J. Pharm. Tech. Res. Management Vol. 8, No. 2 (2020), pp.85–89

CHITKARA

Journal of Pharmaceutical Technology Research and Management
Journal homepage: https://jptrm.chitkara.edu.in/

Pharmacokinetic Studies of Curcumin Based Pyrazoline MAO Inhibitors

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ARTICLE INFORMATION

Received: July 16, 2020
Revised: September 22, 2020
Accepted: November 11, 2020
Published Online: November 17, 2020

Keywords:
Structure-based-drug design, Curcumin based Pyrazoline analogues, Ferulic acid amides, MDCK-II permeability studies, Liver microsomal metabolic stability studies

DOI: 10.15415/jptrm.2020.82012

ABSTRACT

Background: Curcumin is a natural phenolic compound obtained from *Curcuma longa*, with proven human monoamine oxidase (MAO) inhibitory activity, but due to its poor oral bioavailability, blood-brain barrier permeability and extensive metabolism in the liver, it has never been recognized as a drug candidate.

Purpose: In this study, the structure-based-drug design (SBDD) was adopted to incorporate the structural features of Curcumin with an aim to improve drug permeability and metabolic stability.

Method: A series of ferulic amides (half portion of curcumin) (1-3) and curcumin based pyrazolinescompounds (4-6) were designed and Curcumintested for their membrane permeability and liver microsomal metabolic stability in a various animal in an in-vitro assay system.

Conclusion: All the designed compounds showed a significant enhancement in permeability and metabolic stability is achieved through chemical modification.

Abbreviations:
hMAO: human Monoamine Oxidase; MDCK-II: Madin-Darby Canine Kidney; Cl_{int}: Intrinsic clearance; $t_{1/2}$: Half-life; LC-MS/MS

1. Introduction

Curcumin is a natural phenolic compound obtained from *Curcuma longa*, with proven MAO inhibitory activity (Badavath, Baysal, Ucar, Sinha & Jayaprakash, 2016). The poor oral bioavailability (Wahlang, Pawar & Bansal, 2011), bloodbrain barrier permeability and extensive metabolism in the liver (Pan, Huang & Lin, 1999) of curcuminnever allowed this scaffold to be recognized as a drug candidate. Literature review suggests very few attempts have been made to improve its bioavailability through a novel delivery system (Dagar, Dahiya & Bhambi, 2014; Prasad, Tyagi & Aggarwal, 2014; Singh, Wani, Kaul-Ghanekar, Prabhune & Ogale, 2014; Yallapu, Jaggi & Chauhan, 2012). Curcumin is a symmetrical molecule having two aryl rings (4-hydroxy-3-methoxy phenyl) connected to the central methylene group through an α, β-unsaturated carbonyl linker. The aryl unsaturated carbonyl group is an attractive synthon for the synthesis of many heterocycles. Recent studies indicate that pyrazoline and derivatives are amongst largely explored chemical scaffold for MAO-inhibitory activity (Badavath & Jayaprakash, 2021; Nayak, Ciftci-Yabanoglu, Jadav, Jagrat, Sinha, Ucar & Jayaprakash, 2013)Moclobemide (Ki MAO-A; 5.01 AE 0.13 nM. Guided by latter described literature , we have designed a few Ferulic acids/ and its amides 1-3, with half of the curcumin, (i.e., an aryl-α, β-unsaturated carbonyl portion attached with amines) and curcumin based pyrazolines (4-6), to improve its permeability, and metabolic stability. In this SBDD curcumin based pyrazolines (4-6) were designed by incorporating the structural features of curcumin (Figure 1) into the designed compounds (Figure 2). In an *in-vitro* assay system they were then tested for membrane permeability, and liver microsomal metabolic stability in animals (Rat, mouse, Dog and Bovine).

ISSN No.: 2321-2217(Print) ISSN No.: 2321-2225(Online); Registration No. : CHAENG/2013/50088

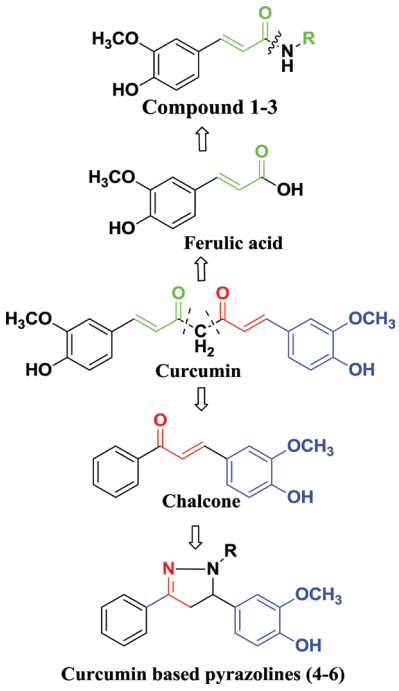


Figure 1: Structure-based drug design strategy adopted to enhance permeability andmetabolic stability.

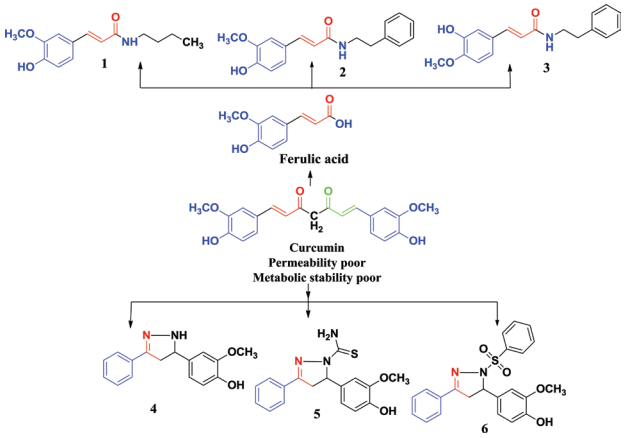
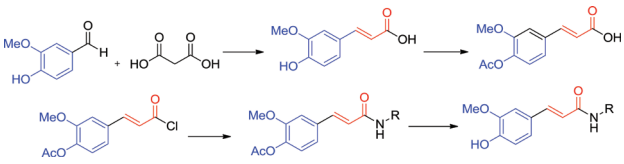


Figure 2: Designed ferulic acid amides and curcumin based pyrazolines (4-6) using a rationale design strategy to access membrane permeability and metabolic stability.

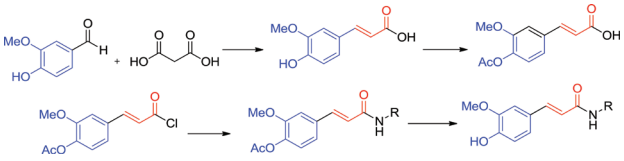
2. Results and Discussion

2.1. Chemistry

Ferulic amides (Vishnu N. Badavath et al., 2016; Yasmin et al., 2020) and curcumin based pyrazolines were synthesized according to the protocol (Badavath et al., 2016; Badavath, Ucar, Sinha, Mondal & Jayaprakash, 2016; Nath et al., 2018). Ferulic acid was synthesized by the reaction of malonic acid with 4-hydroxy-3-methoxy benzaldehyde in the presence of toluene and aniline, pyridine used as a base. The hydroxyl group was protected with acetic anhydride and chlorinated with oxalyl chloride. Further, amidation of acid chlorides and deprotection of acetyl group provide desired ferulic acid amides (1-3). Compounds (3, 4, 5 and 6) were synthesized from chalcone using Claisen condensation (Badavath et al., 2017; Jadav et al., 2015; Badavath et al., 2016; Narender, Venkateswarlu, Nayak & Sarkar, 2011; Nayak et al., 2015)



Scheme 1: Synthesis of Ferulic acid amides (1-3) (Vishnu N. Badavath et al., 2016).



Scheme 1: Synthesis of curcumin based pyrazolines (Badavath et al., 2016).

2.2. Pharmacokinetic Studies

2.2.1. In-vitro permeability studies and metabolic stability studies

In-vitro permeability and metabolic stability tests (on different animal liver microsomal enzymes) were carried out as described previously (Di et al., 2011; Irvine et al., 1999), (Di et al., 2003; Mondal, Mazumdar, Mondal & Banerjee, 2008)metabolic stability was evaluated at a later stage of drug discovery and required laborious manual manipulations. With the advance of high-throughput screening, combinatorial chemistry, and early profiling of drug-like properties, automated and rapid stability assays are needed to meet the increasing demand of throughput, speed, and reproducibility at earlier stages of drug discovery. The authors describe optimization of a simple, robust, high-throughput microsomal stability assay developed in a 96-well format. The assay consists of 2 automated components: robotic sample preparation for incubation and cleanup and rapid liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS to select the best acceptable drug-like candidate/s. LC-MS/MS was used for quantification. P-gp substrate activity was determined using the efflux ratio. All compounds reported in this study were identified to exhibit an asymmetric transport across MDCK-II monolayers (MDCK-II; Papp, B–A (Table 1). All the tested compounds had a good $t_{1/2}$ with an in vitro Clint, app,

which is a sign of strong metabolic stability (Table 2). Thus, suggesting that the tested compounds were absorbed orally in mouse and human models. The *in-vitro* microsomal stability studies of compounds (4-6) were in progress, will be communicated in our subsequent communication.

Table 1: In-vitro permeability Study (MDCK-II).

Compound	Papp ×10-6 cm/sec		Ratio	MAO-Selectivity
	Avg A>B	Avg B>A		
1	13.59	14.61	1.1	Non-selective (Badavath et al., 2016)
2	24.79	23.41	0.9	MAO-B (Badavath et al., 2016)
3	5.68	2.67	0.5	MAO-B (Badavath et al., 2016)
4	8.10	5.59	0.7	MAO-B (Badavath et al., 2016)
5	22.26	23.80	1.1	Non-selective (Badavath et al., 2016)
6	12.33	4.08	0.3	MAO-A (Badavath et al., 2016)
Ferulic acid	5.38	3.07	0.6	MAO-A (Badavath et al., 2016)
Digoxin	0.59	6.55	11.1	-
Fenoterol	0.45	0.37	0.8	-
Prazosin	13.57	22.86	1.7	-
Quinidine	8.69	29.52	3.4	-

A permeability with efflux ratio (Papp, B–A/Papp, A–B) of >2 to glycoprotein (P-gp) indicates a potential compounds and with efflux ratios < 2.0 are not potential P-gp compounds, indicating that they have better oral absorption potential. The apparent permeability coefficients (Papp) (Kellard & Engelstein, 2007) from A-to-B (apical to basolateral) and B-to-A (apical to basolateral) of the cell monolayers. Cell growth media: MEM-alpha with MEM NEAA+ Glutamine-Penicillin-Streptomycin, Test concentration: 2 µM, Incubation period: 150 min, Apical/ Donor pH: 7.4 /7.4: Digoxin, Fenoterol, Prazosin and Quinidine were represented for comparison purposes.

Table 2: In-vitro liver microsomal stability study in different animals.

Compound	Dog		Rat		Bovine		Mouse	
	$t_{1/2}$	Clint	$t_{1/2}$	Clint	$t_{1/2}$	Clint	$t_{1/2}$	Clint
1	21.0	66.1	37.2	37.2	3.0	462.0	7.1	194.69
2	81.0	17.2	37.8	36.7	16.4	84.7	31.1	44.5
3	120.0	11.6	9.2	150.4	4.3	323.7	10.4	133.57
4	48.7	28.5	8.0	172.8	17.7	78.3	6.2	224.0
Ferulic acid	120.0	11.6	107.4	12.9	120.0	11.6	120.0	11.60
Curcumin	12.0	115.5	11.1	125.0	4.4	315.6	9.0	153.30
Desipramine	6.3	218.3	6.6	210.6	3.7	375.7	-	-
Metoprolol	120.0	11.6	36.5	38.0	14.7	94.2	-	-
Verapamil	14.5	95.6	5.3	260.5	3.2	434.1	4.4	316.02
Atenolol	-	-	-	-	-	-	120.0	11.60
Propranolol	-	-	-	-	-	-	11.5	121.02

Cl_{int}, app range: 11.6-462.0 µL/min/mg; t1/2:3-120 min, LM conc: 0.5mg/mL. For to asses oral bioavailability(Mondal et al., 2008), the compounds were incubation with human liver microsomes at 37.5°C and evaluated for their and apparent intrinsic clearance and intrinsic half-life ($t_{1/2}$) in in-vitro.

Conclusion

The membrane permeability and liver metabolic stability of the compounds are some of the most adopted methods to show blood-brain barrier permeability and therapeutic actions. Earlier, due to poor oral bioavailability (Wahlang, Pawar & Bansal, 2011), blood-brain barrier permeability and extensive metabolism in the liver (Pan, Huang &

Lin, 1999) curcumin and its derivatives have never been recognized as a drug candidate. A significant enhancement in permeability, metabolic stability and inhibitory activity was achieved for curcumin-based compounds (ferulic acid amides and pyrazoline) through chemical modification. Thus, allowing us to propose a lead curcumin-based compound (ferulic acid amides and pyrazoline derivatives) to treat depressive illness and neurodegenerative disorders (depression and Parkinson's).

Acknowledgment

The first author acknowledges Chitkara University for providing infrastructure to carry out the research. Also acknowledge to BIT-Mesra, India for Spectral Characterization.

Authorship Contribution

Vishnu Nayak Badavath: Conceptualization, administration
Venkatesan Jayaprakash: Methodology, Software
Susanta Kumar Mondal: Writing - Original Draft
Sandeep Arora: Supervision
Orlando Acevedo: Validation, visualization
Abhishek Thakur: Writing - Review & Editing, investigation

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

Declaration

It is an original data and has neither been sent elsewhere nor published anywhere.

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Journal of Pharmaceutical Technology, Research and Management

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Volume 8, Issue 2 **November 2020** **ISSN 2321-2217**