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 IC₅₀ 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 sulfone 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 CY3A4 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.

Keywords: CYP inhibition potential, drug-drug interaction, Phase I enzymes, LC-MS-MS method, metabolic fate, metabolism.

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.
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 quinoline-imine metabolite of acetaminophen and trifluoracety 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-desalkylitraconazole (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 IC50 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: Hydroxy-warfarin, 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, Canada. Testosterone propionate was purchased from Ipca Laboratories Ltd., Mumbai, India. Rosiglitazone maleate and ketoconazole were purchased from Piramal Healthcare Ltd., Mumbai, India. Hydroxybupropion and 6a-hydroxydiclofenac were purchased from GRK Research Laboratories Pvt. Ltd., Hyderabad, Telangana, India. 6j-hydroxytestosterone was purchased from Elina Biosciences LLC., Wynnewood, PA, USA. 1-Hydroxymidazolam was purchased from SPI-Bio, (Montigny le Bretonneux, France). Midazolam hydrochloride, ziprasidone sulphone were purchased from Clearsynth, Mumbai, India. Reduced β-nicotinamide adenosine dinucleotide phosphate tetrasodium salt (NADPH) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Ketoconazole, S-mephentanyl, 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. Reconstituent 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.
In Vitro Drug-Drug Interaction Potential of Sulfoxide and/or Sulfone Metabolites

Fig. (1). Structures of parent drugs and sulfoxide/sulfone metabolites of methiocarb, aldicarb, montelukast, ziprasidone, albendazole, and triclabendazole.
2.2. CYP 450 Interaction Studies

All sulfoxide and sulfone metabolites of six drugs namely, albendazole, triclabendazole, aldicarb, methiocarb, montelukast and ziprasidone 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 inhibition in human liver microsomes at 2 and 10 µM.

 Tier 1

In vitro CYP1A2, 2C8, 2C9, 2C19, 2D6 & 3A4 inhibition in human liver microsomes at 2 and 10 µM

≥40% CYP inhibition at 10 µM: IC_{50} determination

 Tier 2

In vitro incubations with human liver microsomes for phase 1 metabolism

If >10% metabolised, then incubations with rhCYP (1A2, 2C8, 2C9, 2C19, 2D6 and 3A4) for CYPs mediated metabolism

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 were diluted with PBS pH 7.4 for anticipated protein concentration (Table 1) and CYP isoform-specific substrate was added (Table 1). For each isoyme, 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.

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, IC_{50}: inhibitor concentration causing 50% inhibition of the enzyme activity].

[CYP: Cytochrome P450, IC_{50}: inhibitor concentration causing 50% inhibition of the enzyme activity].
(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 by 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). IC50 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 Micromones

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, Alaidcarb, 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 parent sulfoxide and sulfone metabolites Tier 4 were assessed concurrently.

Serotonin and tryptamine were used as positive controls (substrate used: testosterone). Aldicarb sulfoxide showed inhibitory potential against CYP1A2, 2C8, 2C9, 2C19 and 3A4 with IC₅₀ 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 IC₅₀ 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 IC₅₀ values of 1.05 and 0.69 µM, respectively. Montelukast showed inhibitory potential against 2C8, 2C9 and 3A4 with IC₅₀ 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 IC₅₀ 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 IC₅₀ values 0.02 and 2.15 µM, respectively. Ziprasidone sulfone showed inhibition for CYP1A2 with IC₅₀ 17.14 µM.

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 IC₅₀ 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 IC₅₀ was found to be 0.71 µM for methiocarb. Triclabendazole showed inhibitory potential against CYP isoforms 1A2, 2C8, 2C9, 2C19 and 3A4 with IC₅₀ 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 IC₅₀ 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 IC₅₀ values of 1.05 and 0.69 µM, respectively. Montelukast showed inhibitory potential against 2C8, 2C9 and 3A4 with IC₅₀ 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 IC₅₀ 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 IC₅₀ values 0.02 and 2.15 µM, respectively. Ziprasidone sulfone showed inhibition for CYP1A2 with IC₅₀ 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<sup>1</sup> | CYP3A4<sup>2</sup> |
| 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     | 2.69    | 1.83   | NI     | 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      | 5.73   | NI     | 9.10   |
|            | 10 µM                      | 7.66   | NI     | 4.82   | NI      | NI     | 9.10   | NI     |

(Table 2a) Contd...
### Table 2b. CYP IC₅₀ data of parents and their sulfoxide and sulfone metabolites.

| Compound                          | CYP Isoform | IC₅₀ (µM) |
|-----------------------------------|-------------|-----------|
| Methiocarb                        | 1A2         | 0.71      |
| Triclabendazole                   | 1A2         | 1.07      |
|                                  |             | 2C8       | 3.31      |
|                                  |             | 2C9       | 1.17      |
|                                  |             | 2C19      | 0.25      |
|                                  |             | 3A4¹      | 15.11     |
| Triclabendazole sulfoxide         | 1A2         | 4.19      |
|                                  |             | 2C8       | 8.95      |
|                                  |             | 2C9       | 1.95      |
|                                  |             | 2C19      | 0.22      |

1: substrate, testosterone; 2: substrate, midazolam; NI: No Inhibition, NA: Not Applicable.
| Compound                        | CYP Isoform | IC<sub>50</sub> (µM) |
|--------------------------------|-------------|----------------------|
| Triclabendazole sulfone        | 2C8, 2C9    | 1.05, 0.69           |
| Montelukast                    | 2C8, 2C9    | 0.08, 1.34           |
| Montelukast sulfoxide          | 2C8, 2C9    | 0.05, 3.61, 9.33     |
| Montelukast sulfone            | 2C8, 2C9    | 0.02, 2.15           |
| Ziprasidone sulfone            | 1A2         | 17.14                |

CYP: Cytochrome P450, IC<sub>50</sub>: 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<sup>1</sup> | CYP3A4<sup>2</sup> |
|-------------------|--------|--------|--------|---------|--------|---------------------|---------------------|
| Concentration tested | 20 nM  | 25 µM  | 1 µM   | 2 µM    | 100 nM | 50 nM               | 50 nM               |
| α-Nepthoflavone   | 62.43  | -      | -      | -       | -      | -                   | -                   |
| Ticlopidine       | -      | -      | -      | 83.87   | -      | -                   | -                   |
| Rosiglitazone     | -      | 69.23  | -      | -       | -      | -                   | -                   |
| Sulfaphenazole    | -      | -      | -      | 71.03   | -      | -                   | -                   |
| Ticlopidine       | -      | -      | -      | -       | -      | 67.43               | -                   |
| Quinidine         | -      | -      | -      | -       | -      | -                   | 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

Methiocarb 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, 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 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%, 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, 2C9, 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 | Cytoisol | Mitochondrial Preparation |
|---------------------------|-----------------------------------------------------------------------|---------|---------------------------|
|                           | HLM         | rCYP1A2   | rCYP2C8    | rCYP2C9    | rCYP2C19   | rCYP2D6   | rCYP3A4   |                 |
| 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 | rCYP3A1 | Cytosol | Mitochondrial Preparation |
| Phenacetin        |    |  -     | 91.43   |  -      |  -      |  -      |  -      |  -      |  -                        |
| Paclitaxel        |    |  -     | 11.21   |  -      |  -      |  -      |  -      |  -      |  -                        |
| Diclofenac        |    |  -     |  -      | 98.61   |  -      |  -      |  -      |  -      |  -                        |
| S-Mephenytoin     |    |  -     |  -      |  -      | 74.18   |  -      |  -      |  -      |  -                        |
| Dextromethorphan  |    |  -     |  -      |  -      | 99.81   |  -      |  -      |  -      |  -                        |
| Testosterone      |    | 90.74  |  -      |  -      |  -      | 85.13   | 90.89   |  -      |  -                        |
| Phthalazine       |    |  -     |  -      |  -      |  -      |  -      |  -      |  -      |  -                        |
| Methotrexate      |    |  -     |  -      |  -      |  -      |  -      | 3.18    |  -      |  -                        |
| Serotonin         |    |  -     |  -      |  -      |  -      |  -      |  -      |  -      |  -                        |
| Tryptamine        |    |  -     |  -      |  -      |  -      |  -      |  -      |  -      | >99.99                    |
| Clozapine         |    | 25.19  |  -      |  -      |  -      |  -      |  -      |  -      |  -                        |
| Benzodammine      |    | 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 (nmCYP) 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 Sulfone** | 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 Sulfoxide** | 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 3b).

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 Monoxygenases (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 organizogenesis period [22]. Thirdly, the major metabolite sutezolid sulfoxide was believed to have a higher propensity of bacterial 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 antihelminthic 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) (IC50: 0.71 μM) suggesting a possibility for clinically meaningful drug-drug interaction potential with CYP1A2 substrates. In light of the newly established 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 blockade 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 Cmax values for triclabendazole, sulfoxide and sulfone metabolites were 1.16, 38.6 and 2.29 μM, respectively. Therefore, in relation to the observed Cmax values, the IC50 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, IC50: 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 sulfone 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.

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, IC50 values were generated and reported.

CONCLUSION

The research work evaluated the potential for CYP enzyme inhibition and in vitro metabolic fate of select samples of the parent drug with respective sulfoxide/sulfone metabolites. The chosen drugs were albendazole, triclabendazole, aldicarb, methiocarb, albedazone, triclabendazole, metilukast 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.

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.
In Vitro Drug-Drug Interaction Potential of Sulfoxide and/or Sulfone Metabolites

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.

REFERENCES

[1] Ogura, A.; Masa, J.L. Drug interactions due to cytochrome P450. Proc. Bayl Univ. Med. Cent., 2000, 13, 421-423.

[2] Nettleton, D.O.; Einolf, H.J. Assessment of cytochrome p450 enzyme inhibition and inactivation in drug discovery and development. Curr. Top. Med. Chem., 2011, 11, 382-403.

[3] Palleria, C.; Di Paolo, A.; Giofrè, C.; Caglioti, C.; Leuzzi, G.; Siniscalchi, A.; De Sarro, G.; Gallelli, L. Pharmacokinetic drug-drug interaction and their implication in clinical management. J. Res. Med. Sci., 2013, 18, 601-610.

[4] Yu, H.; Tweedie, D. A perspective on the contribution of metabolites to drug-drug interaction potential: the need to consider both circulating levels and inhibition potency. Drug Metab. Dispos., 2013, 41, 536-540.

[5] Ma, S.; Chowdhury, S.K. A tiered approach to address regulatory drug metabolite-related issues in drug development. Bioanalysis., 2014, 6, 587-590.

[6] Zamek-Gliszczynski, M.J.; Chu, X.; Polli, J.W.; Paine, M.F.; Galetin, A. Understanding the Transport Properties of Metabolics: Case Studies and Considerations for Drug Development. Drug Metab. Dispos., 2014, 42, 650-664.

[7] Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications Guidance for Industry https://www.fda.gov/downloads/drugs/guidances/ucm292362.pdf. (Last accessed July 18th, 2018).

[8] Liu, C.; He, X. Role of Drug Metabolism and its Mediated DDI in Drug Efficacy and Safety Part 2. Curr. Drug Metab., 2015, 16, 848-849.

[9] Smith, H.S. Opioid Metabolism. Mayo Clin Proc., 2009, 84, 613-624.

[10] Sprouse, J.; Clarke, T.; Reynolds, L.; Heym, J.; Rollema, H. Comparison of the effects of sertraline and its metabolite desmethylsertraline on blockade of central 5-HT receptor in vivo. Neuropsychopharmacol., 1996, 14, 225-231.

[11] Davies, R.O.; Gomez, H.J.; Irvin, J.D.; Walker, J.F. An overview of the clinical pharmacology of enalapril. Br. J. Clin. Pharmacol., 1984, 18, 215B-229S.

[12] Frey, H.H.; Göbel, W.; Lösch, W. Pharmacokinetics of primidone and its active metabolites in the dog. Arch. Int. Pharmacodyn. Ther., 1979, 212, 14-30.

[13] Potter, W.Z. Active metabolites of tricyclic antidepressants. In: Udsin E., Dahl S.G., Lingjærde O. Ed. Clinical Pharmacology in Psychiatry. Palgrave Macmillan, London, 1981, 139-153.

[14] Eliasson, E.; Gardner, I.; Hume-Smith, H.; de Waziers, I.; Beaune, P.; Kenna, J.G. Intravascular variability in P450-dependent generation of neontigens in halothane hepatitis. Chem. Biol. Inter., 1998, 116, 123-141.

[15] Isoherranen, N.; Kunze, K.L.; Allen, K.E.; Nelson, W.L.; Thummel, K.E. Role of iraconazole metabolites in CYP3A4 inhibition. Drug Metab. Dispos., 2004, 32, 1121-1131.

[16] Obach, R.S. Pharmacologically Active Drug Metabolites: Impact on Drug Discovery and Pharmacotherapy. Pharmaco Rev., 2013, 65, 578-640.

[17] Yamaori, S.; Takami, K.; Shiozawa, A.; Sakuyama, K.; Matsu-zawa, N.; Ohmori, S. In vitro inhibition of CYP2C9-mediated warfarin 7-hydroxylation by iguratimod: possible mechanism of iguratimod-warfarin interaction. Biol. Pharm. Bull., 2015, 38, 441-447.

[18] Ohyanwa, K.; Nakajima, M.; Suzuki, M.; Shimada, N.; Yamazaki, H.; Yokoi, T. Inhibitory effects of amiodarone and its N-deethylated metabolite on human cytochrome P450 activities: prediction of in vivo drug interactions. Br. J. Clin. Pharmacol., 2000, 49, 244-253.

[19] Giri, P.; Naidu, S.; Patel, N.; Srinivas, N.R. Evaluation of in vitro cytochrome P450 inhibition and in vitro fate of structurally diverse n-oxide metabolites: Case Studies with Clozapine, Levofloxacin, Rosforamil, Viconazole and Zopiclone. Eur. J. Drug Metab. Pharmacokinet., 2017, 42, 677-688.

[20] Khatri, D.K.; Juveka, A.R. Kinetics of Inhibition of Monoamine Oxidase Using Curcumin and Ellagic Acid. Pharmacog. Mag., 2016, 2, S116-120.

[21] Strickland, E.C.; Cummings, O.T.; Morris, A.A.; Clinkscales, A.; McIntire, G.L. Quetiapine Carboxylic Acid and Quetiapine Sulfoxide Prevalence in Patient Urine. J. Anal. Toxicol., 2016, 40, 687-693.

[22] Boix, N.; Teixido, E.; Vila-Cejudo, M.; Ortiz, P.; Ibáñez, E.; Llobet, J.M.; Barenys, M. Triclabendazole sulfone causes stage-dependent embryolethality in zebrafish and mouse in vitro. PLoS One, 2015, 10(3), e0121308.
[23] Zhu, T.; Friedrich, S.O.; Diacon, A.; Wallis, R.S. Population phar-
macamokinetic/pharmacodynamic analysis of the bactericidal activ-
ities of sutezolid (PNU-100480) and its major metabolite against in-
tracellular Mycobacterium tuberculosis in ex vivo whole-blood cul-
tures of patients with pulmonary tuberculosis. Antimicrob. Agents Che-
motherr., 2014, 58, 3306-3311.

[24] Miranda, G.; Molina, A.J.; García, J.L.; Pulido, M.M.; Prieto, J.G.; 
Alvarez, A.I. Intestinal elimination of albendazole sulfoxide: phar-
macamokinetic effects of inhibitors. Int. J. Pharm., 2003, 263, 123-
132.

[25] Nagy, J.; Schipper, H.G.; Koopmans, R.P.; Butter, J.J.; Van Bexel, 
C.J.; Kager, P.A. Effect of grapefruit juice or cimetidine coadmi-
nistration on albendazole bioavailability. Am. J. Trop. Med. Hyg., 
2002, 66, 260-263.

[26] Ozden, S.; Catalgo, B.; Gecen-Okkayoglu, S.; Karatug, A.; 
Bolkent, S.; Alpertunga, B. Acute effects of methiocarb on oxida-
tive damage and the protective effects of vitamin E and taurine in 
the liver and kidney of Wistar rats. Toxicol. Ind. Health., 2013, 29, 
60-71.

[27] Padilla, S.; Marshall, R.S.; Hunter, D.L.; Lowit, A. Time course of 
cholinesterase inhibition in adult rats treated acutely with carbaryl, 
carbofuran, formetanate, methomyl, methiocarb, oxamyl or pro-
proxar. Toxicol. Appl. Pharmacol., 2007, 219, 202-209.

[28] Fujino, C.; Tamura, Y.; Tange, S.; Nakajima, H.; Sanoh, S.; Wata-
nabe, Y.; Uramaru, N.; Kojima, H.; Yoshinari, K.; Ohta, S.; Kita-
mura, S. Metabolism of methiocarb and carbaryl by rat and human 
livers and plasma, and effect on their PXR, CAR and PPAR 
activities. J. Toxicol. Sci., 2016, 41, 677-691.

[29] Lecaillon, J.B.; Godbillon, J.; Campestrini, J.; Naquira, C.; 
Miranda, L.; Pacheco, R.; Mull, R.; Poltera, A.A. Effect of food on 
the bioavailability of triclabendazole in patients with fascioliasis. 
Br. J. Clin. Pharmacol., 1998, 45, 601-604.

[30] Barrera, B.; Otero, J.A.; Egido, E.; Prieto, J.G.; Seelig, A.; Álvarez, 
A.I.; Merino, G. The anthelmintic triclabendazole and its metabol-
ilites inhibit the membrane transporter ABCG2/BCRP. Antimicrob. 
Agents Chemother., 2012, 56, 3535-3543.

[31] Walsky, R.L.; Obach, R.S.; Gaman, E.A.; Gleeson, J.P.; Proctor, 
W.R. Selective inhibition of human cytochrome P4502C8 by mont-
elukast. Drug Metab. Dispos., 2005, 33, 413-418.

[32] Walsky, R.L.; Gaman, E.A.; Obach, R.S. Examination of 209 drugs 
for inhibition of cytochrome P450 2C8. J. Clin. Pharmacol., 2005, 
45, 68-78.

[33] Cardoso Jde, O.; Oliveira, R.V.; Lu, J.B.; Desta, Z. In vitro me-
tabolism of montelukast by cytochrome P450s and UDP-
gluconosyltransferases. Drug Metab. Dispos., 2015, 43, 1905-
16.

[34] Balani, S.K.; Xu, X.; Pratha, V.; Koss, M.A.; Amin, R.D.; Du-
fresne, C.; Miller, R.R.; Arison, B.H.; Doss, G.A.; Chiba, M.; 
Freeman, A.; Holland, S.D.; Schwartz, J.I.; Lasserter, K.C.; Gertz, 
B.J.; Isenberg, J.J.; Rogers, J.D.; Lin, J.H.; Bailie, T.A. Metabolic 
profiles of montelukast sodium (Singulair), a potent cysteiny1 leu-
kotriene1 receptor antagonist, in human plasma and bile. Drug Me-
tab. Dispos., 1997, 25, 1282-1287.

[35] Prakash, C.; Kamel, A.; Cui, D.; Whalen, R.D.; Miceli, J.J.; 
Tweedie, D. Identification of the major human liver cytochrome 
P450 isoform(s) responsible for the formation of the primary me-
tabolites of ziprasidone and prediction of possible drug interac-
tions. Br. J. Clin. Pharmacol., 2000, 49, 355-425.

[36] Prakash, C.; Kamel, A.; Gummerus, J.; Wilner, K. Metabolism and 
excretion of a new antipsychotic drug, ziprasidone, in humans. 
Drug Metab. Dispos., 1997, 25, 863-872.

[37] Srinivas NR. Drug-drug interaction studies in preclinical species: 
should metabolite(s) kinetics be studied? Xenobiotica, 2009, 39, 
193-196.