DIMETHYL FUMARATE AND ITS COCRYSTALS: POTENTIAL BIOLOGICAL APPLICATIONS

Thesis submitted in partial fulfillment for the Award of Degree

Doctor of Philosophy

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Date:

Place: IIT(BHU), Varanasi **Mr. Qadir Alam**

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List of Abbreviations

ACN	: Acetonitrile
ADP	: Adenosine diphosphate
Amber	: Assisted model building with energy refinement)
AMP	: Adenosine monophosphate
AM-BCC1	: Austin model with bond and charge correction
AMPK	: Adenosine monophosphate-activated protein kinase
ANOVA	: Analysis of variance
API	: Active pharmaceutical ingredient
ATP	: Adenosine triphosphate
ATR	: Attenuated total reflectance
AUC	: Area under the curve
BBB	: Blood-brain barrier
CAT	: Catalase
CD	: Cluster of differentiation
CIT	: Citric acid
CSD	: Cambridge structural database
CSF	: Cerebrospinal fluid
CT	: Computed tomography
CL	: Clearance
C_{max}	: Maximum plasma concentration
CNS	: Central nervous system
Cyt. C	: Cytochrome C enzyme
DCFH-DA	: Dichlorodihydrofluorescein diacetate

DMSO : Dimethyl sulfoxide

DMF : Dimethyl fumarate

DNA : Deoxy-ribonucleic-acid

DPP1 : Dipeptidyl peptidase

DSC : Differential scanning calorimetry

DTA : Differential thermal analysis

EB : Evans blue

EAE : Experimental autoimmune encephalitis

EDTA : Ethylenediamine tetra acetic acid

ELISA : Enzyme linked immunosorbent assay

FACS : Fluorescence assorted cell sorting

FDA : Food and Drug Administration

FTIR : Fourie transform infrared

GAFF2 : General AMBER force field

GAPDH : Glyceraldehyde 3-phosphate dehydrogenase

GSH : Glutathione

GPSCH : Guinea pig spinal cord homogenate

GrB : Granzyme B

HPLC : High-performance liquid chromatography

H_2O_2 : Hydrogen peroxide

IAEC : Institutional Animal Ethical Committee

ICH : International Council for Harmonisation

IL-6 : Interleukin-6

IL-10 : Interleukin-10

L-1β : Interleukin -1β

I.V. : Intravenous

KCl : Potassium chloride

K_{inact} : Inactivation rate constant

K_i : Inhibition rate constant

K_{obs} : Observed rate constant

LLOD : Lower limit of detection

LLOQ : Lower limit of quantification

LGA : Lamarckian Genetic Algorithm

LPS : Lipopolysaccharide

MAPK : Mitogen activated protein kinase

MD : Molecular dynamics

MDA : Malonaldehyde

$MgCl_2$: Magnesium chloride

MS : Multiple sclerosis

MMF : Monomethyl fumarate

MRT : Mean residence time

MTT : 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

MOG : Myelin oligodendrocyte glycoprotein

NaCl : Sodium chloride

$NaHCO_3$: Sodium bicarbonate

$NaHPO_4$: Sodium monohydrate phosphate

NADH : Nicotine adenine dinucleotide phosphate

NBT : Nitroblue tetrazolium

NFκB : Nuclear factor- κB

NIC : Nicotinamide

p-AMPK : Phosphorylated-adenosine monophosphate-activated protein kinase

PBS : Phosphate buffer saline

PBMC : Peripheral blood mononuclear cells

PGC1-α : Peroxisome proliferative activated receptor-γ co-activator 1-α

PDA : Photodiode array

PK : Pharmacokinetics

PD : Pharmacodynamics

PGE2 : Prostaglandin E2

PDB : Protein data bank

PLP : Proteolipoprotein

PT : Pertussis toxin

PXRD : Powder X-ray diffraction

RH : Relative humidity

ROS : Reactive oxygen species

RMSF : Root mean square fluctuation

RMSD : Root mean square deviation

RRMS : Relapsing-remitting multiple sclerosis

RSD : Relative standard deviation

SD : Standard deviation

SOD : Superoxide dismutase

SUCC : Succinic acid

TGA : Thermogravimetric analysis

TMRM :Tetramethyl rhodamine methyl ester

TLR : Toll like receptor

T_{max} : Time to reach the maximum plasma concentration

TNF-α : Tumor necrosis factor-α

$T_{½}$	: Half-life
UV	: Ultra violet
V_d	: Volume of distribution
2SC	: S-(2-succinyl) cysteine

Preface

The research work of the thesis entitled "**Dimethyl fumarate and its Cocrystals: Potential biological applications**" assessed the novel mechanism of action of dimethyl fumarate (DMF), including the effect of DMF on MOG_{35-55} induced EAE model and enzyme kinetics of DMF/MMF on cathepsin C. Also, we have prepared cocrystals of DMF which overcome its sublimation problem and provide safety against its gastric-related adverse effects. The whole work has been compiled into six chapters: **Chapter 1** describes the introduction and significance of the present study. **Chapter 2** contains the immunological mechanism of DMF as a covalent inhibitor of cathepsin C; the novel mechanism of action has been explored in both *in-vitro* and *in-vivo*. **Chapter 3** discusses the preparation and characterisation of cocrystals to overcome their gastric-related adverse effects. **Chapter 4** includes the preparation, characterization, *in-vitro* and *in-vivo* pharmacokinetic evaluation of thermostable dimethyl fumarate cocrystals. **Chapter 5** summarizes all the experiments with their essential outcomes.