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Designing and Development of Novel Curcumin Analogues/Congeners as Inhibitors of Breast Cancer Stem Cells Growth

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Curcumin, a polyphenolic yellow pigment of the spice turmeric is a unique, multi-targeted molecule having potential for cancer therapy. The inhibition of cancer stem cell growth is an essential step for breast cancer treatment. The main objective of the present work is to identify a potential target of curcumin against breast cancer stem cells and also to predict potency of designed curcumin analogues/congeners on predicted target. The high expression level of P-glycoprotein (P-gp) in the population of breast cancer stem cells promotes the cancer stem cells growth. The anticancer activity of curcumin is directly associated with the inhibition of P-gp mediated efflux process. This efflux mechanism is considered as a major reason for the failure of multidrug resistance in the cancer treatment. Using computational tools, the strong potency of curcumin against P-gp for the inhibition of breast cancer stem cells growth has been determined. Few analogues of curcumin were found to be more effective than curcumin towards cancer stem cells inhibition. In this way, the present study provides the pathway to design and improve the efficacy of novel and therapeutically important herbal drugs for the inhibition of breast cancer stem cells via inhibiting the P-gp mediated efflux mechanism.

1. Introduction

Curcumin is a naturally occurring polyphenolic compound. It is a bis-α,β-unsaturated β-diketone and constitutes the major component of spice turmeric. Due to structural modifications, there are multiple biological activities of curcumin analogues/congeners in the treatment of various health disorders such as diabetes, neurodegenerative diseases, cardiovascular disorders and others as described by Anand et al., (2008).Therapeutically, it is widely used as potential anticancer (Vallianou et al., 2015), antioxidant (Ak and Gulcin, 2008), antimicrobial (Moghadamtousi et al., 2014), anti-inflammatory (Ferreira et al., 2015) and antiangiogenic agent (Sugiyama et al., 2015). According to Liu et al. (2012), curcumin-loaded myristic acid microemulsions inhibit the growth of *Staphylococcus epidermis* which is mainly responsible for many skin infections. This natural product greatly contributes to the inhibition of metastasis in breast cancer stem cells (Charpentier et al., 2014).The cancer stem cells (CSCs) or tumor initiating cells are small population of cells that have capability of self-renewal and differentiation within a tumor (Tang et al., 2007).The role of cancer stem cells is extremely important in breast cancer because these cells control cancer formation, progression and resistance to therapy (Ercan et al., 2011). CSCs are resistant to chemotherapy and apoptosis (He et al., 2014). Multiple molecular targets like protein kinases, transcription factors, enzymes, gene expression factors are well reported targets of curcumin (Shanmugam et al., 2015; Oliveira et al., 2015). Molecular targets like Signal Transducer and Activator of Transcription3 (STAT3), β-catenin, Nuclear Factor-κB (NF-κB) and P-gp are well studied in case of breast cancer stem cells. The STAT3 is a transcription factor that relays biosignals from cell membrane to the nucleus. It is assumed that the STAT3 transcription factor is critically important in tumor invasion for the activation of cancer stem cells by inhibiting the phosphorylation of STAT3 (Zhang et al., 2013). Another transcription factor, NF-κB is considered as a potential inducer of tumorigenesis for breast cancer by inducing epithelial-mesenchymal transition. It has been reported that NF-κB acts as a regulator of self-renewal property of breast cancer stem cells (Shostak and Chariot, 2011). This transcription factor is

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potentially inhibited by curcumin (Olivera et al., 2012). P-gp is a efflux transporter that is responsible for the extrusion of drug molecules through the process of ATP hydrolysis. It has been reported by our research team that the binding of modulators at the nucleotide binding site of P-gp leads to inhibition of efflux of several drugs resulting in enhancement of their bioavailability (Tripathi et al., 2015) . P-gp causes multidrug resistance (MDR) which is considered as major obstacle in chemotherapy treatment (Follit et al., 2015). It has been reported that curcumin acts as a potential inhibitor of P-gp in the treatment of colon (Neerati et al., 2013) and breast cancer (Shukla et al., 2009). In order to impede the cancerous regrowth and self-renewal property of stem cells, the deactivation of Tcf/beta-catenin complex is important. The role of curcumin is considered as an inhibitor for breast cancer stem cell regrowth (Mukherjee et al., 2014).The present work predicts the potential target of multi - targeted curcumin molecule in case of breast cancer stem cells. Based on the potential target identification, the efficacy of few curcumin analogues has provided the pathway to design some new potential curcumin analogues/congeners as shown in Figure 1.

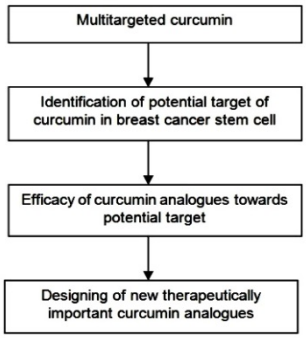


Figure 1: Flowchart for designing novel curcumin analogues/congeners.

2. Material and methods

2.1 Preparation of target proteins and ligands

The three dimensional coordinates of four target proteins namely STAT-3 (PDB ID: 3CWG), NF-κB (PDB ID: 1NFK), P-gp (PDB ID: 2HYD, 4AYT) and Tcf/β-catenin (PDB ID: 1JPW) were retrieved from protein data bank (PDB). On maestro, the downloaded protein structures were prepared by assigning bond orders, adding hydrogens and creating disulfide bonds. Table 1 shows twelve curcumin analogues/congeners that have been taken for the present study. All curcumin analogues/ congeners were prepared on LigPrep. The tautomers were generated at pH 7.0±2.0 by retaining specified chiralities. The broad interval of pH is important for producing a range of ionization states for the docking study. The OPLS-2005 force field was used for preparing curcumin analogues/congeners.

Table 1: List of curcumin analogues/congeners.

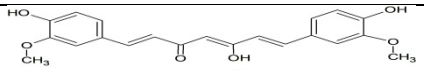
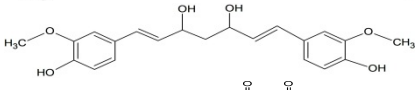
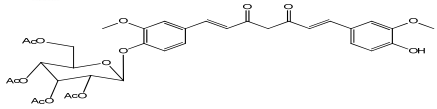
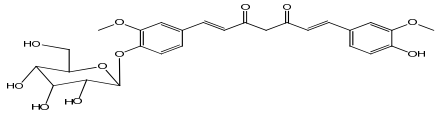
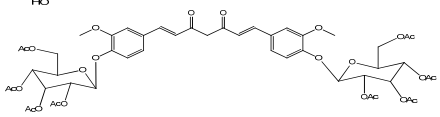
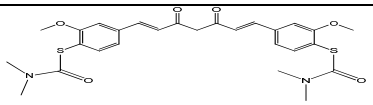
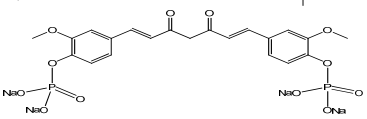
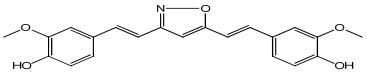
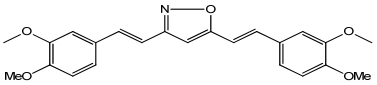
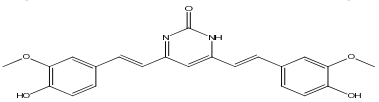
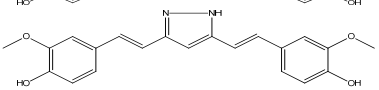
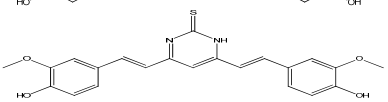
S.No.	Curcumin analogues/congeners	Chemical structures
1.	Curcumin	
2.	3,5-dihydroxycurcumin	
3.	Curcumin-monoglucoside (acetate)	
4.	Curcumin-monoglucoside	
5.	Curcumin-diglucoside (acetate)	

Table 1: List of curcumin analogues/congeners.

S.No.	Curcumin analogues/congeners	Chemical structures
6.	N-dimethyl urea derivative Of 4, 4'-deoxy-curcumin	
7.	Curcumin diphosphate (sodium salt)	
8.	Hydroxyl amine derivative of curcumin	
9.	Hydroxyl amine dimethyl curcumin	
10.	Urea derivative of curcumin	
11.	Hydrazine derivative of curcumin	
12.	Thiourea derivative of curcumin	

2.2 Molecular docking and simulation study of curcumin analogues/congeners on target proteins

The molecular docking and simulation study was carried out with Glide module of maestro (version 9.7). The grids were generated for target proteins by defining receptor with three dimensional coordinates based on van der Waals radius scaling. The docking study was performed for twelve curcumin analogues/congeners on target receptors by using Glide extra precision (XP) descriptor information.

2.3 Generation of new curcumin analogues/congeners

LigBuilder (version 1.2) was used to design new curcumin molecules. It provides structure-based drug design approach and builds up new ligands using cavity detection and fragment-growing strategy. The growing sites were assigned on seed molecule to generate new structures (Wang et al., 2000).

3. Results and discussion

In the present study, the binding affinity of curcumin on different molecular targets was determined. P-gp has been identified as a most potential target of mutitargeted curcumin in order to reduce stem cell growth. Table 2 depicts the efficacy of curcumin against target proteins.

Table 2: Docking scores of curcumin on multiple molecular targets.

S.No.	Target protein	GScore	Hbond
1.	STAT3	-3.0	-1.4
2.	NF-κB	-4.0	-1.5
3.	P-gp		
	a) *Homo sapiens*	-4.1	-1.5
	b) *Staphylococcus aureus*	-8.6	-0.8 -1.9
4.	TCF-4/beta catenin complex	-3.2	-1.2

Table 3 depicts the GScore values of curcumin analogues/congeners on P-gp as a target protein.

Table 3: Docking scores of curcumin analogues/congeners on bacterial P-gp target.

S.No.	Curcumin analogues/congeners	GScore	HBond
1.	Curcumin monoglucoside	-12.8	-5.0
2.	Curcumin mono-Glucoside (acetate)	-10.2	-2.3
3.	Dihydroxy curcumin	-9.1	-2.9
4.	Curcumin diphosphate	-8.7	-1.0

The binding affinity of curcumin monoglucoside was found to be highest than other curcumin analogues/congeners. Figure 2 shows the interaction of curcumin and curcumin monoglucoside with bacterial P-gp.

a)

b)

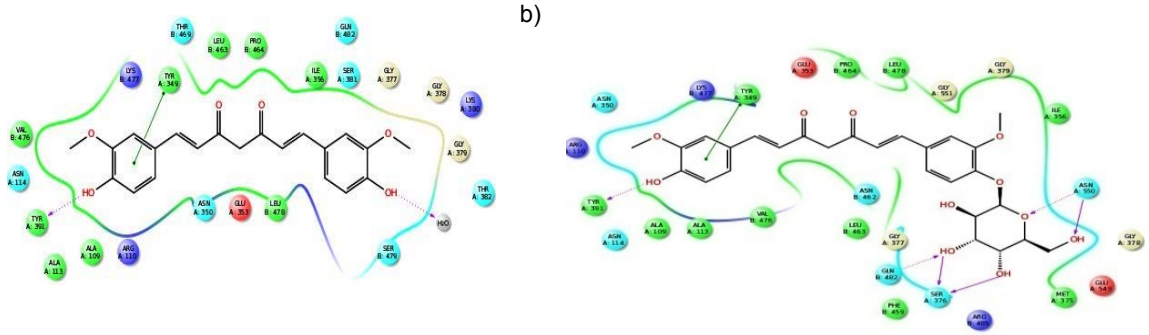


Figure 2: Two dimensional structure of protein ligand interaction of a) Curcumin with bacterial P-gp. b) Curcumin monoglucoside with bacterial P-gp.

In figure 2a, the active site amino acid residues such as Tyr391 and Tyr349 were mainly involved in hydrogen bonding and pi-pi interaction respectively. In addition to Tyr391, other residues like Ser376, Gln482 and Asn550 were involved in the hydrogen bonding interaction of curcumin monoglucoside with bacterial P-gp. The growing sites of curcumin monoglucoside have been taken for generating new molecule as shown in figure 3.

Figure 3: The growing sites (H63 and H64) of curcumin monoglucoside.

Table 4 shows top scoring novel curcumin analogues/congeners that have higher binding affinity than curcumin.

Table 4: Docking scores of novel curcumin analogues/congeners on bacterial P-gp.

S.No.	Novel curcumin analogues/congeners	GScore	HBond
1.	Result_34	-10.9	-3.6
2.	Result_22	-10.8	-3.3
3.	Result_53	-10.6	-4.0
4.	Result_30	-10.4	-3.5
5.	Result_18	-10.3	-3.2
6.	Result_17	-10.0	-2.6
7.	Result_11	-9.9	-2.9
8.	Result_65	-9.8	-3.4
9.	Result_52	-9.6	-2.9
10.	Result_41	-9.5	-2.4

The novel curcumin analogues/congeners in table 4 were found to have higher Gscores than curcumin. Figure 4 depicts the structures of these newly generated curcumin analogues/congeners.

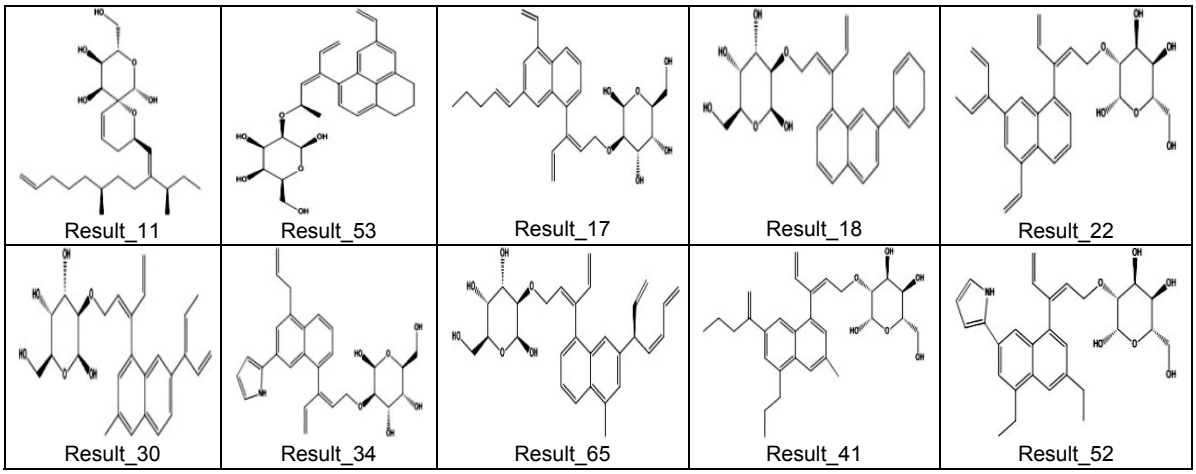


Figure 4: Structures of top scoring novel curcumin analogues/congeners.

4. Conclusion

The identification of potential target for the multitargeted curcumin is one of the most essential aspect to find its therapeutic role. In the present study, P-gp as a potential target of curcumin has been identified. It has been observed that glucoside of curcumin has higher binding affinity towards P-gp in order to reduce the growth of breast cancer stem cells than other analogues. Based on the efficacy of curcumin mono-glucoside with the target P-gp, novel curcumin analogues/congeners have been designed. Few novel curcumin analogues have shown higher affinity than curcumin. This study enables researchers to design, synthesize and develop new curcumin analogues/congeners in such a way that they inhibit growth of breast cancer stem cell growth by reducing P-gp mediated efflux process.

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