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RESEARCH ARTICLE

In Vitro Drug-Drug Interaction Potential of Sulfoxide and/or Sulfone Metabolites of Albendazole, Triclabendazole, Aldicarb, Methiocarb, Montelukast and Ziprasidone

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Abstract: ***Background*:** The use of polypharmacy in the present day clinical therapy has made the identification of clinical drug-drug interaction risk an important aspect of drug development process. Although many drugs can be metabolized to sulfoxide and/or sulfone metabolites, seldom is known on the CYP inhibition potential and/or the metabolic fate for such metabolites.

***Objective*:** The key objectives were: a) to evaluate the *in vitro* CYP inhibition potential of selected parent drugs with sulfoxide/sulfone metabolites; b) to assess the *in vitro* metabolic fate of the same panel of parent drugs and metabolites.

***Methods*:** *In vitro* drug-drug interaction potential of test compounds was investigated in two stages; 1) assessment of CYP450 inhibition potential of test compounds using human liver microsomes (HLM); and 2) assessment of test compounds as substrate of Phase I enzymes; including CYP450, FMO, AO and MAO using HLM, recombinant human CYP enzymes (rhCYP), Human Liver Cytosol (HLC) and Human Liver Mitochondrial (HLMit). All samples were analysed by LC-MS-MS method.

***Results*:** CYP1A2 was inhibited by methiocarb, triclabendazole, triclabendazole sulfoxide, and ziprasidone sulfone with IC_{50} of 0.71 µM, 1.07 µM, 4.19 µM, and 17.14 µM, respectively. CYP2C8 was inhibited by montelukast, montelukast sulfoxide, montelukast sulfone, tribendazole, triclabendazole sulfoxide, and triclabendazole sulfone with IC50 of 0.08 µM, 0.05 µM, 0.02 µM, 3.31 µM, 8.95 µM, and 1.05 µM, respectively. CYP2C9 was inhibited by triclabendazole, triclabendazole sulfoxide, triclabendazole sulfone, montelukast, montelukast sulfoxide and montelukast sulfone with IC50 of 1.17 µM, 1.95 µM, 0.69 µM, 1.34 µM, 3.61 µM and 2.15 µM, respectively. CYP2C19 was inhibited by triclabendazole and triclabendazole sulfoxide with IC50 of 0.25 and 0.22, respectively. CYP3A4 was inhibited by montelukast sulfoxide and triclabendazole with IC50 of 9.33 and 15.11, respectively. Amongst the studied sulfoxide/sulfone substrates, the propensity of involvement of CY2C9 and CYP3A4 enzyme was high (approximately 56% of total) in the metabolic fate experiments.

***Conclusion*:** Based on the findings, a proper risk assessment strategy needs to be factored (*i.e.*, perpetrator and/or victim drug) to overcome any imminent risk of potential clinical drug-drug interaction when sulfoxide/sulfone metabolite(s) generating drugs are coadministered in therapy.

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1. INTRODUCTION

Clinical Drug-Drug Interaction (DDI) studies are intended to identify potential safety issues due to altered drug pharmacological activity and/or pharmacokinetic disposition. These DDI studies are important during a drug development program to guide dose selection, co-medication exclusion strategy and appropriate patient enrolment in clinical trials. Cytochrome P450 (CYP) enzymes are considered to be of paramount importance for DDIs due to their involvement in the metabolic clearance of the majority of prescribed drugs [1-5]. Typically clinical DDI studies involve comparative safety and pharmacokinetic assessment of the investigational drug alone versus investigational drug plus co-administered drug(s); such studies are intended to assess whether or not the investigational drug is a victim drug or a perpetrator.

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Traditionally, Phase 1 studies conducted in healthy subjects help us to understand the drug disposition parameters of the parent drug along with safety and tolerability profile. Nowadays, importance is also given to the measurement of metabolites during early Phase 1 studies. This growing trend is important because patients who participate in clinical trials are exposed not only to the parent drug but also to the metabolite(s) generated by drug-metabolizing enzymes. Despite the increased awareness of the role of metabolites, the degree of assessment of inhibition of CYP enzymes by metabolite(s) is still not routinely performed in drug discovery/development programs. However, the customary evaluation of time-dependent CYP inhibition during clinical candidate nomination may suggest possible involvement, if any, of metabolites in CYP inhibition.

It should be anticipated that metabolic profiles can vary across species used in preclinical evaluation, and that certain clinically relevant metabolites may escape scrutiny during preclinical drug safety assessment. In this regard in the last several years, extensive attention has been focused on the safety evaluation of drug metabolites generated by novel drug candidates [6, 7]. Additionally, there has been a suggestion for consideration of drug metabolites in preclinical DDI studies [8]. In accordance with changing times, regulatory agencies, have clearly established strategies for the evaluation of toxicity of circulating metabolites of a potential human relevance at a predefined threshold [7]. In majority of the cases since metabolites have undergone chemical alterations from the parent drug, the potential to bind and engage target receptors may be significantly diminished [9]. However, in some cases, metabolite(s) maintain sufficient intrinsic activity at the target receptor level. Some notable examples of pharmacologically active metabolites encountered in clinical therapy include morphine, N-desmethylsertraline, enalaprilat, phenobarbital, and desipramine from the respective parent drugs: codeine, sertraline, enalapril, primidone, and imipramine [9-13]. Sometimes drug metabolites may also represent an avenue for manifestation of the drug-related toxicity and Adverse Drug Reactions (ADRs) such as the quinone-imine metabolite of acetaminophen and trifluoroacetyl chloride of halothane [14]. Whereas the requirement and appropriate timing for establishing metabolite(s) role in CYP inhibition remain a topic of debate, it is evident that in certain cases, metabolite(s) may be a significant contributor for the CYP inhibitions: a Itraconazole (ITZ):, The metabolism of ITZ by CYP3A4 was studied and three metabolites were detected: hydroxy-itraconazole (OH-ITZ), keto-itraconazole (keto-ITZ) and N-desalkyl-itraconazole (ND-ITZ). Inhibition of CYP3A4 by ITZ, OH-ITZ, keto-ITZ, and ND-ITZ was evaluated using hydroxylation of midazolam as a probe reaction, and unbound IC_{50} values were 6.1 nM, 4.6 nM, 7.0 nM, and 0.4 nM, respectively. Itraconazole metabolites are equally or more potent CYP3A4 inhibitors than parent itself, and therefore are responsible for *in vitro* versus *in vivo* discrepancy observed in CYP3A4 inhibition by ITZ [15, 16]; b) Warfarin: Hydroxywarfarin, the key metabolite showed inhibition of CYP2C9 *in vitro* (human liver microsomes and recombinant enzymes) [17]; c) Amiodarone: Desethylamiodarone metabolite was shown to inhibit CYP1A1, CYP1A2, CYP2B6 and CYP2D6 *in vitro* (human B-lymphoblastoid cell microsomes) and *ex vivo* (human plasma) [18]; d) Voriconazole: The voriconazole N-oxide metabolite was shown to inhibit both CYP2C9 and CYP3A4 enzyme in a competitive fashion in both human liver microsomes and hepatocytes [19]. From the ensuing topic discussed above, an independent assessment for *in vitro* CYP inhibition potential of metabolite(s), in addition to parent drug, may be warranted to make an informed risk assessment decision of drugs in development.

We chose sulfoxide/sulfone metabolites of six drugs namely, albendazole, triclabendazole, Aldicarb, methiocarb, montelukast and Ziprasidone (sulfone metabolite only) that manifested diversity in the structures (Fig. **1**) in this evaluation. Also, the observed heterogeneity in the structures provided an opportunity to evaluate the complete spectrum of CYP enzymes. From the experimental design perspective, the planned *in vitro* evaluation was considered relevant from an *in vivo* consideration for the chosen drug and the metabolites.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Acetaminophen, aldicarb, aldicarb sulfone, aldicarb sulfoxide, methiocarb, methiocarb sulfoxide, triclabendazole sulfoxide, ziprasidone, dextrorphan, quinidine, sulfaphenazole, ticlopidine, 4-hydroxy diclofenac, montelukast, phenacetin, phthalazine, monobasic potassium hydrogen phosphate, dibasic potassium hydrogen phosphate and dimethyl sulfoxide were purchased from Sigma-Aldrich, St. Louis, MO, USA. Methiocarb sulfone was obtained from Chem Service Inc, West Chester, PA, USA. Triclabendazole, albendazole sulfoxide, montelukast sulfoxide, albendazole sulfone, albendazole, montelukast sulfone and triclabendazole sulfone were procured from Torrent Research Chemicals, Toronto, Canda. Testosterone propionate was purchased from Ipca Laboratories Ltd., Mumbai, India. Rosiglitazone maleate and ketoconazole were purchased from Piramal Healthcare Ltd., Mumbai, India. Hydroxybupropion and 6α-hydroxypaclitaxel were purchased from GRK Research Laboratories Pvt. Ltd., Hyderabad, Telangana, India. 6β-hydroxytestosterone was purchased from Elina Biosciences LLC., Wynnewood, PA, USA. 1-Hyroxymidazolam was purchased from SPI-Bio Bertin, (Montigny le Bretonneux, France). Midazolam hydrochloride, ziprasidone sulphone were purchased from Clearsynth, Mumbai, India. Reduced β-nicotinamide adenine dinucleotide phosphate tetrasodium salt (NADPH) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Ketoconazole, S-mephenytoin, α-naphthoflavone, bupropion hydrochloride, dextromethorphan, diclofenac sodium were purchased from Cayman Chemical, Ann Arbor, MI, USA. Pooled mixed gender human liver microsomes (50 donor pool; protein content 20 mg/mL), Pooled mixed gender human liver cytosol (protein content 10 mg/mL) and Pooled mixed gender human liver mitochondria (protein content 20 mg/mL) were purchased from Sekisui XenoTech, LLC, Lenexa, Kansas, USA and stored at -80°C. Recombinant human CYP enzymes were purchased from Corning Life Sciences, Tewksbury MA, USA and stored at -80°C. Acetonitrile, isopropanol, and methanol were of HPLC grade quality. All other chemicals were obtained from commercial sources and were of the highest purity available and were used without further purification.

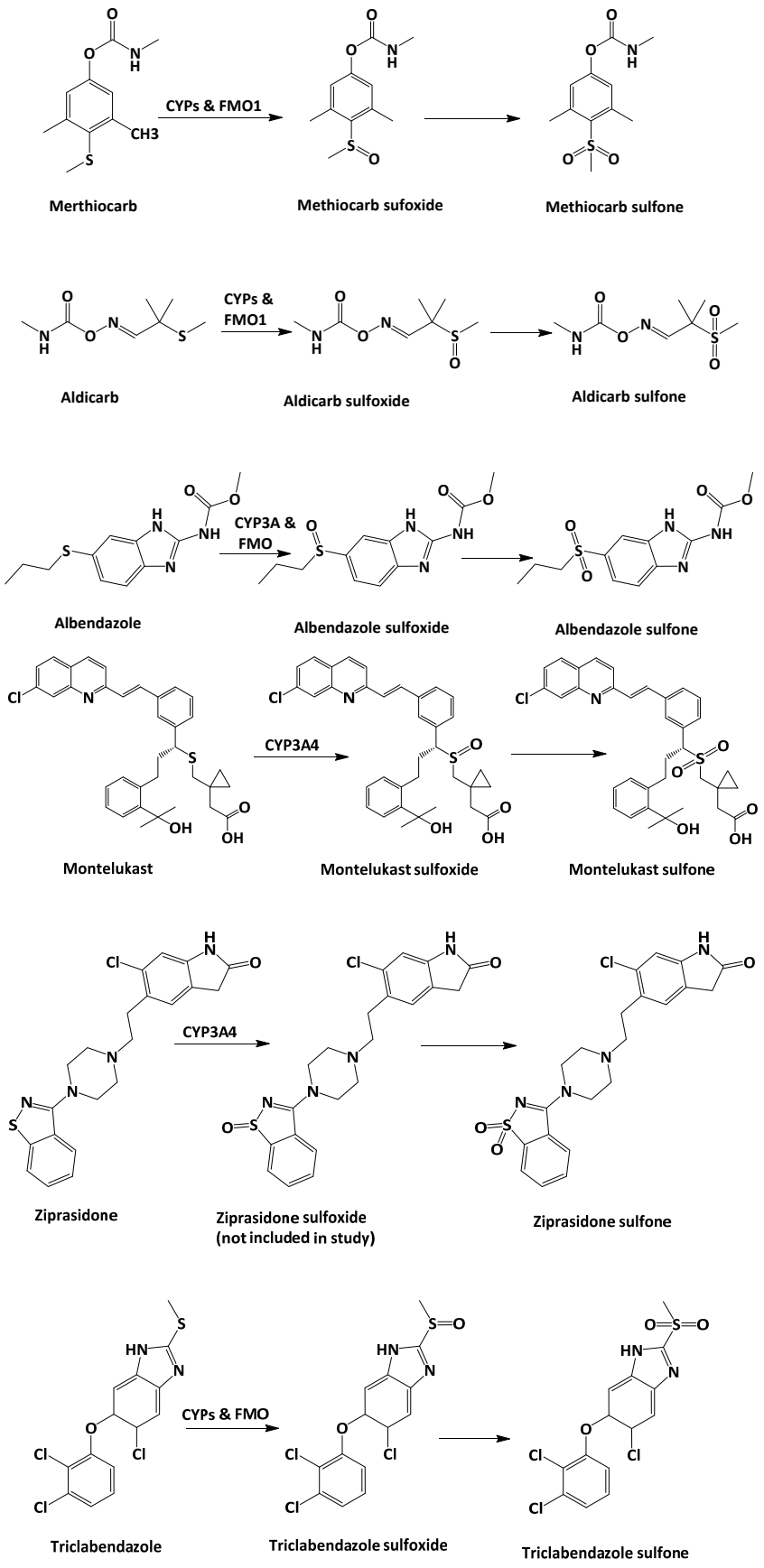

Fig. (1). Structures of parent drugs and sulfoxide/sulfone metabolites of methiocarb, aldicarb, montelukast , ziprasidone, albendazole and triclabendazole.

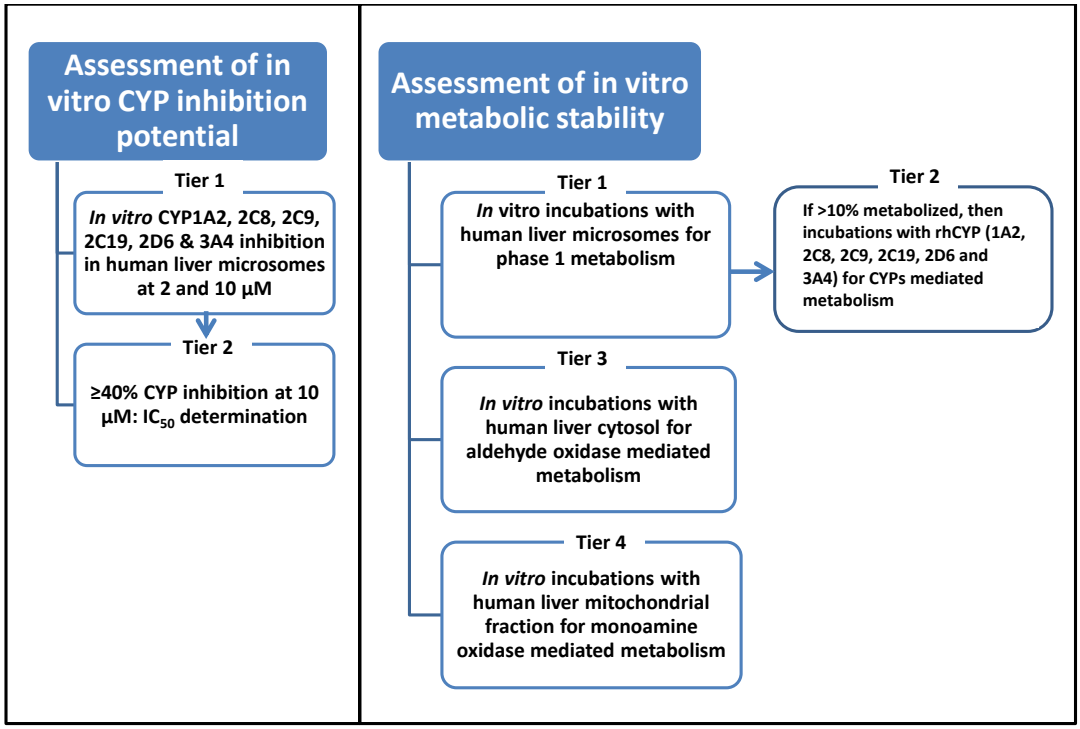

Fig. (2). Schematic presentation of tiered assessment of drug-drug interaction potential of sulfoxide and/or sulfone metabolites of albendazole, triclabendazole, aldicarb, methiocarb, montelukast and ziprasidone.
[CYP: Cytochrome P450, IC50: inhibitor concentration causing 50% inhibition of the enzyme activity].

2.2. CYP 450 Interaction Studies

All sulfoxide and sulfone metabolites of six drugs namely, albendazole, triclabendazole, aldicarb, methiocarb, montelukast and ziprasidone (sulfone metabolite only) along with parent drugs were assessed for CYP inhibition potential using Human Liver Microsomes (HLM) in a 2-tiered approach and for metabolic stability, *in vitro* incubation was performed with Human Liver Microsomes (HLM), recombinant human CYP450 (*rhCYP*) enzymes, Human Liver Cytosol (HLC) and Human Liver Mitochondrial (HLMit) fraction to check further metabolism of sulfoxide and sulfone metabolites in relation with parent drugs using a 4-tiered approach (Fig. **2**).

2.3. *In Vitro* CYP Inhibition

2.3.1. Tier 1 Study- In Vitro Determination of CYP Inhibition Potential at 2 and 10 µM Concentrations

CYP inhibition potential of parent drugs and metabolites was assessed at two concentrations (2 µM and 10 µM) using HLM against each of the CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 (with two substrate testosterone and midazolam) enzymes. Stock solutions were prepared in DMSO for all tested drugs and diluted with DMSO. For each CYP isoform, a separate reaction mixture was prepared. HLM were diluted with PBS pH 7.4 for anticipated protein concentration (Table **1**) and CYP isoform-specific substrate was added (Table **1**). For each isozyme, a 499 µL aliquot of incubation mixture was spiked with 1 µL test compound (sulfoxide/sulfone / parent) working solution in a 96 well plate, mixed gently and preincubated for 5 min. Thereafter, an aliquot of 117 µL was drawn in duplicate and transferred to another 96 well plate. Reactions were initiated by addition of 13 µL of 10 mM NADPH and incubated at 37°C and 80 rpm for CYP isoform-specific time period (Table **1**). At pre-set times, the incubation reactions were stopped with the addition of 1:5 volume of ice-cold acetonitrile containing analytical internal standard alprazolam. Non-inhibitor incubations were prepared similarly except that blank DMSO was spiked in place of test compounds working solution. All samples were centrifuged at 2500 rpm for 15 min. The supernatant was analysed for peak area ratio using LC-MS/MS to determine the formation of CYP-specific metabolites (Table **1**). The CYP inhibition potential of test compounds *vs*. CYP isoforms was determined following transformation of the metabolite response data as percent of control (containing no added inhibitor). Inhibition by positive control inhibitors (Table **1**) was similarly determined.

2.3.2. Tier 2 Study- In Vitro Determination of Half Maximal Inhibitory Concentration (IC_{50})

CYP IC_{50} of test compounds (*i.e.*, parent drugs and metabolites) which has shown ≥40% CYP activity inhibition against control in Tier 1 experiment were determined using HLM at eight different concentration level; 0, 0.01, 0.1, 1, 3, 10, 30 and 100 µM. Stock solutions were prepared in DMSO for test compounds and serially diluted with DMSO. For each CYP isoform, separate reaction mixture was prepared. HLM was diluted with PBS pH 7.4 for anticipated protein concentration (Table **1**) and CYP isoform-specific substrate (Table **1**) was added and pre-incubated for 5 min. For each isozyme, a 499 µL aliquot of incubation mixture at each concentration level was spiked with 1 µL test compound

Table 1. Summary of CYP inhibition assay conditions.

CYP Isoforms	Probe Substrate	Substrate Conc.	Marker Metabolites	HLM (mg/mL)	Incubation Time	Positive Control Inhibitor
CYP1A2	Phenacetin	50 µM	Acetaminophen	0.025	20 min,	α-Naphthoflavone
CYP2C8	Paclitaxel	5 µM	6'-OH Taxol	0.15	10 min,	Rosiglitazone
CYP2C9	Diclofenac	5 µM	4' OH- Diclofenac	0.025	10 min,	Sulfaphenazole
CYP2C19	S-Mephenytoin	35 µM	4' OH- S- Mephenytoin	0.2	40 min,	Ticlopidine
CYP2D6	Dextromethorphan	5 µM	Dextrorphan	0.2	10 min,	Quinidine
CYP3A4/5	Testosterone	60 µM	6β-OH-Testosterone	0.2	10 min,	Ketoconazole
	Midazolam	2 µM	1-OH-Midazolam	0.05	5 min,	

(parent/ sulfoxide / sulfone) working solution in a 96 well plate and mixed gently. Thereafter, an aliquot of 117 µL aliquot was drawn in duplicate and transferred to separate 96 well plate. Reactions were initiated by the addition of 13 µL of 10 mM NADPH and incubated at 37°C and 80 rpm for CYP isoform-specific time period (Table **1**). At pre-set times, the incubation reactions were stopped with the addition of 1:5 volume of ice-cold acetonitrile containing analytical internal standard alprazolam. The incubation for positive control inhibitors was run concurrently in a similar fashion. Vehicle control (No-inhibitor) incubations were prepared similarly except that blank DMSO was spiked in place of test compounds. All samples were centrifuged at 2500 rpm for 15 min. The supernatant was analyzed for peak area ratio using LC-MS/MS for the formation of CYP-specific metabolites (Table **1**). The CYP inhibition potential of test compounds at different concentration levels against control was determined following transformation of the metabolite response data as percent of control (no inhibitor). IC_{50} was calculated using GraphPad Prism® software, version 7.0.

2.4. *In Vitro* Metabolic Stability

2.4.1. Tier 1 Study- In Vitro Incubations with Human Liver Microsomes

The *in vitro* metabolism of sulfoxide and sulfone metabolites of six drugs namely, albendazole, triclabendazole, aldicarb, methiocarb, montelukast and ziprasidone (sulfone metabolite only) along with parents was investigated using pooled (n=50) mixed gender human liver microsomes. An incubation mixture prepared by diluting HLM with potassium phosphate buffer pH 7.4 and test compound was pre-incubated at 37°C for 5 min. Incubation was performed in duplicate in 96 well plate and total organic concentration was 0.1%. A 120 µL aliquot of incubation mixture containing 0.5 mg/mL protein and 1 µM test compound in potassium phosphate buffer pH 7.4 was incubated at 37°C for 0, 5, 10, 15 and 30 min and 30 min (no NADPH. The reactions were initiated by addition of NADPH (final concentration 1 mM). Reactions were terminated by addition of 1: 6 volume of acetonitrile containing internal standard alprazolam. After vortexing and centrifugation at 2500 rpm for 15 min, the supernatant was analyzed by LC-MS/MS analysis. An NADPH-free incubation was used as a negative control for chemical instability/ non-NADPH dependent metabolism. The metabolic stability (% remaining of test compounds) in incubated samples was assessed with respect to control samples (0 min incubation). Testosterone was used as a positive controls compound and was assessed concurrently.

2.4.2. Tier 2- Study- In Vitro Incubations with Recombinant Human CYP Enzymes

The *in vitro* metabolism of sulfoxide and sulfone metabolites of six drugs namely, albendazole, triclabendazole, aldicarb, methiocarb (sulfoxide metabolite only), montelukast and ziprasidone (sulfone metabolite only) along with parent drugs was investigated with a panel of rhCYP isoforms, including CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4. An incubation mixture (100 µL) contained individual protein (100 pmol/mL) and test compound (1 µM) in 100 mM potassium phosphate buffer pH 7.4. The reactions were initiated by addition of 1 mM NADPH and incubated at 37°C for 0, 30 min and 30 min (no NADPH). Reactions were terminated by addition of 1: 3 volume of acetonitrile. After vortexing and centrifugation at 10,000 rpm for 5 min, the supernatant was analysed by LC-MS/MS analysis. An NADPH-free incubation was used as a negative control for chemical instability/ non-NADPH dependent metabolism. The metabolic stability (% remaining of test compounds) in incubated samples was assessed with respect to control samples (0 min incubation). Positive controls compounds were concurrently assessed and included phenacetin (1A2), paclitaxel (2C8), diclofenac (2C9), S-mephenytoin (2C19), dextromethorphan (2D6) and testosterone (3A4) to check for appropriate incubation conditions and enzyme activity.

2.4.3. Tier 3- Study- In Vitro Incubations with Human Liver Cytosol

The *in vitro* metabolism of sulfoxide and sulfone metabolites of six drugs namely, albendazole, triclabendazole, Aldicarb, methiocarb, montelukast and ziprasidone (sulfone metabolite only) along with parent drugs were investigated using mixed gender HLC. HLC was diluted in 100 mM potassium phosphate buffer pH 7.4 to achieve 1 mg/mL protein and preincubated for 5 min at 37°C. Test compound (final concentration 1 µM) was added to the diluted cytosol and incubated at 37°C for 0, 10, 20 and 30 min in duplicate. Re-

actions were terminated by addition of 1: 5 volume of acetonitrile containing internal standard alprazolam. After vortexing and centrifugation at 10,000 rpm for 5 min, the supernatant was analyzed by LC-MS/MS analysis. The metabolic stability (% remaining of test compounds) in incubated samples was assessed with respect to control samples (0 min incubation). Methotrexate and phthalazine were used as positive controls compound and were assessed concurrently.

2.4.4. Tier 4- Study- In Vitro Incubations with Human Liver Mitochondrial Preparation

The *in vitro* metabolism of sulfoxide and sulfone metabolites of six drugs namely, albendazole, triclabendazole, aldicarb, methiocarb, montelukast (sulfoxide metabolite only) and ziprasidone (sulfone metabolite only) along with parents were investigated using mixed gender HLMit preparation. HLMit was diluted in 100 mM potassium phosphate buffer pH 7.2 to achieve 0.5 mg/mL protein and preincubated for 5 min at 37°C [20]. Test compound (final concentration 1 µM) was added to diluted mitochondria and incubated at 37°C for 0, 10, 20 and 30 min in duplicate. Reactions were terminated by addition of 1: 5 volume of acetonitrile containing internal standard alprazolam. After vortexing and centrifugation at 10,000 rpm for 5 min, the supernatant was analysed by LC-MS/MS analysis. The metabolic stability (% remaining of test compounds) in incubated samples was assessed with respect to control samples (0 min incubation). Serotonin and tryptamine were used as positive controls and were assessed concurrently.

2.5. Bioanalysis

Samples were analysed using an LC-MS/MS method; instrumentation included a HPLC system (Shimadzu, Kyoto, Japan) comprising of LC-30AD NexeraX2 pump, DGU-20A5R vacuum degasser, CTO-20 AC prominence column oven (ambient temperature), CBM 20A communication bus module and Nexera X2 30 SIL ACMP autosampler (maintained at 10°C) coupled to an API 5500 Q-Trap system mass spectrometer from AB Sciex (Foster City, CA, USA) equipped with an electrospray ionization (ESI) source . The details of liquid chromatography conditions and mass spectrometry parameters used for sample analysis are summarized in Supplementary Table **S1a** and Table **S1b**.

3. RESULTS

3.1. *In Vitro* CYP Inhibition Potential of Sulfoxide/Sulfone Metabolites and Parent Drugs

CYP inhibition potential of the various drugs/metabolites was assessed in tiered approach. Tier-1 assessment of CYP inhibition potential of test compounds at 2 and 10 µM is presented in Table **2a**. The IC_{50} determination for drugs/metabolites Tier-2 assessment is shown in Table **2b**. Positive control inhibitors data are presented in Table 2c to illustrate the validity of the test systems employed in the assessment. The observed CYP1A2 IC_{50} was found to be 0.71 µM for methiocarb. Triclabendazole showed inhibitory potential against CYP isoforms 1A2, 2C8, 2C9, 2C19 and 3A4 with IC_{50} values for CYP1A2 – 1.07 µM, 2C8 – 3.31 µM, 2C9 – 1.17 µM, 2C19 – 0.25 µM and 3A4 – 15.11 µM (substrate used: testosterone). Triclabendazole sulfoxide showed inhibitory potential against CYP1A2, 2C8, 2C9 and 2C19 with IC_{50} values for CYP1A2 – 4.19 µM, 2C8 – 8.95 µM, 2C9 – 1.95 µM and 2C19 – 0.22 µM. Triclabendazole sulfone showed inhibitory potential against CYP2C8 and 2C9 with IC_{50} values of 1.05 and 0.69 µM, respectively. Montelukast showed inhibitory potential against 2C8, 2C9 and 3A4 with IC_{50} values for 2C8 – 0.08 µM; 2C9 – 1.34 µM, and 3A4 – 10.43 µM (substrate used: midazolam). Montelukast sulfoxide showed inhibitory potential against 2C8, 2C9 and 3A4 with IC_{50} values for CYP2C8 – 0.05 µM, 2C9 – 3.61 µM and 3A4 – 9.33 µM (substrate used: midazolam). Montelukast sulfone showed inhibitory potential against 2C8 and 2C9 with IC_{50} values 0.02 and 2.15 µM, respectively. Ziprasidone sulfone showed inhibition for CYP1A2 with IC_{50} 17.14 µM.

Table 2a. Summary of two concentration CYP inhibition potential of parents and their sulfoxide and sulfone metabolites.

Inhibitors	Inhibitors Concentrations	% Inhibition of Control Activity						
		CYP1A2	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4[1]	CYP3A4[2]
Aldicarb	2 µM	6.19	2.63	NI	2.78	2.45	NI	NI
	10 µM	5.89	NI	0.11	4.00	5.47	NI	NI
Aldicarb sulfoxide	2 µM	NI	NI	7.71	NI	NI	NI	NI
	10 µM	NI	2.32	13.98	NI	6.74	NI	NI
Aldicarb sulfone	2 µM	1.41	2.77	NI	NI	2.69	1.83	NI
	10 µM	0.21	4.10	6.09	5.22	4.23	NI	NI
Methiocarb	2 µM	77.49	NI	NI	7.34	NI	8.34	NI
	10 µM	88.89	NI	16.31	32.01	NI	29.71	NI
Methiocarb sulfoxide	2 µM	1.52	NI	NI	NI	NI	5.73	NI
	10 µM	7.66	NI	4.82	NI	NI	9.10	NI

(Table 2a) Contd…

Inhibitors	Inhibitors Concentrations	% Inhibition of Control Activity						
		CYP1A2	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4[1]	CYP3A4[2]
Methiocarb sulfone	2 μM	2.14	NI	NI	0.91	NI	4.83	NI
	10 μM	15.32	NI	5.31	5.12	NI	7.70	NI
Albendazole	2 μM	31.12	NI	NI	NI	NI	11.22	NI
	10 μM	35.54	NI	12.40	4.20	NI	13.06	NI
Albendazole sulfoxide	2 μM	3.17	NI	6.08	NI	NI	2.48	NI
	10 μM	22.20	NI	9.49	NI	NI	4.82	NI
Albendazole sulfone	2 μM	2.25	NI	6.06	NI	NI	7.51	NI
	10 μM	7.84	NI	11.87	3.27	NI	21.40	NI
Triclabendazole	2 μM	68.23	7.09	74.36	81.71	NI	13.53	NI
	10 μM	86.11	79.66	99.50	94.21	4.19	52.86	NI
Triclabendazole sulfoxide	2 μM	39.42	11.20	49.60	84.33	1.63	0.18	NI
	10 μM	69.42	53.67	86.62	88.87	3.13	14.43	NI
Triclabendazole sulfone	2 μM	NI	65.36	78.78	NI	NI	4.09	NI
	10 μM	8.60	92.88	96.15	5.05	NI	8.63	NI
Montelukast	2 μM	NI	93.74	68.84	NI	NI	0.79	20.00
	10 μM	24.96	97.32	97.48	22.70	NI	11.15	47.95
Montelukast sulfoxide	2 μM	6.14	93.68	33.10	0.00	NI	NI	10.87
	10 μM	1.35	97.35	76.47	25.67	NI	6.67	48.64
Montelukast sulfone	2 μM	NI	94.63	44.50	NI	NI	NI	0.42
	10 μM	NI	96.51	91.12	18.54	NI	NI	32.76
Ziprasidone	2 μM	NI	NI	3.68	NI	NI	8.30	22.00
	10 μM	NI	12.00	1.44	3.00	11.70	33.06	31.08
Ziprasidone sulfone	2 μM	11.73	NI	0.77	NI	NI	NI	NI
	10 μM	45.57	NI	15.65	1.70	NI	6.74	NI

1: substrate, testosterone; 2: substrate, midazolam; NI: No Inhibitin, NA: Not Applicable.

Table 2b. CYP IC_{50} data of of parents and their sulfoxide and sulfone metabolites.

Compound	CYP Isoform	IC_{50} (μM)
Methiocarb	1A2	0.71
Triclabendazole	1A2	1.07
	2C8	3.31
	2C9	1.17
	2C19	0.25
	3A4[1]	15.11
Triclabendazole sulfoxide	1A2	4.19
	2C8	8.95
	2C9	1.95
	2C19	0.22

(Table 2b) Contd...

Compound	CYP Isoform	IC_{50} (μM)
Triclabendazole sulfone	2C8 2C9	1.05 0.69
Montelukast	2C8 2C9 3A4[2]	0.08 1.34 10.43
Montelukast sulfoxide	2C8 2C9 3A4[2]	0.05 3.61 9.33
Montelukast sulfone	2C8 2C9	0.02 2.15
Ziprasidone sulfone	1A2	17.14

CYP: Cytochrome P450, IC_{50}: inhibitor concentration causing 50% inhibition of the enzyme activity,
1: Testosterone was used as substrate;
2: Midazolam was used as substrate

Table 2c. Percent inhibition data for positive control inhibitors.

Isoforms	CYP1A2	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4[1]	CYP3A4[2]
Concentration tested	20 nM	25 μM	1 μM	2 μM	100 nM	50 nM	50 nM
α-Nepthoflavone	62.43	-	-	-	-	-	-
Ticlopidine	-		-	-	-	-	-
Rosiglitazone	-	69.23		-	-	-	-
Sulfaphenazole	-	-	83.87		-	-	-
Ticlopidine	-	-	-	71.03		-	-
Quinidine	-	-	-	-	67.43		-
Ketoconazole	-	-	-	-	-	69.17	
Ketoconazole	-	-	-	-	-	-	80.06

CYP: Cytochrome P450, 1: Testosterone was used as substrate, 2: Midazolam was used as substrate, (-): Not applicable.

3.2. *In vitro* Metabolic stability of Parents and Sulfoxide and Sulfone Metabolites

3.2.1. Aldicarb and Metabolites

Aldicarb showed 13.41%, 13.26%, 6.93%, 21.08% and 10.94% metabolism *in vitro* using HLM, rhCYP enzymes (2C8, 2C19), human liver cytosol (HLC) and human liver mitochondria (HLMit), respectively (Table **3a**). The contribution of CYP2C8 and 2C9 was 56.91% and 43.09% in CYP enzymes mediated metabolism of aldicarb, respectively (Table **4**).

Aldicarb sulfoxide showed 21.18%, 0.85%, 5.91% and 5.58% metabolism *in vitro* using HLM, rhCYP enzymes (2C19, 3A4) and HLMit, respectively (Table **3a**). The contribution of CYP2C19 and 3A4 was 2.34% and 97.66% in CYP enzymes mediated metabolism of aldicarb sulfoxide, respectively (Table **4**).

Aldicarb sulfone showed 1.05% and 6.43% metabolism *in vitro* using HLM and HLMit, respectively (Table **3a**).

3.2.2. Methiocarb and Metabolites

Methicarb showed 78.39%, 46.94%, 41.44%, 6.50%, 19.42%, 99.43%, 98.38%, 6.93% and 1.83% metabolism *in vitro* using HLM, rhCYP enzymes (1A2, 2C8, 2C9, 2C19, 2D6 and 3A4), HLC and HLMit, respectively Table **3a**). The contribution of CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 was 4.75%, 4.94%, 0.93%, 0.59%, 7.46% and 81.34% in CYP enzymes mediated metabolism of methiocarb, respectively (Table **4**).

Methiocarb sulfoxide showed 36.86%, 10.85%, 9.15%, 10.25%, 2.27%, 4.16%, 3.37%, 20.66% and 5.51% metabolism *in vitro* using HLM, rh CYP enzymes (1A2, 2C8, 2C9, 2C19, 2D6 and 3A4), HLC and HLMit, respectively (Table **3a**). The contribution of CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 was 21.99%, 22.61%, 38.22%, 1.61%, 1.56% and 14.01%, respectively, in CYP enzymes mediated metabolism of methiocarb sulfoxide, respectively (Table **4**).

Methiocarb sulfone showed *in vitro* metabolism of 54.62%, 67.56% and 33.06% in HLM, HLC and HLMit, respectively (Table **3a**).

3.2.3. Albendazole and Metabolites

Albendazole showed *in vitro* metabolism of 50.06%, 98.92%, 2.43%, 5.96%, 95.76%, 64.92% and 1.25% in HLM and rhCYP enzymes (1A2, 2C8, 2C9, 2C19, 2D6 and 3A4), respectively (Table **3a**). The contribution of CYP1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 was 74.79%, 0.50%, 1.87%,

19.06%, 3.33% and 0.44%, respectively, in CYP enzymes mediated metabolism of albendazole (Table **4**).

Albendazole sulfoxide showed *in vitro* metabolism of 4.23% and 0.2% in HLM and HLC, respectively (Table **3a**).

Albendazole sulfone showed *in vitro* metabolism of 24.28%, 18.46%, 11.43%, 3.21%, 0.99% and 11.13% in HLM and rhCYP enzymes (1A2, 2C8 and 3A4), respectively (Table **3a**). The contribution of CYP1A2, 2C8 and 3A4 was 48.23%, 35.31% and 16.46%, respectively, in CYP enzymes mediated metabolism of albendazole sulfone. (Table **4**).

3.2.4. Triclabendazole and Metabolites

Triclabendazole showed *in vitro* metabolism of 51.68%, 97.14%, 55.00%, 94.55%, 73.66%, 11.70% and 12.40% in HLM, rhCYP enzymes (1A2, 2C9, 2C19, 2D6, 3A4) and HLC, respectively (Table **3a**). The contribution of CYP1A2, 2C9, 2C19, 2D6 and 3A4 was 53.74%, 22.29%, 16.07% and 3.88% , respectively, in CYP enzymes mediated metabolism of triclabendazole (Table **4**).

Triclabendazole sulfoxide showed *in vitro* metabolism of 18.08%, 20.46%, 83.19%, 63.84%, 3.68% and 4.30% in HLM, rHCYP enzymes (1A2, 2C9, 2C19, 2D6 and 3A4), respectively (Table **3a**). The contribution of CYP1A2, 2C9, 2C19, 2D6 and 3A4 was 5.73%, 82.43%, 9.31%, 0.18% and 2.35%, respectively, in CYP enzymes mediated metabolism of triclabendazole sulfoxide (Table **4**).

Triclabendazole sulfone showed *in vitro* metabolism of 52.20%, 0.40%, 98.31%, 3.53% and 9.25% in HLM, rHCYP enzymes (1A2, 2C9, 2C19, and 3A4), respectively (Table **3a**). The contribution of CYP1A2, 2C9, 2C19 and 3A4 was 0.05%, 97.11%, 0.17% and 2.67%, respectively, in CYP enzymes mediated metabolism of triclabendazole sulfone (Table **4**).

3.2.5. Montelukast and Metabolites

Montelukast showed *in vitro* metabolism of 25.71%, 9.37%, 52.29%, 10.85%, 7.46%, 40.25% and 10.13% in HLM, rhCYP enzymes (1A2, 2C8, 2C9, 2D6, 3A4) and HLC, respectively (Table **3a**). The contribution of CYP1A2, 2C8, 2C9, 2D6 and 3A4 was 4.21%, 39.00%, 9.08%, 0.64 and 47.07%, respectively, in CYP enzymes mediated metabolism of montelukast (Table **4**).

Montelukast sulfoxide showed *in vitro* 18.50%, 0.57%, 32.54%, 37.31 and 9.87% in HLM, rhCYP enzymes (1A2, 2C19, 2D6) and HLC, respectively (Table **3a**). The contribution of CYP2C9, CYP2C19 and CYP2D6 was 4.32%, 58.90%, and 36.78%, respectively, in CYP enzymes mediated metabolism of montelukast sulfoxide (Table **4**).

Table 3a. Summary of metabolic stability of parents and their sulfoxide and sulfone metabolites.

Compounds	% Metabolized at 30 min Incubation with Human Liver Fractions/Enzymes								
	HLM	rCYP1A2	rCYP2C8	rCYP2C9	rCYP2C19	rCYP2D6	rCYP3A4	Cytosol	Mitochondrial Preparation
Aldicarb	13.41	0.00	13.26	0.00	6.93	0.00	0.00	21.08	10.94
Aldicarb Sulfoxide	21.18	0.00	0.00	0.00	0.85	0.00	5.91	0.00	5.58
Aldicarb Sulfone*	1.05	-	-	-	-	-	-	0.00	6.43
Methiocarb	78.39	46.94	41.44	6.50	19.42	99.43	98.38	6.93	1.83
Methiocarb Sulfoxide	36.86	10.85	9.15	10.25	2.27	4.16	3.37	20.66	5.51
Methiocarb Sulfone#	54.62	-	-	-	-	-	-	67.56	33.06
Albendazole	50.06	98.92	2.43	5.96	95.76	64.92	1.25	0.00	0.00
Albendazole Sulfoxide*	4.23	-	-	-	-	-	-	0.20	0.00
Albendazole Sulfone	24.28	18.46	11.43	0.00	0.00	0.00	3.21	0.99	11.13
Triclabendazole	51.68	97.14	0.00	55.00	94.55	73.66	11.70	12.40	0.00
Triclabendazole Sulfoxide	18.08	20.46	0.00	83.19	63.84	3.68	4.30	0.00	0.00
Triclabendazole Sulfone	52.20	0.40	0.00	98.31	3.53	0.00	9.25	0.00	0.00
Montelukast	25.71	9.37	52.29	10.85	0.00	7.46	40.25	10.13	0.00
Montelukast Sulfoxide	18.50	0.00	0.00	0.57	32.54	37.31	0.00	9.87	0.00
Montelukast Sulfone	72.10	0.00	7.02	0.00	0.00	0.00	17.17	32.35	0.00
Ziprasidone	83.11	5.98	22.84	0.00	21.17	3.73	76.47	10.86	0.00
Ziprasidone Sulfone*	0.00	-	-	-	-	-	-	39.22	29.52

(-): Not applicable,
*: CYP reaction phenotyping with recombinant human CYP enzymes was not performed because of very low/ nil metabolism in human liver microsomes.
#: Compound could not be extracted from matrix , might be due to strong binding with matrix proteins, further investigation was not performed.

Table 3b. Summary of metabolic stability positive control substrates.

Compounds	% Metabolized at 30 min Incubation with Human liver fractions/ enzymes								
	HLM	rCYP1A2	rCYP2C8	rCYP2C9	rCYP2C19	rCYP2D6	rCYP3A[1]	Cytosol	Mitochondrial Preparation
Phenacetin	-	91.43	-	-	-	-	-	-	-
Paclitaxel	-	-	11.21	-	-	-	-	-	-
Diclofenac	-	-	-	98.61	-	-	-	-	-
S-Mephenytoin	-	-	-	-	74.18	-	-	-	-
Dextromethorphan	-	-	-	-	-	99.81	-	-	-
Testosterone	90.74	-	-	-	-	-	85.13	-	-
Phthalazine	-	-	-	-	-	-	-	90.89	-
Methotrexate	-	-	-	-	-	-	-	3.18	-
Serotonin	-	-	-	-	-	-	-	-	86.99
Tryptamine	-	-	-	-	-	-	-	-	>99.99
Clozapine	25.19	-	-	-	-	-	-	-	-
Benzydamine	16.45	-	-	-	-	-	-	-	-
Imipramine	20.47	-	-	-	-	-	-	-	-

CYP: Cytochrome P450, 1: Testosterone was used as substrate, (-): Not applicable.

Table 4. Summary of contribution of CYP enzymes (fmCYP) in metabolism of parents and their sulfoxide and sulfone metabolites.

Compound	CYP 450 Enzymes Responsible for Metabolism	rCYP Clint (µL/min/pmol)	Predicted Microsomal Clint (µL/min/mg Protein)	% Contribution of CYP Enzymes (fmCYP) in Metabolism
Aldicarb	CYP2C8	0.047	3.035	56.91
	CYP2C9	0.024	2.298	43.09
Aldicarb Sulfoxide	CYP2C19	0.003	0.054	2.34
	CYP3A4	0.020	2.254	97.66
Methiocarb	CYP1A2	0.211	10.987	4.75
	CYP2C8	0.178	11.416	4.94
	CYP2C9	0.022	2.151	0.93
	CYP2C19	0.072	1.367	0.59
	CYP2D6	1.724	17.239	7.46
	CYP3A4	1.694	188.076	81.34
Methiocarb Sulfoxide	CYP1A2	0.038	1.991	21.99
	CYP2C8	0.032	2.047	22.61
	CYP2C9	0.036	3.460	38.22
	CYP2C19	0.008	0.145	1.61
	CYP2D6	0.014	0.142	1.56
	CYP3A4	0.011	1.268	14.01

(Table 4) Contd..

Compound	CYP 450 Enzymes Responsible for Metabolism	rCYP Clint (µL/min/pmol)	Predicted Microsomal Clint (µL/min/mg Protein)	% Contribution of CYP Enzymes (fmCYP) in Metabolism
Albendazole	CYP1A2	1.510	78.510	74.79
	CYP2C8	0.008	0.525	0.50
	CYP2C9	0.020	1.966	1.87
	CYP2C19	1.053	20.011	19.06
	CYP2D6	0.349	3.491	3.33
	CYP3A4	0.004	0.465	0.44
Albendazole Sulfone	CYP1A2	0.068	3.537	48.23
	CYP2C8	0.040	2.589	35.31
	CYP3A4	0.011	1.207	16.46
Triclabendazole	CYP1A2	1.185	61.600	53.74
	CYP2C9	0.266	25.548	22.29
	CYP2C19	0.969	18.415	16.07
	CYP2D6	0.445	4.448	3.88
	CYP3A4	0.041	4.604	4.02
Triclabendazole Sulfoxide	CYP1A2	0.076	3.968	5.73
	CYP2C9	0.594	57.057	82.43
	CYP2C19	0.339	6.442	9.31
	CYP2D6	0.012	0.125	0.18
	CYP3A4	0.015	1.626	2.35
Triclabendazole Sulfone	CYP1A2	0.001	0.069	0.05
	CYP2C9	1.359	130.447	97.11
	CYP2C19	0.012	0.228	0.17
	CYP3A4	0.032	3.591	2.67
Montelukast	CYP1A2	0.033	1.705	4.21
	CYP2C8	0.247	15.789	39.00
	CYP2C9	0.038	3.675	9.08
	CYP2D6	0.026	0.258	0.64
	CYP3A4	0.172	19.054	47.07
Montelukast Sulfoxide	CYP2C9	0.002	0.183	4.32
	CYP2C19	0.131	2.493	58.90
	CYP2D6	0.156	1.557	36.78
Montelukast Sulfone	CYP2C8	0.024	1.553	18.22
	CYP3A4	0.063	6.970	81.78
Ziprasidone	CYP1A2	0.021	1.069	1.73
	CYP2C8	0.086	5.532	8.96
	CYP2C19	0.079	1.507	2.44
	CYP2D6	0.013	0.127	0.21
	CYP3A4	0.482	53.530	86.67

Montelukast sulfone showed *in vitro* metabolism of 72.10%, 7.02%, 17.17% and 32.35% in HLM, rhCYP enzymes (2C8 and 3A4) and HLC, respectively (Table **3a**). The contribution of CYP2C8 and 3A4 was 18.22% and 81.78% , respectively, in CYP enzymes mediated metabolism of montelukast sulfone (Table **4**).

3.2.6. Ziprasidone and Metabolite

Ziprasidone showed *in vitro* metabolism of 83.11%, 5.98%, 22.84%, 21.17%, 3.73%, 76.47% and 10.86% in HLM, rhCYP enzymes (1A2, 2C8, 2C19, 2D6, 3A4) and HLC, respectively (Table **3a**). The contribution of CYP1A2, 2C8, 2C19, 2D6 and 3A4 was 1.73%, 8.96%, 2.44%, 0.21% and 86.67%, respectively, in CYP enzymes mediated metabolism of ziprasidone (Table **4**).

Ziprasidone sulfone showed *in vitro* metabolism of 39.22% and 29.52% in HLC and HLMit, respectively (Table **3a**).

All positive controls substrate data are presented in Table **3b** which illustrate the validity of the test systems.

4. DISCUSSION

Previously we have executed *in vitro* experiments to unambiguously evaluate the role of N-oxide metabolites of diversified chemical structures such as clozapine, levofloxacin, roflumilast, voriconazole and zopiclone for the potential CYP inhibition [19]. Accordingly, it was summarized that certain N-oxide metabolites such as clozapine-N-oxide and voriconazole-N-oxide may potentially play a role as a perpetrator drug despite having a higher potency (> 1 μM but < 10 μM) for certain CYP enzymes because the N-oxide metabolites are long-lived and therefore, may potentially accumulate after multiple doses in patients who may further manifest impaired clearance of such N-oxide metabolite(s) [19].

In lieu of the gaining importance of the assessment of any risk posed by metabolite(s), we designed experiments with the following objectives: (a) to evaluate the potential of select parent drugs and corresponding sulfoxide/sulfone metabolites on the inhibition of several CYP enzymes that play an important role in clinical DDI using Human Liver Microsomes (HLM), which contains cytochromes P450 (CYP), Flavin Monooxygenases (FMO) and Uridine glucuronide Transferase (UGT) enzymes predominately); (b) to assess biotransformation of parent drugs, sulfoxide and sulfone metabolites using human liver microsomes, recombinant human CYP (rhCYP) enzymes, Human Liver Cytosol (HLC) (to investigate whether Aldehyde Oxidase (AO), a molybdenum-containing enzyme is involved in the metabolism of tested compounds and human liver mitochondria (HLMit) (to investigate whether monoamine oxidase (MAO), is involved in the metabolism of the selected drug substrates).

In the present work, we have performed systematic tiered experiments, to evaluate the potential of sulfoxide/sulfone metabolites of various drugs to inhibit CYP enzymes of interest that are implicated in clinical drug-drug interactions. Our report assumes particular significance since such an endeavour has not been performed hitherto and given the current thinking around the possible role of circulatory metabolite(s), it is timely that the CYP liability potential of certain sulfoxide/sulfone metabolites be clarified. In order to check the quantitative aspects of the reported CYP inhibition data in the present work including the generated IC50 values, we have tabulated the available CYP inhibition literature data for the parent drugs/metabolite(s) in relation to the corresponding data from our study (Supplementary Table **2a**). Examination of the Supplementary Table **2a** suggested that our reported values were generally in the proximity of the literature reported values, wherever such comparisons were possible. Likewise, we found that enzymes responsible for metabolism for the chosen substrates in the present work was generally qualitatively similar to those reported in the literature (Supplemental Table **2b**).

One of the questions introspected during the planning of this work was pertaining to the real utility of such an exercise; which was rationalized as follows: Firstly, the recent work of Strickland *et al.* has suggested the usefulness of monitoring sulfoxide metabolite in the urine samples to measure the adherence of mental health of patients undergoing therapy with quetiapine. The inclusion of quetiapine sulfoxide measurement in urine along with other analytes improved the adherence from 31% to 48%. Therefore, quetiapine sulfoxide due to its longer residence and larger systemic availability may provide an opportunity for a possible drug-drug interaction [21]. Secondly, in the case of triclabendazole sulfoxide, it was noted that the circulatory concentrations of the metabolite (peak concentration: 38.6 uM) far exceeded the observed concentration of the parent drug triclabendazole (peak concentration: 1.16 uM) following a standard dose of triclabendazole of 10 mg/kg in humans to treat trematode infestations such as Fascioliasis and Paragonimiasis [22]. Therefore, given such a high concentration triclabendazole sulfoxide, it will not only become relevant for CYP liability potential but was demonstrated to exhibit a very strong teratogenic potential *in vitro* during the organogenesis period [22]. Thirdly, the major metabolite sutezolid sulfoxide was believed to have a higher propensity of bactericidal activities against *Mycobacterium spp*. In comparison to sutezolid as judged by the *ex vivo* study performed in cultures of pulmonary tuberculosis patients, the PK-PD modelling of the data suggested that sutezolid metabolite may be relevant in different Mycobacterial sub-populations supporting the combination of the two analytes to cover a broad range *Mycobacterium spp*. [23]. The metabolite to parent (*i.e*., sutezolid sulfoxide/ sutezolid) median concentration ratio was 7.1 suggesting higher circulatory levels of the sulfoxide metabolite [23]. The enhanced circulatory sulfoxide metabolite may carry risks associated with a possible drug-drug interaction in the patient population who are on other co-medications for the management of co-morbidity associated with tuberculosis. Fourthly, with respect to albendazole, the formation of albendazole sulfoxide metabolite not only enhances its anthelmintic activity but also exposes the drug for possible clinical drug-drug interaction. For instance, transport inhibitors such as verapamil, quinidine and ivermectin increased the AUC of the albendazole sulfoxide by >50% [24] and CYP3A4 inhibitor such as grapefruit juice increased the exposure of albendazole by 3.2-fold [25].

In the present work, out of the tested parent drugs and sulfoxide/sulfone metabolites showing structural diversity,

all CYP enzymes with the exception of CYP2D6 were inhibited by the chosen substrates (Table **2a** and **b**). CYP1A2 was inhibited very potently by methiocarb and triclabendazole; strongly by triclabendazole sulfoxide; and modestly by ziprasidone sulfone. CYP2C8 was inhibited very potently by montelukast, montelukast sulfoxide, montelukast sulfone, triclabendazole sulfone; strongly by tribendazole and moderately by triclabendazole sulfoxide. CYP2C9 was inhibited very potently by triclabendazole sulfone, montelukast and triclabendazole; strongly by triclabendazole sulfoxide, montelukast sulfoxide, and montelukast sulfone. CYP2C19 was very potently inhibited by triclabendazole and triclabendazole sulfoxide. CYP3A4 was inhibited moderately by montelukast sulfoxide and modestly by triclabendazole.

4.1. Methiocarb

Being a potent inhibitor of acetylcholinesterase activity, methiocarb can cause excessive cholinergic stimulation resulting in acute toxicity including oxidative damage to kidneys [26, 27]. Because methiocarb is used as a pesticide and insecticide, accidental exposure to humans through skin and/or inhalation is possible. The parent methiocarb showed potent inhibition of CYP1A2 (Fig **3**) (IC_{50}: 0.71 μM) suggesting a possibility for clinically meaningful drug-drug interaction potential with CYP1A2 substrates. In light of the newly es-tablished metabolic pathway in the human/rat liver micro-somes of interconversion between methiocarb and methio-carb sulfoxide metabolites [28], potent CYP1A2 inhibition may pose a safety risk if any accidental exposure to methio-carb occurs in addition to its pharmacological activity of the blockage of acetylcholinesterase [27].

4.2. Triclabendazole and Sulfoxide/Sulfone Metabolites

The clinical pharmacokinetics of triclabendazole and its two metabolites have been adequately defined in human patients at therapeutic doses [29]. The higher circulatory levels of the sulfoxide metabolite have been attributed to the first pass metabolism of triclabendazole in humans. The C_{max} values for triclabendazole, sulfoxide and sulfone metabolites were 1.16, 38.6 and 2.29 uM, respectively. Therefore, in relation to the observed C_{max} values, the IC_{50} values for the inhibition of various CYP enzymes were several fold higher; therefore, there is a potential for clinical drug-drug interaction for drugs whose metabolism is governed by CYP1A2,

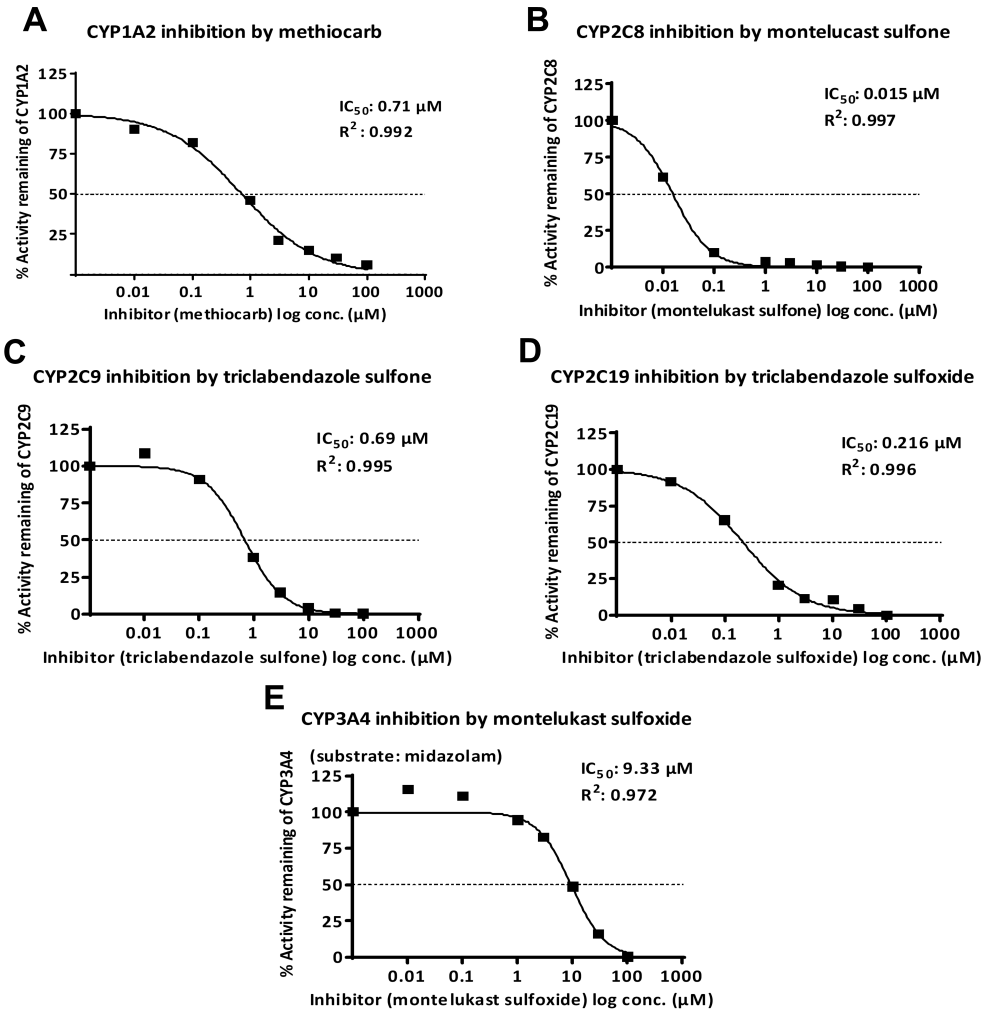

Fig. (3). Representative inhibition curves obtained using selective CYP isoform probe substrates. (**A**) Inhibition of acetaminophen by methiocarb; (**B**) Inhibition 6'-OH paclitaxel by montelukast sulfone; (**C**) inhibition of 4' OH- diclofenac by triclabendazole sulfone; (**D**) inhibition of 4' OH- S- mephenytoin by triclabendazole sulfoxide; (**E**) inhibition of 1-OH-midazolam by montelukast sulfoxide. [CYP: Cytochrome P450, IC_{50}: inhibitor concentration causing 50% inhibition of the enzyme activity].

2C8, 2C9 and 2C19, when human subjects are dosed triclabendazole. Because both sulfoxide and sulfone metabolites have a half-life value of approximately 11-12 hours, there may be an opportunity of accumulation after repeated dosing in humans and hence, even after the triclabendazole is tapered off one should be vigilant of potential drug-drug interaction for the above mentioned CYP enzymes. While the potential to inhibit CYP enzymes by triclabendazole and metabolites has been confirmed for the first time, previously Barrera *et al.* have reported that both sulfoxide and sulfone metabolites are involved in the inhibition of the Abcg2/ABCG2-mediated transport of danofloxacin and nitrofurantoin, antibacterial drugs. It was also suggested that these metabolites may have the potential to participate in drug-drug interactions mediated by transporters [30].

4.3. Montelukast and Sulfoxide/Sulfone Metabolites

The observed potent inhibition of CYP2C8 by montelukast in our study was comparable to the reported literature values [31, 32]. We also found that montelukast was a strong inhibitor of CYP2C9 in adherence to the multiple pathways involved in the metabolism of montelukast involving both CYP enzymes and glucuronosyl transferases [33]. To the best of our knowledge, this is the first report showing potent to strong inhibition of both CYP2C8 and CYP2C9 by the two metabolites of montelukast, which is of particular importance in clinical therapy. In this regard, montelukast sulfone showed potent CYP2C8 inhibition relative to the parent or montelukast sulfoxide metabolite. There has been no pharmacokinetic data published on either sulfoxide or sulfone metabolite of montelukast to judge the clinical impact of the observed CYP2C8 or CYP2C9 inhibition. However, earlier work on the metabolic profiling in humans has suggested the presence of sulfoxide as a minor metabolite along with numerous other metabolites and the circulatory plasma concentrations of such metabolites were <2% of the plasma radioactivity [34]. Since the two metabolites exhibited high *in vitro* potency for the CYP inhibition (Table **2a** and **2b**), there appears to be a slight chance that sulfoxide/sulfone metabolites of montelukast may be relevant in the observed clinical drug-drug interaction of montelukast.

4.4. Ziprasidone Sulfone

Our data suggested that ziprasidone had negligible impact on the inhibition of various CYPs examined in the panel which was in agreement with the reported value [35]. However, ziprasidone sulfone metabolite showed modest inhibition of only CYP1A2 (Table **2a** and **2b**). Because ziprasidone undergoes extensive metabolism with sulfone/sulfoxide regarded as major metabolites [35], one needs to consider the risk potential, if any, of the observed CYP1A2 inhibition in clinical therapy. Due to the unavailability of ziprasidone sulfoxide, the CYP inhibitory experiment with this metabolite was not performed.

The experiments carried out to understand the *in vitro* reaction phenotyping of the sulfoxide/sulfone metabolites of various drugs suggested the importance of CYP related pathway in further disposition of the metabolites. In the limited studied examples, it was noted that the propensity of the contribution appeared to be distributed mainly between CYP2C9 and CYP3A4 accounting for approximately 60% of the studied substrates, as illustrated in Fig. (**4**). Hence, inhibition of these two specific CYP enzymes may need to be considered in the relevant patient pool that are co-administered with such drugs for the assessment of clinical drug-drug interaction risk.

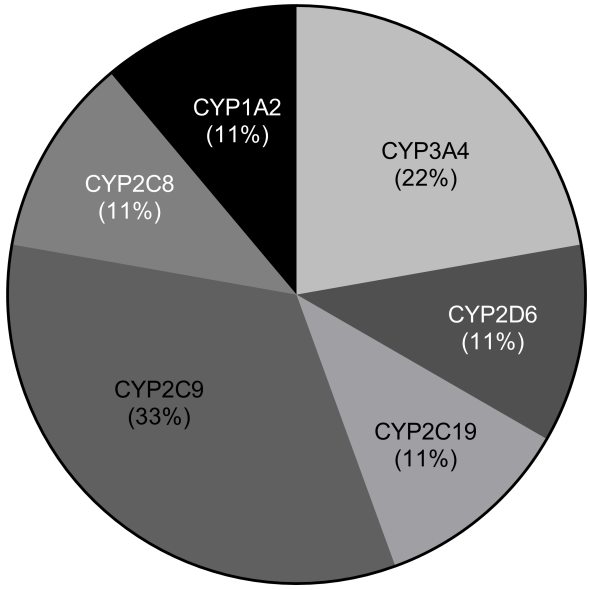

Fig. (4). Percentage of CYP isoforms involved in metabolism of sulfoxide and/or sulfone metabolites of aldicarb, methiocarb, albendazole, triclabendazole, montelukast and ziprasidone. CYP isoforms, which showed greater than 30% contribution in CYP mediated metabolism of metabolites was considered for this compilation. Test compounds metabolized by each CYP isoforms were counted and percentage calculated for representation in the pie distribution chart.

A dilemma in carrying out our CYP inhibition work, which is largely applicable for similar CYP inhibition work carried out elsewhere, needs to be pointed out. There may be a possibility of a physical interaction between the probe(s) *vs*. test (inhibitor) drug(s) and also, the test drug(s) may be a substrate for the specific CYP enzyme assessed for inhibitory potential. However, despite such limitations, IC_{50} values were generated and reported.

CONCLUSION

The research work evaluated the potential for CYP enzyme inhibition and *in vitro* metabolic fate of select examples of the parent drug with respective sulfoxide/sulfone metabolites. The chosen drugs were albendazole, triclabendazole, aldicarb, methiocarb, montelukast and ziprasidone. With the exception of CYP2D6, other CYP enzymes were modestly to potently inhibited by one or more of the chosen substrates. CYP1A2 was inhibited very potently by methiocarb (IC_{50}: 0.71 μM) and triclabendazole (IC_{50}: 1.07 μM); strongly by triclabendazole sulfoxide (IC_{50}: 4.19 μM); and modestly by ziprasidone sulfone (IC_{50}: 17.14 μM). CYP2C8 was inhibited very potently by montelukast (IC_{50}: 0.08 μM), montelukast sulfoxide (IC_{50}: 0.05 μM), montelukast sulfone (IC_{50}: 0.02 μM), triclabendazole sulfone (IC_{50}: 1.05 μM);

strongly by triclabendazole (IC_{50}: 3.31 μM) and moderately by triclabendazole sulfoxide (IC_{50}: 8.95 μM). CYP2C9 was inhibited very potently by triclabendazole sulfone (IC_{50}: 0.69 μM), montelukast (IC_{50}: 1.34 μM) and triclabendazole (IC_{50}: 1.17 μM); strongly by triclabendazole sulfoxide (IC_{50}: 1.95 μM), montelukast sulfoxide (IC_{50}: 3.61 μM), and montelukast sulfone (IC_{50}: 2.15 μM). CYP2C19 was very potently inhibited by triclabendazole (IC_{50}: 0.25 μM) and triclabendazole sulfoxide (IC_{50}: 0.22 μM). CYP3A4 was inhibited moderately by montelukast sulfoxide (IC_{50}: 9.33 μM) and triclabendazole (IC_{50}: 15.11 μM). The CYP reaction phenotyping work revealed the propensity of CY2C9 and CYP3A4 enzymes in the metabolic fate for a proportionally higher number of sulfoxide/sulfone substrates considered in this evaluation. In summary, several sulfoxide and/or sulfone metabolites of the investigated drugs showed dual role of both being a perpetrator drug (based on IC_{50} values for CYP inhibition) and/or victim drug (based on CYP reaction phenotyping). Hence, there is a need to consider proper risk assessment strategy to possibly overcome potential clinical drug-drug interaction when sulfoxide/sulfone metabolite(s) generating drugs are coadministered in therapy.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's web site along with the published article.

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