WCK 4873 (Nafithromycin): Assessment of *in vitro* Human CYP Inhibitory Potential of a Novel Lactone-ketolide

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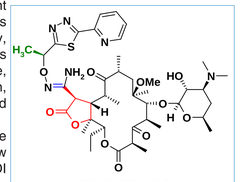
INTRODUCTION

- WCK 4873 (INN: Nafithromycin) is a novel Lactone-ketolide antibiotic developed (Wockhardt, India) as both oral and intravenous formulation for the treatment of Community Acquired Bacterial Pneumonia (CABP). It shows promising antibacterial activity against a wide range of pathogens causing CABP including Telithromycin (TEL) non-susceptible strains of *Streptococcus pneumoniae*[1-3]. It also retains high potency against TEL-resistant *Streptococcus pyogenes*, methicillin-sensitive *Staphylococcus aureus*, *Moraxella catarrhalis* and *Hemophilus influenzae* and atypical RTI pathogens[4-8]. Oral and intravenous Phase I pharmacokinetic studies of Nafithromycin have shown best-in-class pharmacokinetic profile commensurate to once-a-day dosing potential in addition to encouraging tolerability and safety profile as observed in single ascending and multiple ascending dose studies[9-11].
- Many of the members of the macrolide/ketolide class of antibiotics have been widely recognized for their potential to induce clinically relevant drug-drug interactions (DDIs) due to their potent CYP inhibitory activity. These DDIs may cause undesired adverse reactions such as cardiac dysrhythmias, hypoglycemia, nephrotoxicity, neurotoxicity, neutropenia, and rhabdomyolysis or therapeutic failures. For instance, TEL is known to cause cardiac adverse events when co-administered with cisapride, midazolam, triazolam or statins. In contrast, CYP-inducers like rifampin, carbamazepine etc., induce the metabolism of TEL, resulting in its reduced efficacy[12].
- Elderly patients are more often on concomitant medications and are therefore vulnerable to such drug-drug interactions[13]. Drug-interaction studies of a new investigational drugs are essential as they help predicting the clinical DDI potential and thus manage patient safety and drug efficacy. In this study, we examined *in vitro* human CYP inhibition potential of Nafithromycin, including comparator ketolides for all key CYP enzymes recommended by FDA/EMEA.



LACTONE-KETOLIDE (NAFITHROMYCIN)

METHODS

Materials

- Human Liver Microsomes (HLM) were purchased from Xenotech. All CYP substrates, inhibitors and NADPH were purchased from Sigma Aldrich™. Solithromycin (SOL), Cethromycin (CET) and TEL (purity of all compounds >95%) employed in these studies were synthesized in-house and were internally referred to as WCK 4224, WCK 4212 and WCK 4210, respectively.

CYP inhibition assay

- The *in vitro* CYP inhibitory potential of Nafithromycin along with SOL, CET, TEL and reference positive inhibitors were evaluated as per FDA/EMEA guidelines by LC MS/MS method. CYP isoform specific inhibitory effect was evaluated for both test compounds and reference inhibitors. Briefly, CYP isoform-specific marker reactions were performed using pooled HLM (n=200) and appropriate CYP isoform specific probe substrate (substrate concentrations employed around Km): CYP1A2 (Phenacetin O-dealkylation), CYP2B6 (Bupropion hydroxylation), CYP2C8 (Amodiaquine N-Deethylation), CYP2C9 (Diclofenac 4-hydroxylation), CYP2C19 (Mephenytoin 4- hydroxylation), CYP2D6 (Dextromethorphan demethylation), CYP3A4/5 (midazolam 1-hydroxylation), CYP3A4/5 (Testosterone 6-β hydroxylation), CYP3A4/5 (Nifedipine oxidation). All experiments were performed in triplicate.

CYP 3A isoenzyme DI, TDI/MBI potential assay:

- Direct Inhibition (DI), Time Dependent and/or Metabolism Based Inhibition (TDI/MBI) of CYP 3A isoenzyme was further investigated by IC_{50} shift approach. Different concentrations of ketolides and reference positive and negative control were pre-incubated (0.5h) with HLM, and ± NADPH to test CYP3A (Testosterone 6-β hydroxylation and Midazolam 1-hydroxylation) activity. The reactions were initiated by addition of a co-factor NADPH and were terminated by the addition of acetonitrile. The samples were centrifuged at 10000 rpm for 10 min,prior to LC-MS/MS analysis. IC_{50} values were calculated using Graph Pad Prism 6.0.

Table 1. Summary of incubation conditions for CYP450 isoform inhibition assays

CYP450 isoform	Substrate (μM)	HLM (mg/mL)	Reaction Time (min)	Reference Inhibitor
CYP1A2	Phenacetin (50)	0.10	20	α Napthoflavone
CYP2B6	Bupropion HCL (80)	0.05	20	Ticlopidine
CYP2C8	Amodiaquine (2)	0.025	10	Montelukast
CYP2C9	Diclofenac (10)	0.05	20	Sulphafenazole
CYP2C19	S- Mephenytoin (60)	0.20	40	Ticlopidine
CYP2D6	Dextromethorphan (5)	0.10	10	Quinidine
CYP3A4	Testosterone (100)	0.10	10	Ketoconazole
CYP3A4	Midazolam (5)	0.05	5	Ketoconazole
CYP3A4	Nifedipine (25)	0.05	20	Ketoconazole

HLM: Human Liver Microsomes.

RESULTS AND DISCUSSION

- Similar to other macrolides and ketolides, Nafithromycin is primarily metabolized in the liver by CYP450 enzyme, particularly by 3A superfamily. Our studies have shown that Nafithromycin at a higher concentration is a weak inhibitor of CYP3A isoform. The main distinguishing feature of Nafithromycin over other ketolides like SOL, CET and TEL is its weak inhibitory potency towards CYP3A isoform and absence of inhibitory potential towards other key CYP isoforms like 1A2, 2B6, 2C8, 2C9, 2C19 and 2D6 (IC_{50}: >300 μM). Unlike Nafithromycin, SOL and TEL were observed to be moderate inhibitors of another key CYP2D6 isoform **(Table 2 and Figure 1)**.
- Importantly, N-Desmethyl metabolite of Nafithromycin which constitutes about 10% of the parent drug in systemic circulation does not demonstrate increased inhibitory potential unlike the metabolites of other ketolides which have even higher inhibitory potential than their respective parent drugs. Moreover, neither Nafithromycin nor its metabolites showed TDI or MBI- a feature desirable for lower DDI potential whereas metabolites of other macrolides/ ketolide are likely to be associated with higher CYP3A inhibition potential as evidenced by metabolite based inhibition associated with them[14].

Table 2 : Mean CYP inhibitory potential of Nafithromycin and comparators.

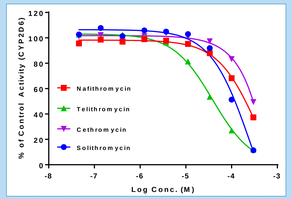
CYP450 iso form	Probe substrate	IC_{50} (μM)				
		Ref. Inhibitor	Nafithromycin	TEL	SOL	CET
CYP1A2	Phenacetin	0.015-0.025	>300	>300	>300	>300
CYP2B6	Bupropion HCL	0.168	224.4	163.9	224	77.8
CYP2C8	Amodiaquine	0.069-0.159	>300	>300	100-200	100-200
CYP2C9	Diclofenac	0.45-0.50	>300	>300	>300	>300
CYP2C19	S- Mephenytoin	1.10-1.50	>300	>300	>300	>300
CYP2D6	Dextromethorphan	0.059-0.074	224	39	95	>300
CYP3A4	Testosterone	0.042	>300	103	5.5	45.7
CYP3A4	Midazolam	0.061	83.8	19.4	6	15.1
CYP3A4	Nifedipine	0.013	70.2	30.3	1.2	14.9

TEL: telithromycin; SOL: solithromycin; CET: cethromycin.

- In IC_{50} shift assays, unlike other ketolides SOL, CET and TEL, no left side shift (lowering) in IC_{50} values was observed for Nafithromycin towards CYP3A isoenzyme using Testosterone and Midazolam substrates, indicating that the metabolites of Nafithromycin do not contribute to additional inhibition liability. Whereas,significant left side shift in IC_{50} (lowering) values was observed for TEL, CET and SOL (IC_{50} shift: 2, 5 and 11 and 11, 22 and 49 times, respectively for the two substrates) suggesting the metabolites of these ketolides are more potent inhibitor of CYP 3A4 than the parent drugs **(Table 3 and Figure 2).**

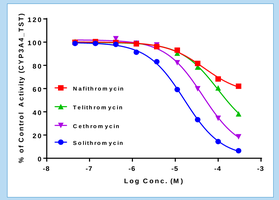
Figure 1 : Mean IC_{50} graphs of Nafithromycin vs comparators for CYP2D6 and CYP3A4

1a: CYP2D6 (Substrate: Dextromethorphan)



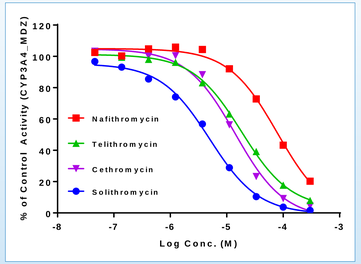
Gradation of ketolides based on CYP 3A inhibition: TEL>SOL>CET = Nafithromycin

1b : CYP3A4 (Substrate : Testosterone)



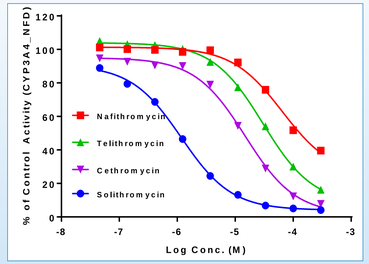
Gradation of ketolides based on CYP 3A inhibition : SOL > CET >TEL>Nafithromycin

1c : CYP3A4 (Midazolam Substrate)



Gradation of ketolides based on CYP 3A inhibition : SOL > CET=TEL >Nafithromycin

1d: CYP3A4 (Nifedipine Substrate)



Gradation of ketolides based on CYP 3A inhibition: SOL > CET > TEL >Nafithromycin

2c. : Telithromycin

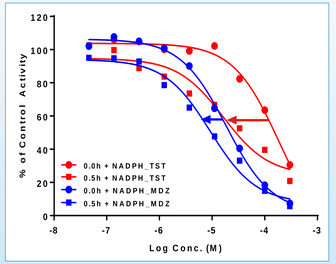


Table 3 : Inhibition (DI, TDI, and MBI) of CYP3A isoform by Nafithromycin vs comparators

CYP450 isoform	Probe substrate	Inhibition type	IC_{50} (μM)				
			Ref. Inhibitor	Nafithromycin	TEL	SOL	CET
HLM (CYP3A)	Testosterone	DI	39.5	>300	178.0	48.9	37.0
		TDI	30.2	>300	184.6	52.3	44.4
		MBI	3.9	>300	16.3	1.0	1.7
		IC_{50} shift (TDI)	No shift	No shift	No shift	No shift	No shift
		IC_{50} shift (MBI)	10.3	No shift	11.0	49.0	21.8
HLM (CYP3A)	Midazolam	DI	40.8	67.6	18.5	1.7	6.3
		TDI	42.4	87.4	26.0	1.4	4.6
		MBI	6.5	59.8	9.3	0.2	1.3
		IC_{50} shift (TDI)	No shift	No shift	No shift	No shift	No shift
		IC_{50} shift (MBI)	6.3	1.1	2.0	11.2	4.8

HLM: human liver microsomes; TEL: telithromycin; SOL: solithromycin; CET: cethromycin.
Note: Verapamil was used as a reference inhibitor.

2d. Cethromycin

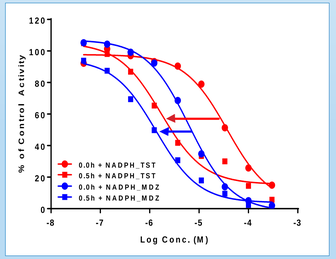
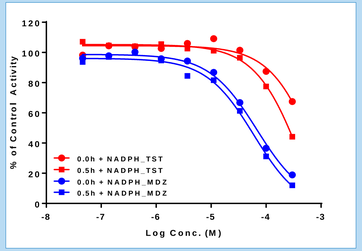
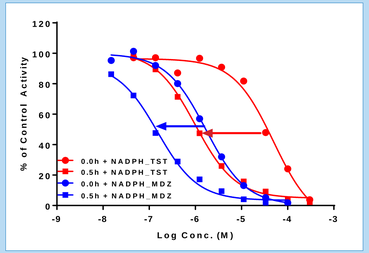


Figure 2 : Mean MBI IC_{50} graphs for CYP3A (midazolam and testosterone substrate)

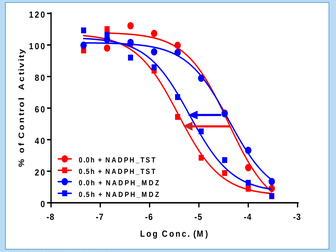
2a. Nafithromycin



2b. Solithromycin



2e. Verapamil



TST: Testosterone; MDZ: Midazolam

CONCLUSION

- **Nafithromycin shows a weaker CYP 3A enzyme inhibition potential and also lacks metabolism based inhibition potential compared to other ketolides like TEL, SOL and CET.**
- **The drug-drug interaction risk for Nafithromycin would be significantly lower compared to other ketolides and hence it would provide convenient/safer therapeutic option for RTI infections in community.**

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DISCLOSURES
All authors are employees of Wockhardt Research Centre, Aurangabad, India.

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