JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2022/29686

RESEARCH ARTICLE

Antimicrobial Activity of Curcumin and Deuterated Curcumin

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Abstract

Deuterium (D) and hydrogen (H) are bioisosteres because they are similar in size and shape with alike physiochemical properties and hence expect similar biological activity. The purpose of replacing H with D is to extend the time the active drug species spends in plasma, resulting in increased effectiveness and/or the avoidance of unwanted side effects. Deutetrabenazine was the first deuterated medicinal molecule, recently authorized by the FDA for the treatment of chorea "an involuntary movement disorder" associated with tardive dyskinesia and Huntington's disease. Curcumin is found to have a long history of use in human disorders such as inflammation, metabolic syndrome, arthritis, anxiety, antimicrobial, hyperlipidemia, etc., Curcumin's oral bioavailability and water solubility are low, resulting in poor absorption, rapid metabolism, and systemic elimination. To overcome curcumin's drawbacks, H/D exchange was performed in curcumin, which was then characterized and tested for antibacterial, antifungal, and anti-tubercular activities. The deuterated compound showed equipotent antibacterial activity when compared with the non-deuterated compound and had better anti-fungal, anti-tubercular activity compared to its parent compound.

Keywords: Antibacterial Antifungal Anti-TB Activity, Deuterated Curcumin

1. Introduction

The H/D exchange is an attempt to introduce deuterium into the existing drug molecules through the replacement of hydrogen atoms (-H) with deuterium (-D). D is a stable, nonradioactive hydrogen isotope that occurs naturally. Deuterium has a neutron and has a mass of 2.014 atomic mass units (AMU), while hydrogen has one electron and one proton with a mass of 1.008 AMU[1–3]. Deuterium resembles hydrogen and hence deuterium-containing compounds are predicted to preserve the therapeutic activity of their hydrogen analogs. With the retention of biological activity, deuterium can be explored for improving the physicochemical property and thereby altering the pharmacokinetic profile of a drug. The substitution with D in a molecule is the smallest structural alteration, and the incorporation is mainly useful for substitutions in positions that do not endure changes in electronic properties or steric hindrance[4–7]. The exchange of D for H can stabilize stereoisomers, prolongation of the half-life, lower toxicity, reduce drug-drug interactions, etc. The selective H-D exchange of biologically active compounds offers huge application opportunities, as drugs due to the stronger chemical bond acquired through selective deuterium modifications would possibly enhance the drug's metabolic properties. This Deuterium-hydrogen replacement can have a significant impact on enhancing the pharmacokinetic properties (ADMET), by reducing metabolic rate, thus resulting in a lower drug dose and improved drug tolerance. The Deuterium exchange can also result

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Article Received on: 04.05.2022 Revised on: 04.06.2022 Accepted on: 16.06.2022

in decreasing the drug's clearance rate leading to an increased biological half-life, higher safety, tolerability, and efficacy[8-13]. The deuterium-labelled drugs are more resistant to enzymatic or chemical degradation as the C-D bond cleavage is slower when compared to the C-H bond cleavage. Deuteration of bioactive compounds has become more popular and in high demand as numerous deuterium-labelled entities have entered into clinical trials. As a result, scientists are working on new synthetic ways to suit the demand. The deuterated and its parent compound should show comparable biological activity before determining the pharmacokinetic property. Hence scientists are still interested in substituting C-H with C-D to extend the lifetime of active drugs, with improved pharmacokinetics and toxicological aspects[14–17].

Curcumin "((1E,6E)-17-bis(4-hydroxy-3-methoxyphenyl) hepta-16-diene-35-dione)" is a lipophilic polyphenol present in three Indian traditional herbs "Curcuma Longa, Curcuma Wenyujin, and Curcuma Zedoaria". Curcumin, demethoxycurcumin, and bisdemethoxycurcumin are prevalent components in the traditional herb. Curcumin is a popular spice, food preservative, flavoring, and coloring ingredient and it is a diarylheptanoid that belongs to the category of curcuminoids, which are phenolic pigments accountable for the yellow color in turmeric[18]. In the last few decades, curcumin has gained a lot of attention for its various therapeutic properties like anti-diabetic, anti-inflammatory, anti-aging, antimicrobial, and anti-cancer agents. Curcumin shows therapeutic benefits in Alzheimer's disease, metabolic syndrome, arthritis, cardiovascular disease, anxiety, neurodegenerative disorders, multiple sclerosis, allergies, AIDS, inflammatory bowel disease, diabetes, nephrotoxicity, psoriasis, lung fibrosis, hyperlipidemia, and antibacterial property[19,20]. Curcumin is metabolized in the body by reducing dihydro-, tetrahydro-, and hexahydro-derivatives, as well as Michael's addition to form glucuronide, sulfate, and a GS-adduct.

Despite curcumin being a safe natural product, preclinical studies showed that curcumin cannot be used to treat any disease because of its low bioavailability, and inadequate solubility in aqueous solvents, rapid metabolism and systemic elimination[21–23]. The hydrophobic characteristic (low absorption capacity)[24], rapid metabolism (short half-life)[25], and rapid elimination by the liver are the main reasons for the limited bioavailability[26] of curcumin. As a result, researchers have made significant efforts and extensive studies to overcome the disadvantage and improve the bioavailability of curcumin using a variety of strategies, including improving curcumin solubility, developing curcumin nanoparticles and formulations in liposomal, phospholipid complexes or micellar, combining adjuvants, developing new derivatives, analogs, and designing hybrid curcumin molecules. Several research works proved that deuterated compounds improve the pharmacokinetic profile of a drug[27–29]. The objective of our study is to explore the D exchange for H in curcumin without loss of its activity and to evaluate physical-chemical properties.

2. Methods and Materials

2.1 *In silico* Studies

The physicochemical characteristics of curcumin and deuterated curcumin were determined using Molinspiration (www. molinspiration.com) and Swiss ADME. The MiLog P parameter is used to determine cell membrane permeability. The Partition coefficient (Log P) is used to calculate the hydrophobicity of a molecule. Drug absorption, bioavailability, drug-receptor interactions, metabolism, and toxicity are all affected by the hydrophilic or lipophilic characteristics of drug molecules. Total polar surface area (TPSA) is an excellent interpreter of drug transport qualities such as intestinal absorption, bioavailability, and blood-brain barrier penetration because it is closely connected to a molecule's hydrogen bonding potential. The Log S distribution suggests that a value between -1 to -4 is optimal for improved medication absorption and distribution in the body. Molecular flexibility is measured by the number of rotatable bonds. A molecule with more rotatable bonds is more flexible and has a better binding affinity. A molecule's molecular properties and structural features can be compared to known drugs using drug-likeness data (Lipinski's rule of five). The online software Molinspiration drug-likeness score is used to determine the bioactivity score for deuterated curcumin and curcumin like GPCR

ligand, nuclear receptor ligand, a kinase inhibitor, and ion channel modulator (www.molinspiration.com)[30–33].

2.2 H/D Exchange in Curcumin

Deuteration of curcumin can be accomplished by treating with D_2O and metal catalysts such as Pd, Pt, Rh, at high temperature and pressure. Deuterium oxide (D_2O) is easily available and hence used for H/D exchange in curcumin. Curcumin (0.2 mmol), PhCOOH (0.04 mmol), and 0.2 mL of D_2O were refluxed for 48–50 hours at 120 °C under nitrogen atmosphere. TLC was used to monitor the reaction's progress. After completion, the mixture was neutralized with a saturated $NaHCO_3$ solution (3 mL) and extracted three times with ethyl acetate(3 mL). The extracted organic layers were combined and dried with Na_2SO_4 and fdistilled under reduced pressure to obtain the target compound. (Scheme 1).

2.3 Biological Evaluation

The biological evaluations were carried out in Maratha Mandal's Central Research Laboratory in Belgaum.

Test microorganisms

The test microorganisms tused for the study includes Gram-negative (*P. aeruginosa and E. coli*), Gram-positive (*S. aureus and E. faecalis*), and Mycobacterium tuberculosis strain *H37Rv*. *C. albicans,* and *A. niger* were also used to define the antifungal activity of the deuterated compound.

2.4 Antibacterial and Antifungal Activity

Antibacterial and antifungal activities were performed as per the procedures cited in the literature[35–37].

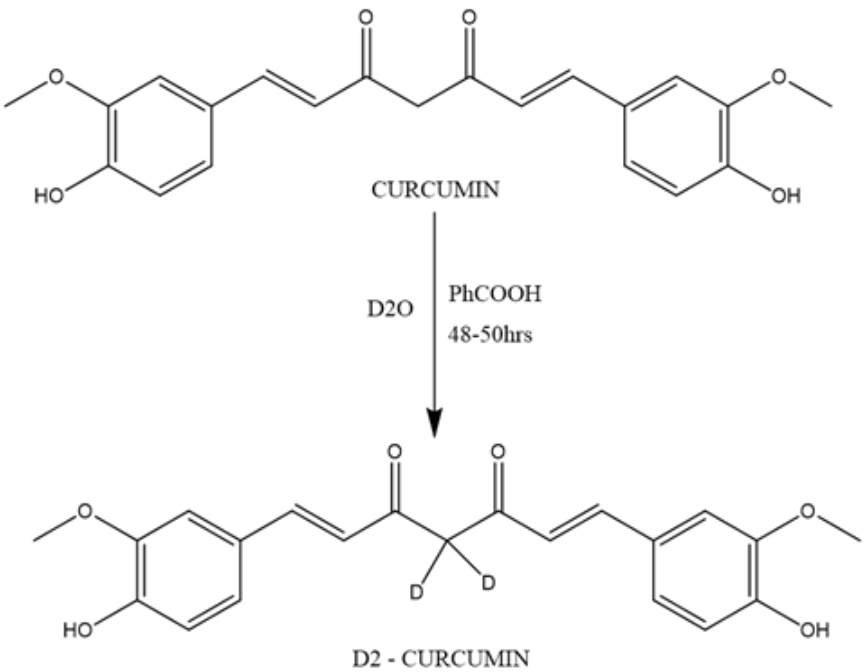
2.5 Anti-tubercular Activity

Anti-tubercular activity were performed as per the procedures cited in the literature[38].

3. Results and Discussion

3.1 *In silico* Studies

Deuterated compounds can lead to alteration of the pharmacokinetic profile of the drug, and reduce toxicity as it increases chemical stability. The *in-silico* drug activity prediction by molinspiration of deuterated curcumin and curcumin followed Lipinski 'Rule of



Scheme 1. H/D exchange in curcumin.
Reagents and conditions: PhCOOH, D_2O reflux for 48-50 hrs at 120°C

Five,' and Swiss ADME results proved that the Log P and Log S value showed a slight difference as shown in Table 1.

Both curcumin and its deuterated molecule had equal bioactivity scores for GPCR ligand, Kinase inhibitor, Nuclear Receptor Ligand, Ion channel modulator, enzyme inhibitor, and Protease inhibitor, and all were judged to be within the range of activity. The bioactivity score profile of the deuterated curcumin and curcumin are given in Table 2.

The bioactivity score for curcumin and deuterated curcumin against enzyme inhibitors and nuclear receptors indicated that both the compounds are active and falls in the range of -0.50 to 0.00.

3.2 H/D Exchange in Curcumin

Metal-catalyzed or acid/base promoted C–H/C–D exchanges are the most common techniques for deuterium incorporation since they enable direct deuterium incorporation without the need to pre-functionalize the starting materials or significantly modify the structure of the molecules. The H/D exchange in curcumin was carried out using commercially available curcumin as the starting material. The deuterated curcumin was obtained as a brown solid with 90% yield; mp:183-186^{0}C; and R_f value of 0.83 (n-hexane: Ethyl acetate: 2.5:2.5). The synthesized deuterated curcumin showed an m/z of 370. The small difference in the pharmacokinetic profile is due to the bond character difference between C-H and C-D. Hence, the exchange of hydrogen with deuterium at the appropriate part of the chemical structure will significantly slow down the metabolism and thereby can increase the plasma drug concentration.

3.3 Antibacterial Activity

Deuterium and hydrogen are bioisosteres and as a result, substituting deuterium for a hydrogen atom might result in analogs with similar biological activity. The metabolism of some bacteria is disrupted by the presence of the deuterium in metabolites The deuterium antibiotics will have modified physicochemical properties disrupting enzyme substrates recognition processes. The biodegradation of antibiotics in the resistant bacterial strain is one of the most common causes of drug resistance. One of the assumptions is that the deuterated drugs may be resistant to the bacterial enzyme, so there is a possibility of enhancing the intracellular concentration of antibiotics inside the cytoplasm.

The antibacterial activity of deuterated curcumin and curcumin against two Gram-positive bacteria (S. aureus *and E. faecalis*) and two Gram-negative bacteria (*P. aeruginosa and E. coli*) are represented in Table 3. The antibacterial activity of deuterated curcumin and curcumin displayed MIC values of 25 µg/ml against *P. aeruginosa*, 50 µg/ml against *E. coli*, and 50 µg/ml against *E. faecalis*, respectively. In the case of S. aureus, deuterated curcumin had a MIC of 25 µg/ml, compared with 12.5 µg/ml of curcumin. Thus, deuterated curcumin and curcumin exhibited similar activity against *P. aeruginosa*, *E. coli* and *E. faecalis* whereas deuterated curcumin showed lesser activity against S. aureus when compared with curcumin.

3.4 Antifungal Activity

The minimum inhibitory concentration (MIC) of deuterated curcumin and curcumin against *C. albicans* and A. niger was determined using Brain Heart Infusion Broth. Table 3 represents the MIC values for antifungal activity of deuterated curcumin and curcumin. From the results, it is clear that deuterated curcumin exhibited better antifungal activity against *C. albicans* with a MIC of 6.25 µg/ml compared to 50µg/ml of curcumin. In A. niger, deuterated curcumin exhibited minimal antifungal activity with a MIC of 100 µg/ml against 50 µg/ml of curcumin. It is reasonable to predict that the mass differences associated with the substitution of hydrogen for deuterium in a molecule will have a significant impact on its physical and chemical characteristics. Changes in the biological activity of deuterium compounds can be predicted as a result of structural changes.

3.5 Activity Against Tuberculosis

The Microplate Alamar Blue Assay (MABA) was used to screen deuterated curcumin and curcumin against M. tuberculosis H37Rv. Table 3 clearly explains that Deuterated curcumin has shown better anti TB activity with a MIC of 25 µg/ml, compared with 50 µg/ml of curcumin. The increased activity of deuterated

Table 1. Pharmacokinetics parameters for curcumin and its deuterated compound

Name	Molecular weight	Log P	TPSA	n OH	nOHNH	nrotb	volume	Log S
Curcumin	368.38	3.03	93.07	6	2	8	332.18	-3.94
Deuterated curcumin	370.37	3.01	93.07	6	2	8	332.18	-3.95

Table 2. Bioactivity score for curcumin and its deuterated compound

Name	GPCR Ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor	Ligand Protease inhibitor	Enzyme inhibitor
Curcumin	-0.06	-0.20	-0.26	0.12	-0.14	0.08
Deuterated curcumin	-0.06	-0.20	-0.26	0.12	-0.14	0.08

Table 3. Antibacterial activity of deuterated curcumin and curcumin against different microbes

S. No	Microorganism	Minimum Inhibitory concentration (µ g/ml)	
		Deuterated Curcumin	Curcumin
	Antibacterial activity		
1	*P. aeruginosa*	25	25
2	*E. coli*	50	50
3	*E. faecalis*	50	50
4	S. aureus	25	12.5
	Antifungal activity		
5	*C. albicans*	6.25	50
6	A. niger	100	50
	Anti-tubercular activity		
7	M. tuberculosis *H37Rv*	25	50

curcumin could be due to a primary isotope effect in which the rupture of C-D bonds is directly involved, or the increased stability of the deuterated molecule could play a role in the bacteria's inability to metabolize deuterated curcumin.

4. Conclusion

Curcumin was subjected to D exchange in order to overcome the disadvantages and alter physicochemical properties without loss of bioactivity. The physicochemical properties determined using molinspiration and Swiss ADME proved that both curcumin and its deuterated derivative showed similar bioactivity scores. Curcumin and its deuterated derivative were tested for antibacterial efficacy against Gram-positive, Gram-negative, and fungus, as well as the M. tuberculosis *H37Rv* bacterium. Deuterated curcumin showed an MIC of 6.25 µg/ml against 50 µg/ml of curcumin towards *C. albicans*. Deuterated curcumin's greater antifungal effectiveness might be related to higher efficacy of action.

The increased stability of the molecule may alter the rate of deuterated curcumin metabolism by the

fungus. This boost in efficiency might be due to a primary deuterium isotope effect, in which the target site involves the direct breaking of a C-D bond. Similarly, deuterated curcumin exhibited MIC of 25 μg/ml against 50 μg/ml of curcumin towards M. tuberculosis *H37Rv*. Also, it was found that deuterated derivatives had nearly equal activity to that of curcumin against the tested gram-positive and gram-negative bacterial strains. From the results, it is interesting to note that Deuterated curcumin provides a useful starting point for the rational design toward antimicrobial activity. Further, pharmacokinetic and pharmacodynamic studies have to be carried out to prove the efficacy of the deuterated compound.

In spite of research and development of numerous deuterated drugs, their efficiency, safety, and thorough knowledge of the exact mechanisms of the deuterated drugs still remain unaddressed. Beyond all, the design and development of a successful deuterated drug with acceptable efficacy is still a challenge. Also, long-term drug stability and toxicity studies for individual drugs are to be studied which may vary from patient to patient. The cost and intellectual property rights of the deuterated drug will also have a great impact on the launch of deuterated drugs. In spite of all these challenges, these deuterated drugs give us one more opportunity to combat the antimicrobial resistance problem.

5. Acknowledgment

We are thankful to the Research Council of SRMIST for constant encouragement and support.

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