in Table. Figure 4 represents the docking positions and interactions with amino acids, bond distance, and type of bond.

T a b l e

Ligands with their binding energy

Sr. No.	Ligands	Target	Binding energy (kcal/mol)
1.	Folic acid (reference drug)	Folate receptor (PDB ID: 4LRH)	–10.6
2.	BER-FA conjugate		–11.2
3.	QC- FA conjugate 1		–12.0
4.	QC- FA conjugate 2		–10.3
5.	CUR- FA conjugate1		–10.4
6.	CUR- FA conjugate 2		–10.2
7.	CUR- FA conjugate 3		–10.2

Pharmacokinetic property prediction: QC-FA 1conjugate and BER-FA conjugate showed high binding energy and greater binding affinity to folate receptor. These conjugates were compared with respective phytochemicals for pharmacokinetic profile and analyzed to assess whether the conjugates showed enhanced pharmacokinetic activity than individual phytochemicals.

ADME/T Prediction: Both QC and QC-FA 1 conjugate showed impermeability to the Blood Brain Barrier (BBB). BER showed permeability to BBB while BER-FA conjugate demonstrated impermeability to the BBB, indicating absence of Central Nervous System (CNS) side effects. Both the conjugates showed non-substrate activity to CYP450 2C9 and 2D6 enzymes, non- inhibitor activity to CYP450 2C9, 2C19, and 3A4 enzymes indicating slower metabolism and increased half life. CYP Inhibitory Promiscuity (capacity for a drug to bind to and decrease or diminished the activity of multiple different CYP450 isoform) of both the conjugates was found to be satisfactory. All of them showed no AMES toxicity and were non-carcinogenic. QC exhibited type II acute oral toxicity which was not exhibited by its conjugate indicating its safety.

Prediction of the P450 site of metabolism: P450 metabolism prediction of QC and BER was compared with their conjugates, QC-FA 1 and BER-FA conjugate respectively. Carbon 13, 15 and 2 of QC were the most prominent atoms with low enzyme score for all three enzymes of CYP450 family, which gives strong knowledge of being catalyzed by using 3A4, 2D6 and 2C9 enzyme. On other hand, carbon 28, nitrogen 37 and 27 of QC-FA 1 conjugate molecule shows lowest enzyme score. However, the site for metabolism of QC-FA 1 involved carbon atoms present on FA structure indicating the metabolite stability of amide linkage in the complex. For BER, carbon 22, 1 and 25 represent lowest enzyme score. Considering enzyme score, energy and 2D solvent accessible surface area (2DSASA), these atoms are responsible for BER metabolism. Carbon 20, 29 and 46 of BER-FA conjugate shows lowest enzyme score of which carbon 46 is present in BER structure and carbon 20, 29 presents in folic acid structure. While, carbon 1 and 25 were absent in conjugate's metabolism data indicating metabolic stability of conjugate. This supports the hypothesis that the designed conjugates will internalize into the tumor cells through endocytosis after binding to the receptor, thereby improving specificity to the tumor cells.

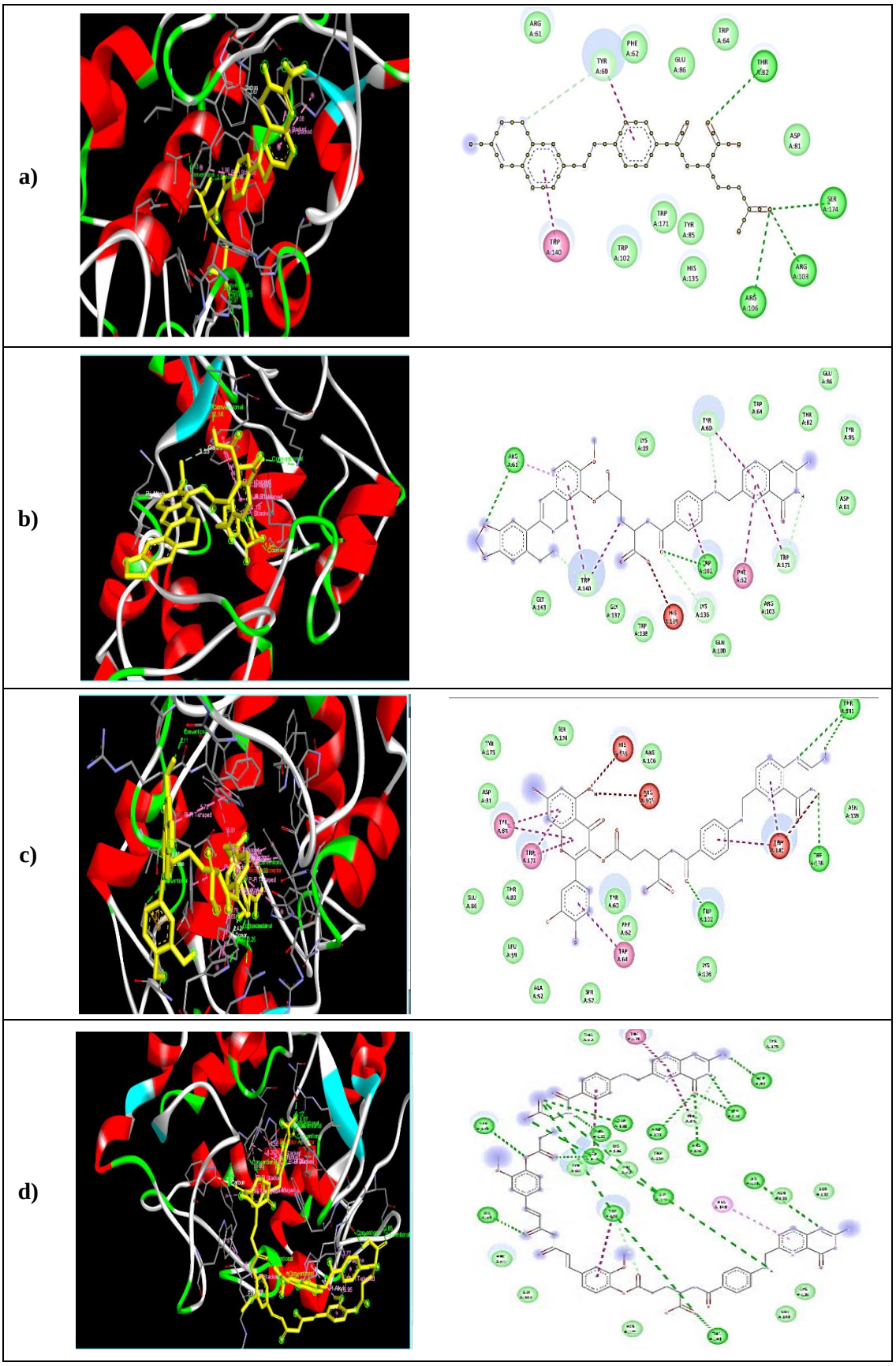

Figure 4. *a*) Standard FA docked to Human folate receptor alpha; *b*) Human folate receptor alpha is docked with a BER-FA conjugate; *c*) Human folate receptor alpha is docked with QC-FA conjugate 1; *d*) Human folate receptor alpha is docked CUR- FA conjugate 1

Conclusions

The conventional chemotherapeutic drugs exhibit undesirable side effects that may damage the healthy cells, including those in the gastrointestinal tract, bone marrow, and hair follicles. However, phytomolecules like curcumin, quercetin and berberine with proven anti-cancer potential can be employed for cancer therapeutics. However, their utilization is limited due to their poor bioavailability. Therefore, higher doses of phytomolecules required to achieve a therapeutic response may lead to toxicity and other side effects. To achieve improved bioavailability with improved tumor cells specificity, quercetin, berberine and curcumin were complexed with folic acid. The conjugates demonstrated favorable affinity for the human folate receptor, with the QC-FA1 conjugate showing higher binding affinity (–12 kcal/mol) than standard folic acid (–10.6 kcal/mol). The QC-FA1 conjugate also displayed satisfactory pharmacokinetic and safety predictions with reduced cytochrome susceptibility indicating stability of complex, supporting the concept of receptor mediated endocytosis in tumor cells. Further QC-FA1 conjugate was successfully synthesized, characterized using suitable chromatographic and spectroscopic tools and will be subsequently tested for its anti-cancer potential.

The successful design and synthesis of the QC-FA1 conjugate may act as a novel pro-drug, improving specificity to cancer cells, thereby reducing the undesirable side effects and bioavailability problems associated with its use. This study open development prospects for the identification and synthesis of newer phytomolecules based therapeutics for addressing the rising concerns associated with chemical-based agents. This novel but simplified approach of design of vitamin-phytomolecule conjugates can be further explored and studied to better understand its effective role in revolutionizing cancer therapeutics.

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References

1 Debela, D.T., Muzazu, S.G., Heraro, K.D., Ndalama, M.T., Mesele, B.W., Haile, D.C., Kitui, S.K., & Manyazewal, T. (2021) New approaches and procedures for cancer treatment: Current perspectives. *SAGE open medicine, 9.* https://doi.org/10.1177/20503121211034366

2 Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., et al. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin. 71*, 209–249. https://doi.org/10.3322/caac.21660

3 What is Cancer? https://www.cancer.gov/about-cancer/understanding/what-is-cancer, Accessed on October 10, 2023.

4 Allen, T.M. (2002). Ligand-targeted therapeutics in anticancer therapy. *Nature Reviews Cancer, 2(10)*, 750–763. https://doi.org/10.1038/nrc903

5 Chari, R.V.J. (1998). Targeted delivery of chemotherapeutics: Tumor-activated prodrug therapy. *Advanced Drug Delivery Reviews, 31(1)*, 89–104. https://doi.org/10.1016/S0169-409X(97)00095-1

6 Ojima, I., Geng, X., Wu, X., Qu, C., Borella, C.P., & Xie, H.et al. (2002). Tumor-Specific Novel Taxoid−Monoclonal Antibody Conjugates. *Journal of Medicinal Chemistry, 45(26)*, 5620–5623. https://doi.org/10.1021/jm025540g

7 Jaracz, S., Chen, J., Kuznetsova, L. V., & Ojima, I. (2005). Recent advances in tumor-targeting anticancer drug conjugates. *Bioorganic & Medicinal Chemistry, 13(17)*, 5043–5054. https://doi.org/10.1016/j.bmc.2005.04.084

8 Yan, Y., Dong, Y., Yue, S., Qiu, X., Sun, H., & Zhong, Z. (2019). Dually Active Targeting Nanomedicines Based on a Direct Conjugate of Two Purely Natural Ligands for Potent Chemotherapy of Ovarian Tumors. *ACS Applied Materials & Interfaces, 11(50)*, 46548–46557. https://doi.org/10.1021/acsami.9b17223

9 Zhong, Y., Meng, F., Deng, C., & Zhong, Z. (2014). Ligand-Directed Active Tumor-Targeting Polymeric Nanoparticles for Cancer Chemotherapy. *Biomacromolecules, 15(6)*, 1955–1969. https://doi.org/10.1021/bm5003009

10 Zagami, R., Rapozzi, V., Piperno, A., Scala, A., Triolo, C., & Trapani, M.et al. (2019). Folate-Decorated Amphiphilic Cyclodextrins as Cell-Targeted Nanophototherapeutics. *Biomacromolecules, 20(7)*, 2530–2544. https://doi.org/10.1021/acs.biomac.9b00306

11 Sun, H., Dong, Y., Feijen, J., & Zhong, Z. (2018). Peptide-decorated polymeric nanomedicines for precision cancer therapy. *Journal of Controlled Release, 290*, 11–27. https://doi.org/10.1016/j.jconrel.2018.09.029

12 Jiang, Z., Guan, J., Qian, J., & Zhan, C. (2019). Peptide ligand-mediated targeted drug delivery of nanomedicines. *Biomaterials Science, 7(2)*, 461–471. https://doi.org/10.1039/C8BM01340C

13 Srinivasarao, M., Galliford, C.V., & Low, P.S. (2015). Principles in the design of ligand-targeted cancer therapeutics and imaging agents. *Nature Reviews Drug Discovery, 14(3)*, 203–219. https://doi.org/10.1038/nrd4519

14 Srinivasarao, M., & Low, P.S. (2017). Ligand-Targeted Drug Delivery (world) [Review-article]. *American Chemical Society.* https://doi.org/10.1021/acs.chemrev.7b00013

15 Muro, S. (2012). Challenges in design and characterization of ligand-targeted drug delivery systems. *Journal of Controlled Release, 164(2)*, 125–137. https://doi.org/10.1016/j.jconrel.2012.05.052

16 Ojima, I. (2008). Guided Molecular Missiles for Tumor-Targeting Chemotherapy — Case Studies Using the SecondGeneration Taxoids as Warheads. *Accounts of Chemical Research, 41(1)*, 108–119. https://doi.org/10.1021/ar700093f

17 Russell-Jones, G., McTavish, K., McEwan, J., Rice, J., & Nowotnik, D. (2004). Vitamin-mediated targeting as a potential mechanism to increase drug uptake by tumours. *Journal of Inorganic Biochemistry, 98(10)*, 1625–1633. https://doi.org/10.1016/j.jinorgbio.2004.07.009

18 Chen, S., Zhao, X., Chen, J., Chen, J., Kuznetsova, L., Wong, S.S., & Ojima, I. (2010). Mechanism-Based Tumor-Targeting Drug Delivery System. Validation of Efficient Vitamin Receptor-Mediated Endocytosis and Drug Release. *Bioconjugate Chemistry, 21(5)*, 979–987. https://doi.org/10.1021/bc9005656

19 Leamon, C.P., & Reddy, J.A. (2004). Folate-targeted chemotherapy. *Advanced Drug Delivery Reviews, 56(8)*, 1127–1141. https://doi.org/10.1016/j.addr.2004.01.008

20 Lu, Y., & Low, P.S. (2002). Folate-mediated delivery of macromolecular anticancer therapeutic agents. *Advanced Drug Delivery Reviews, 54(5)*, 675–693. https://doi.org/10.1016/S0169-409X(02)00042-X

21 Reddy, J.A., Westrick, E., Vlahov, I., Howard, S.J., Santhapuram, H.K., & Leamon, C.P. (2006). Folate receptor specific antitumor activity of folate–mitomycin conjugates. *Cancer Chemotherapy and Pharmacology, 58(2)*, 229–236. https://doi.org/10.1007/s00280-005-0151-z

22 Leamon, C.P., Reddy, J.A., Vlahov, I.R., Vetzel, M., Parker, N., Nicoson, J.S., Xu, L.-C., & Westrick, E. (2005). Synthesis and Biological Evaluation of EC72: A New Folate-Targeted Chemotherapeutic. *Bioconjugate Chemistry, 16(4)*, 803–811. https://doi.org/10.1021/bc049709b

23 Shi, H., Guo, J., Li, C., & Wang, Z. (2015). A current review of folate receptor alpha as a potential tumor target in non-smallcell lung cancer. *Drug Design, Development and Therapy, 9*, 4989–4996. https://doi.org/10.2147/DDDT.S90670

24 Zhao, X., Li, H., & Lee, R.J. (2008). Targeted drug delivery via folate receptors. *Expert Opinion on Drug Delivery, 5(3)*, 309–319. https://doi.org/10.1517/17425247.5.3.309

25 Low, Philip Stewart, & Kularatne, S.A. (2009). Folate-targeted therapeutic and imaging agents for cancer. *Current Opinion in Chemical Biology, 13(3)*, 256–262. https://doi.org/10.1016/j.cbpa.2009.03.022

26 Bhole, R. P., Yogita, S., Bonde, C. G., & Wavhale, R. D. (2021). Vitamin Drug conjugate: a systematic review of pharmacological potential. *Bulletin of the University of Karaganda — Chemistry, (1)*, 27–52. https://doi.org/10.31489/2021Ch1/27-52

27 Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of natural products*, *79*(3), 629-661.https://doi.org/10.1021/acs.jnatprod.5b01055

28 Shaikh, S., Shaikh, J., Naba, Y. S., Doke, K., Ahmed, K., & Yusufi, M. (2021). Curcumin: Reclaiming the lost ground against cancer resistance. *Cancer Drug Resistance*, *4*(2), 298. http://dx.doi.org/10.20517/cdr.2020.92

29 Kashyap, A., Umar, S. M., Mendiratta, M., & Prasad, C. P. (2022). In vitro anticancer efficacy of a polyphenolic combination of Quercetin, Curcumin, and Berberine in triple negative breast cancer (TNBC) cells. *Phytomedicine Plus*, *2*(2), 100265. https://doi.org/10.1016/j.phyplu.2022.100265

30 Shaikh, S., Shaikh, J., Naba, Y. S., Doke, K., Ahmed, K., & Yusufi, M. (2021). Curcumin: Reclaiming the lost ground against cancer resistance. *Cancer Drug Resistance*, *4*(2), 298. https://doi.org/10.20517 %2Fcdr.2020.92

31 Vyas, A., Dandawate, P., Padhye, S., Ahmad, A., & Sarkar, F. (2013). Perspectives on new synthetic curcumin analogs and their potential anticancer properties. *Current pharmaceutical design*, *19*(11), 2047–2069. https://doi.org/10.2174/138161213805289309

32 Dandawate, P. R., Vyas, A., Ahmad, A., Banerjee, S., Deshpande, J., Swamy, K. V.,... & Sarkar, F. H. (2012). Inclusion complex of novel curcumin analogue CDF and β-cyclodextrin (1: 2) and its enhanced in vivo anticancer activity against pancreatic cancer. *Pharmaceutical research*, *29*, 1775-1786. https://doi.org/10.1007/s11095-012-0700-1

33 De Paz, E., Martin, A., Every, H., & Cocero, M. J. (2015). Production of water-soluble quercetin formulations by antisolvent precipitation and supercritical drying. *The Journal of Supercritical Fluids*, *104*, 281–290.https://doi.org/10.1016/j.supflu.2015.07.006

34 Wong, M. Y., & Chiu, G. N. (2011). Liposome formulation of co-encapsulated vincristine and quercetin enhanced antitumor activity in a trastuzumab-insensitive breast tumor xenograft model. *Nanomedicine: Nanotechnology, Biology and Medicine*, *7*(6), 834-840. https://doi.org/10.1016/j.nano.2011.02.001

35 Xiong, R. G., Huang, S. Y., Wu, S. X., Zhou, D. D., Yang, Z. J., Saimaiti, A., ... & Li, H. B. (2022). Anticancer effects and mechanisms of berberine from medicinal herbs: An update review. *Molecules*, *27*(14), 4523. https://doi.org/10.3390/molecules27144523

36 Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, *31*(2), 455-461. https://doi.org/10.1002/jcc.21334

37 Spessard, G.O. (1998) ACD Labs/LogP dB 3.5 and ChemSketch 3.5. *Journal of Chemical Information and Computer Sciences, 38*, 1250–1253. https://doi.org/10.1021/ci980264t

38 Ullah, A., Prottoy, N. I., Araf, Y., Hossain, S., Sarkar, B., & Saha, A. (2019). Molecular docking and pharmacological property analysis of phytochemicals from Clitoria ternatea as potent inhibitors of cell cycle checkpoint proteins in the cyclin/CDK pathway in cancer cells. *Computational Molecular Bioscience, 9(03)*, 81.http://dx.doi.org/10.4236/cmb.2019.93007

39 Zeleke, D., Eswaramoorthy, R., Belay, Z., & Melaku, Y. (2020). Synthesis and antibacterial, antioxidant, and molecular docking analysis of some novel quinoline derivatives. *Journal of Chemistry*, 1–16. https://doi.org/10.1155/2020/1324096

40 Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports*, *7(1)*, 42717. https://doi.org/10.1038/srep42717

41 Lipinski, C.A. (2004) Lead-and Drug-Like Compounds: The Rule-of-Five Revolution. *Drug Discovery Today: Technologies*, *1*, 337-341. https://doi.org/10.1016/j.ddtec.2004.11.007

42 Li, A.P. (2001) Screening for human ADME/Tox drug properties in drug discovery. *Drug Discovery Today*, *6*, 357–366. https://doi.org/10.1016/S1359-6446(01)01712-3

43 Guengerich, F.P. (1999) Cytochrome P-450 3A4: Regulation and role in drug metabolism. *Annual Review of Pharmacology and Toxicology*, *39*, 1-17. https://doi.org/10.1146/annurev.pharmtox.39.1.1

44 Geersing, A., de Vries, R. H., Jansen, G., Rots, M. G., Roelfes, G. (2019) Folic acid conjugates of a bleomycin mimic for selective targeting of folate receptor positive cancer cells. *Bioorganic & medicinal chemistry letters*, *29(15*), 1922–1927. https://doi.org/10.1016/j.bmcl.2019.05.047

45 Figliola, C., Marchal, E., Groves, B. R., Thompson, A. (2019) A step-wise synthetic approach is necessary to access γ-conjugates of folate: folate-conjugated prodigiosenes. *RSC advances*, *9(25)*, 14078–14092. https://doi.org/10.1039/c9ra01435g

46 Pechinskii, S.V., Kuregyan, A. G., Oganesyan, E. T. (2023) Regioselective synthesis of quercetin and myricetin derivatives. *Russ J Gen Chem*. *93*, 245-252. https://doi.org/10.1134/s1070363223020032

47 Elmegerhi, S., Su, C., Buglewicz, D. J., Aizawa, Y., Kato, T. A. (2018) Effect of hydroxyl group position in flavonoids on inducing single-stranded DNA damage mediated by cupric ions. *International journal of molecular medicine*, *42(1)*, 658–664. https://doi.org/10.3892/ijmm.2018.3615

48 Dueñas, M., Surco-Laos, F., González-Manzano, S., González-Paramás, A. M., Gómez-Orte, E., Cabello, J., Santos-Buelga, C. (2013) Deglycosylation is a key step in biotransformation and lifespan effects of quercetin-3-O-glucoside in Caenorhabditis elegans. *Pharmacological research*, *76*, 41–48. https://doi.org/10.1016/j.phrs.2013.07.001

49 Panda, S., Kar, A. (2007) Antidiabetic and antioxidative effects of Annona squamosa leaves are possibly mediated through quercetin-3-O-glucoside. *BioFactors*, *31(3-4)*, 201–210. https://doi.org/10.1002/biof.5520310307

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