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IN VITRO ASSESSMENT OF REVERSIBLE AND METABOLISM-DEPENDENT INHIBITORY EFFECTS OF PROPOXAZEPAM ON CYP2C8 ACTIVITY

Background. In oncology, drug-drug interactions (DDIs) are particularly relevant due to the complex medication regimens of cancer patients. These patients often require multiple drugs to manage both their disease and treatment-related side effects. Evaluating potential DDIs via the inhibition of CYP enzymes is crucial in drug discovery. This study **aimed** to assess the effect of propoxazepam on CYP2C8 activity in vitro by amodiaquine N-deethylation in human liver microsomes and to predict the likelihood of DDI through CYP activity reduction. **Materials and Methods.** Amodiaquine N-deethylation was used as a marker of CYP2C8 activity. The positive controls included montelukast (1 μM) for reversible inhibition and gemfibrozil O-glucuronide (40 μM) for metabolism-dependent inhibition. Propoxazepam was tested in both reversible and metabolism-dependent inhibition conditions being added with the substrate or pre-incubated with microsomes and NADPH, respectively. The metabolite formation was quantified by LC-MS/MS in a multiple reaction monitoring mode using the electrospray ionization technique. **Results.** Propoxazepam inhibited CYP2C8 activity in a concentration-dependent manner, with IC_{50} values of 20.5 ± 2.2 μM for reversible inhibition and 23.1 ± 3.2 μM for metabolism-dependent inhibition. Positive controls montelukast and gemfibrozil O-glucuronide showed expected inhibition (4.4% and 12.2% of control, respectively). Propoxazepam showed low binding to microsomal protein under the experimental conditions. **Conclusion.** Based on the indicators used (K_i, IC_{50}, IC_{50} shift, and $[I]/K_i$ ratios), propoxazepam is not expected to be a significant CYP2C8 inhibitor in vitro.

Keywords: cancer, propoxazepam, CYP2C8, montelukast, gemfibrozil *O*-glucuronide, reversible inhibition, metabolism-dependent inhibition, DDI prediction.

Pain management remains a cornerstone of supportive care in oncology as pain significantly impacts the quality of life and functional status of cancer patients. Both during antineoplastic therapy and in palliative care, effective analgesia is paramount to reduce suffering [1]. Despite the availability of various analgesics [2, 3], limitations such as inadequate pain relief, adverse effects, and the development of tolerance necessitate the development of new more effective analgesic agents. These new drugs must not only exhibit potent analgesic effects but also demonstrate safety in terms of their pharmacokinetic and pharmacodynamic profiles. Cancer patients are at particularly high risk of

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drug-drug interactions (DDIs) because they usually receive a large number of drugs simultaneously, including cytotoxic agents, hormonal agents, targeted agents, and supportive care agents among drugs prescribed for the treatment of co-morbidities [4]. DDIs of this drug class are very common due to their narrow therapeutic index. There is a strong relationship between the therapeutic effect of these drugs on cancer cells and toxicity on normal cells [5—7]. There have been tragic deaths from the combination of sorivudine and fluorouracil [8]. This was caused by an abnormal increase in plasma fluorouracil levels due to the inhibition of fluorouracil metabolism by sorivudine.

In oncology, the complexity of treatment regimens, which often include multiple agents with narrow therapeutic windows, underlines the importance of evaluating the interactions of new analgesics with other drugs, particularly antitumor agents [9]. The problem of DDI is especially relevant for tyrosine kinase inhibitors (TKIs) since among them, there are substrates (brigatinib, cabozantinib, dabrafenib, imatinib, lapatinib, nilotinib, pazopanib, and ponatinib) and inhibitors (alectinib, axitinib, bosutinib, cabozantinib, idelalisib, lapatanib, larotrectinib, lenvatinib, nilotinib, pazopanib, regorafenib, tivozanib, and vismodegib) of CYP2C8 [10].

Nearly 60% of patients undergoing cancer therapy (regardless of the mode of administration of drugs) are estimated to have at least one potential DDI [11].

Pain is one of the most common symptoms in cancer patients. Pain can be caused by cancer, cancer treatment, or a combination of factors. Tumors, surgery, intravenous chemotherapy, radiation therapy, targeted therapy, therapies such as bisphosphonates, and diagnostic procedures may be the causes of pain [12].

Propoxazepam is considered a promising drug, and clinical studies of this drug are ongoing in Ukraine. Similar to gabapentinoid drugs (derivatives of inhibitory neurotransmitter gamma-aminobutyric acid, GABA), which are used in general medical practice in the treatment of neuropathic pain, propoxazepam also has an anticonvulsant effect [13, 14], which is considered a predictor of the analgesic action thus explaining the analgesic component in the pharmacological spectrum of a compound. Propoxazepam successfully passed the first stage of clinical studies in healthy volunteers, in which the safety and proper pharmacokinetics of the compound were proven [15]. The second phase of clinical research involves studying the analgesic effect of the drug on cancer patients with pain.

The study of DDI potential is initiated early in drug discovery with preclinical assessment and characterization using appropriate in vitro tools of human systems in support of labeling requirements and prescribing information. EMA [16] and the Ministry of Health of Ukraine [17] issued guidance documents on DDI which describe a tiered risk assessment strategy for potential perpetrators of CYP DDI that utilizes basic approaches. To date, CYP2C8 has been found to play a major role in the metabolism of several drugs, such as paclitaxel, cerivastatin, repaglinide, rosiglitazone, and pioglitazone [18]. Many drugs, including loperamide, zopiclone, ibuprofen, cerivastatin, repaglinide, and pioglitazone, have been reported to be metabolized by CYP2C8 in vitro [19].

This study evaluated the effect of propoxazepam on CYP2C8 activity in vitro using the amodiaquine N-deethylation reaction as a classical marker in the human liver microsomes (HLM) with the aim to assess the likelihood of DDI caused by propoxazepam via the reduced CYP activity.

Materials and Methods

Chemicals and tissue source. Propoxazepam was synthesized by the method described in [20]. The internal standard (Propoxazepam-D7 ($C_{18}H_9BrClD_7N_2O_2$) was supplied by SLC «INTERCHEM» (purity ≥98.0%, MM 414.73 g/mol). The general-purpose reagents and solvents were of analytical grade (or a suitable alternative) and were obtained principally from VWR International Ltd, Rathburn Chemicals Ltd, Sigma Aldrich Chemical Company Ltd, and Fisher Scientific UK Limited. HLMs were obtained from Corning Ultra Pool HLM 150 (Lot 38292).

Reversible CYP inhibition. Activities of HLM CYP2C8 were determined according to the standard assay. The formation of the metabolite of amodiaquine was quantified by ultra-performance liquid chromatography (UPLC) with mass spectrometry (MS) detection. To determine the inhibitory potential of propoxazepam, HLM were incubated, in triplicate, with isoform-selective probe substrates, NADPH, and propoxazepam at concentrations between 0.1 and 100 μM. After equilibra-

tion, the reactions were initiated by the addition of NADPH. Incubations were performed at 37 °C and terminated after the relevant incubation time by the addition of an appropriate stop reagent containing an internal standard. The samples were then centrifuged for 5 min to sediment the precipitated protein. Montelukast was used as a positive control.

Metabolism-dependent CYP inhibition. The metabolism-mediated inhibitory potential was investigated using a similar procedure, except that the HLM were pre-incubated for 30 min at 37 °C, in triplicate, with propoxazepam (over the same 0.1—100 μM concentration range) and NADPH before the addition of the CYP marker substrate amodiaquine at a concentration which approximated to the K_m. The metabolism-dependent inhibitor gemfibrozil *O*-glucuronide was used as a positive control for the effects on CYP2C8. The CYP enzyme activity for the pre-incubated samples in the presence of propoxazepam was compared to the samples incubated without propoxazepam.

Microsomal binding. The microsomal binding of propoxazepam was determined by the equilibrium dialysis using a high throughput equilibrium dialysis device. HLMs were diluted with an assay buffer to protein concentrations of 0.01, 0.05, and 1 mg/mL and spiked with propoxazepam at concentrations of 0.1, 10, and 100 μM. Triplicate spiked microsome samples were then dialyzed against the assay buffer at 37 °C/5% CO_2 for 6 h. The aliquots of the stock spiked microsomes, together with samples from the protein and buffer chambers were then analyzed by using a qualified LC-MS/MS method, and the concentration of propoxazepam was calculated.

Measurement of drug concentration. The formation of metabolites was quantified by LC-MS/MS in a multiple reaction monitoring mode using an electrospray ionization technique. The calibration standard working solutions were used to freshly prepare calibration standards.

Data analysis. The activity of the enzyme in the presence of various concentrations of propoxazepam was expressed as a percentage of the appropriate control activity. When the IC_{50} (the concentration at which the CYP probe substrate activity was reduced by 50%) could be determined, it was calculated by non-linear regression using the validated SigmaPlot software (Version 12.5, Systat Software Inc).

Due to the suboptimal data fits, the data for the CYP2C8 reversible and metabolism-dependent inhibition assays were fitted to a 3-parameter equation without the background function:

$$y = \frac{\text{Range}}{1 + \left(\frac{x}{IC_{50}}\right)^{s}}$$

where Range is the maximum y range, i.e. the control conversion rate (without inhibitor); s — slope factor; y — the conversion rate of probe substrate to metabolite; x — the propoxazepam concentration.

The extent of microsomal binding, determined using the equilibrium dialysis method, was calculated from the following equations:

$$\%\ \text{Bound fraction} = \frac{Cp - Cb}{Cp} \times 100$$

$$\%\ \text{Free fraction} = \frac{Cb}{Cp} \times 100$$

$$\%\ \text{Recovery} = \frac{(CpVp + CbVb)}{CpiVp} \times 100$$

where Cp is the concentration in protein compartment; Cb — the concentration in buffer compartment; Cpi — the initial concentration in spiking solution; Vp — the volume in protein compartment; Vb — the volume in buffer compartment.

Assuming enzyme competitive inhibition (K_i) can be estimated as follows:

$$IC_{50} = K_i\ (1 + S/K_m)$$

$$\text{If } [S] = K_m \text{ then } IC_{50} = 2K_i$$

All substrate concentrations used in the current study were approximated to the K_m.

The results were presented as the mean (M) ± ± standard error of the mean (SEM). Statistical analysis was performed using Student's *t*-test with the significance level $p \le 0.05$.

Results

When CYP2C8 enzyme interactions with inhibitors are of significantly different types of inhibition, different clinical implications follow [17]. The inhibition can be caused directly by the drug (propoxazepam), or it can be caused by the metabolite produced by the CYP catalytic cycle. An inhibition caused directly by propoxazepam can be classified as a reversible inhibition. An inhibition caused by the metabolite can be

classified as a metabolism-dependent inhibition (time-dependent inhibition). Following 4-h incubation with HLM, propoxazepam accounted for 96.0 % of the profile. The most abundant metabolite formed was oxidized propoxazepam (3-hydroxyderivative), which accounted for approximately 2.5% of the total peak response in the sample [22].

In our studies, we used montelukast (a potent inhibitor of the CYP2C8 amodiaquine N-deethylation reaction — reversible inhibition) and glucuronide of gemfibrozil (metabolism-dependent inhibition) as the positive controls. The data [23] support the use of montelukast as a selective CYP2C8 inhibitor to determine the contribution of this enzyme to drug metabolism reactions. The glucuronide of gemfibrozil is a substrate of CYP2C8 that transforms this substance into a potent inhibitor via the formation of a benzyl radical intermediate resulting in an irreversible inhibition and inactivation of the enzyme [24].

For both variants, the used positive controls (montelukast, 1μM, and gemfibrozil *O*-glucuronide, 40 μM) demonstrated the expected CYP2C8 activity inhibition to 4.39% (Table. 1) and to 12.2% (Table 2) compared to control.

To determine the possible effect of propoxazepam on the reversible inhibition of CYP2C8, it was incubated with HLM and amodiaquine, and a concentration-dependent activity inhibition was found (Table 1). Propoxazepam had the most significant effect at concentrations of 30, 60, and 100 μM.

The metabolism-mediated inhibitory potential was investigated using HLM pre-incubated for 30 min at 37 °C with propoxazepam (0.1—100 μM concentration range) and NADPH before the addition of CYP substrate amodiaquine at a concentration which approximated to the K_m. The results of the study are presented in Table 2.

The obtained results (Tables 1 and 2) made it possible to calculate IC_{50}, IC_{50} shift, and K_i (Table 3).

The concentration of plasma-unbound propoxazepam 0.084 μg/mL corresponds to 4264 ng /mL of the total plasma concentration (assuming that the free fraction of propoxazepam in plasma is 1.96 %), which is much higher than the C_{max} of the total propoxazepam plasma concentration after single oral administration.

The regulatory guidance states [16] that drug developers should correct for the nonspecific binding in microsomes if it is expected to influence the analysis of kinetic data. The evaluation of microsomal protein binding provides a better understanding of the relationship between in vitro metabolism and in vivo pharmacokinetics. The nonspecific binding of propoxazepam by human microsomes has been also analyzed because of the need to incorporate the fraction unbound by microsomes to obtain its meaningful concentrations for the prediction of CYP inhibition potential [25].

The results of the microsomal binding experiment are summarized in Table 4. The post-dialysis

Table 1. **Concentration-dependent inhibition of CYP2C8 — catalyzed amodiaquine N-deethylation in HLM by propoxazepam (reversible inhibition)**

Inhibitor	Nominal concentration, μM	Enzyme activity, pmol/min/mg (M ± m)	Calculated activity relative to control, %
Control	—	1737 ± 77	—
Propoxazepam solvent[#]	—	1681 ± 48	—
Propoxazepam	0.1	1657 ± 56	98.6 ± 5.5
	0.3	1705 ± 31	101.5 ± 4.9
	1	1653 ± 29	98.4 ± 4.7
	3	1486 ± 87*	88.4 ± 6.5
	10	1150 ± 11*	68.4 ± 3.1
	30	583 ± 20*	34.7 ± 2.0
	60	252 ± 11*	15 ± 0.9
	100	125 ± 5*	7.4 ± 0.4
Montelukast solvent control[##]	0	1616 ± 128	—
Montelukast	1	70.9 ± 5.2*	4.2 ± 0.4

Notes: [#] dimethyl sulfoxide (DMSO) (0.5% v/v); [##] ethanol (0.5% v/v); * differences are significant ($p \leq 0.05$) compared to the corresponding values for control and solvent.

recoveries are presented in Table 5. These data indicated that microsomal binding was not notably dependent on the propoxazepam concentration but was dependent on the microsomal protein concentration. The mean free fractions at microsomal protein concentrations of 0.01, 0.05, and 1 mg/mL were 103 ± 5%, 88.5 ± 4.7%, and 30.3 ± 5.7%, respectively, over the propoxazepam concentration range used. Given that the binding of propoxazepam to microsomal protein, when incubated under

Table 2. **Concentration-dependent inhibition of CYP2C8 — catalyzed amodiaquine N-deethylation in HLM by propoxazepam (metabolism-dependent inhibition)**

Inhibitor	Nominal concentration, μM	Enzyme activity, pmol/min/mg (M ± m)	Calculated activity relative to control, %
Control	—	1089 ± 5	—
Propoxazepam solvent[#]	—	1147 ± 29	—
Propoxazepam	0.1	1113 ± 18	97 ± 1.6
	0.3	1088 ± 36	94.8 ± 3.1
	1	1061 ± 22	92.5 ± 2.0
	3	1101 ± 44	96 ± 3.9
	10	833 ± 30*	72.6 ± 2.6
	30	452 ± 40*	39.4 ± 3.5
	60	205 ± 27*	17.9 ± 2.4
	100	104 ± 3*	9.1 ± 0.3
Gemfibrozil			
1-O-β-glucuronide solvent control[##]	—	1087 ± 69	—
Gemfibrozil 1-O-β-glucuronide	40	132 ± 22*	11.5 ± 1.9

Notes: [#]dimethyl sulfoxide (DMSO), (0.5% v/v); [##] methanol (0.5% v/v); * differences are significant ($p < 0.05$) compared to the corresponding values for control and solvent.

Table 3. **Parameters (IC_{50} and K_i) of reversible and metabolism-dependent inhibition by propoxazepam in vitro, M ± m**

Substrate	Inhibition concentration, IC_{50}, μM		Calculated IC_{50} shift (IC_{50} reversible/IC_{50} metabolism-dependent)	Inhibition constant, K_i, μM	Unbound plasma concentration	
	Reversible inhibition	Metabolism dependent			μM	μg/mL
Amodiaquine	20.5 ± 2.2	23.1 ± 3.2	0.89 (1.0)	10.3 ± 1.1	0.206	0.084

Table 4. **In vitro determination of binding of propoxazepam (0.1, 10, and 100 μM) following dialysis of spiked human liver microsomes for 6 h**

Group	Nominal conc., μM	HLM mg/mL	Actual conc., μM	Donor conc., μM	Acceptor conc., μM	Fraction bound, %	Fraction unbound, %	Fraction unbound, mean
1	0.1	0.01	0.045 ± 0.002	0.018 ± 0.002	0.019 ± 0.001	–6.2 ± 2.3	106.1 ± 7.1	103.6 ± 3.2
	10		8.2 ± 0.3	3.3 ± 0.2	3.6 ± 0.6	–7.2 ± 2.8	107.4 ± 3.5	
	100		85.9 ± 2.5	36.8 ± 3.4	35.7 ± 2.5	2.7 ± 2.6	97.3 ± 1.4	
2	0.1	0.05	0.046 ± 0.001	0.021 ± 0.001	0.018 ± 0.001	6.8 ± 1.9	93.2 ± 2.3	88.5 ± 2.7*
	10		8.6 ± 0.3	4.2 ± 0.6	3.7 ± 0.6	11.7 ± 3.6	88.3 ± 3.5	
	100		85.5 ± 1.1	39.5 ± 5.1	32.9 ± 2.2	16.1 ± 4.7	83.9 ± 3.3	
3	0.1	1	0.049 ± 0.001	0.035 ± 0.009	0.011 ± 0.002	70.3 ± 4.5	29.7 ± 2.6	30.3 ± 3.3**
	10		8.2 ± 0.2	6.5 ± 0.3	1.6 ± 0.3	75.1 ± 6.4	24.9 ± 3.5	
	100		98.8 ± 4.1	65.1 ± 11.7	22.3 ± 5.7	63.7 ± 16.5	36.3 ± 9.5	

Notes: HLM — human liver microsomes; conc. — concentration; * significant at $p \leq 0.05$ (compared to group 1); ** significant at $p \leq 0.01$ (compared to group 1).

conditions that reflected those in the IC_{50} experiments, was low, no microsomal binding correction factor was applied to the reported IC_{50} values.

Discussion

In clinical oncology, DDI acquires additional relevance. Most anticancer agents have a narrow therapeutic index and more exuberant toxicity profile, which increases the risk of DDI. These interactions can lead to increased side effects, reduced treatment effectiveness, or even harm to the patient [26]. An inhibition of CYP-dependent metabolism is a widespread source of DDI that may lead to serious clinical consequences. An assessment of the potential for new chemical entities to cause DDI via inhibition of CYP-dependent metabolism is important in the drug discovery process [27].

The goal of this study was to assess the effect and metabolism-dependent CYP2C8 inhibition by propoxazepam in HLM, using an amodiaquine N-deethylation reaction. Experimentally, the inhibitory potential of a tested compound is determined by measuring the decrease in metabolite amodiaquine (monodesethyl-amodiaquine) formation by HLM enzymes CYP2C8 using the LC-MS/MS system.

Inhibition of cytochrome CYP2C8 is the most common mechanism leading to DDI and can be categorized as reversible or metabolism-dependent. Each type of interaction involves a distinct clinical management strategy. The first stage of the interaction between a drug and a CYP is the process of binding, which often involves a change in the UV-visible absorbance spectrum, usually observed in the Soret band. A shift of the iron from a resting low-spin state to a high-spin state is termed type I change (λ_{max} ~390 nm). Type II change involves the formation of a low-spin iron bound to a nitrogen atom of the ligand (λ_{max} ~430 nm) [28]. These changes can be used to characterize the binding affinity of CYP and ligands. Earlier, we showed [29] that propoxazepam and 3-hydroxymetabolite when interacting with the rat liver CYP show type II spectral changes in hemoprotein. Their binding constants are significantly different, which indicates the possibility of interaction of substrates with different sites of CYP. The quantitative indicators of the inhibitory activity of propoxazepam and its metabolite determined by differential spectroscopy are only indicative but still may suggest at least a significant possibility of inhibitory interaction in the clinical use of drugs.

Table 5. **Microsomal binding of propoxazepam (0.1, 10, and 100 μM): post dialysis recoveries**

Nominal conc., μM	HLM, mg/mL	Recovery, %
0.1	0.01	83.0 ± 6.6
10		84.1 ± 5.0
100		84.4 ± 6.8
0.1	0.05	82.1 ± 1.7
10		91.8 ± 12.5
100		84.7 ± 8.3
0.1	1	93.6 ± 21.7
10		99.5 ± 0.5
100		88.4 ± 6.5

Results are presented as the mean ± standard deviation from three determinations

HLM — human liver microsomes

To better decipher the interaction between propoxazepam and CYP at the molecular level, we used drug docking to CYP2C8 HLM. Propoxazepam has fairly high values (8.15—9.8 cal/mol) of the free energy of interaction with CYP isozymes 1A2, 2B6, 2C9, 2C19, 2D6, and 3A4, although there is a difference in the number of common amino acid residues involved in the interaction with separate substrates [30]. The results of the analysis of the interaction of propoxazepam with different CYP isoenzymes suggest the possibility of a competitive interaction for 1A2, 2C19, and 2C8 and, to a lesser extent, for 2C9, 3A4, and 2B6.

In the pharmaceutical industry, CYP inhibition studies are typically included in the standard screening strategy for drug candidates. A measurement of the CYP inhibition is always performed by assaying inhibition of substrate metabolism and determining inhibition values (K_i, IC_{50} shift, and IC_{50}). K_i reflects the binding affinity, and IC_{50} more closely reflects the functional potency of the inhibitor for the drug [31]. If K_i is much larger than the maximal plasma drug concentration, a patient is typically exposed to from the typical dosing, then that drug is not likely to inhibit the activity of that enzyme [32].

Propoxazepam in our study showed similar (the absence of significant difference) CYP2C8 amodiaquine N-deethylation activity inhibition with IC_{50} of 20.5 ± 2.2 μM for reversible and 23.1 ± 3.2μM for metabolism-dependent inhibition. Based on the

above judgments, the inhibitory effects of propoxazepam on the CYP enzyme showed more reversible inhibition. A reversible inhibition is a result of the rapid association and dissociation between the substrate drugs and the enzyme. A direct inhibition occurs when the inhibitor and substrate bind to the same site on the enzyme. It occurs without preincubation, so it is immediate. The metabolism-dependent inhibition of the enzyme by a metabolite of the parent drug can occur when the parent is metabolized by CYP enzymes in the presence of the NADPH cofactor.

Prediction of a potential DDI with CYP2C8 is challenging due to an interindividual variability in the abundance and activity in both the liver and small intestine. K_i can be used in determining the $[I]/K_i$ ratio as a tool for predicting DDI. The parameter [I] represents the mean steady-state C_{max} of the inhibitor exposed to the active site of the CYP enzyme. The closer the drug concentration to the K_i, the greater the chance that the medication in question will inhibit that enzyme and cause drug interactions with medications that are substrates of that enzyme. Therefore, as the ratio increases, so does the likelihood of a drug interaction. If the $[I]/K_i$ ratio is < 0.1, the prediction for drug interaction is remote; at $0.1 < [I]/K_i < 1.0$, prediction is possible, and if the $[I]/K_i$ ratio is > 1, it is likely. [33]. A basic assessment of the range of $[I]/K_i$ ratios (0.02— 0.11) would suggest that they are not likely to inhibit CYP2C8.

Based on the estimated K_i (assuming competitive inhibition) and EMA guidelines [16], propoxazepam would be predicted to cause clinically relevant drug interactions with CYP2C8 substrate at unbound plasma C_{max} concentrations of ≥ 0.206 μM (ca. 84 ng/mL). Using the approach outlined in the FDA DDI final guidance document [34], it is suggested that for the direct (reversible) inhibition, a clinically-relevant drug interaction would be likely if the value of R1 is over 1.02, where R1 (the predicted ratio of a victim drug's AUC in the presence and absence of an inhibitor) is equal to $1 + ([I]/K_i)$. The value of [I] can be estimated by the maximum inhibitor (i.e. drug) concentration unbound in plasma. The EMA guidance [16] suggested that an in vivo DDI study with a sensitive probe substrate is recommended when $[I]/K_i \geq 0.02$, where [I] is the unbound mean C_{max} value obtained during treatment with the highest recommended dose. On this basis, the unbound plasma concentration above which propoxazepam would be predicted to cause a clinically relevant drug interaction with co-administered CYP2C8 substrate is IC_{50} 20.5 μM; estimated K_i 10.3 μM; unbound plasma concentration 0.206 μM, where R1 ≥ 1.02* or 0.02** μM. Estimated K_i is based on an assumed competitive mechanism of direct inhibition.

Thus, the highest predicted unbound C_{max} plasma concentration of propoxazepam, above which the interaction can take place is 0.206 μM, or 206 nM, which (with the propoxazepam molecular weight of 407.73 g/mol) gives 84 ng/mL.

According to our data [34], the unbound propoxazepam fraction in human plasma is 1.96%, so its total concentration where the inhibition is prognosed is 4264 ng/mL. Pharmacokinetics study results showed that "the maximum propoxazepam concentration (22.276 ng/mL) was reached in blood by 4 h after oral administration on healthy volunteers" [15], which is much lower than the estimated prognosed inhibition levels. Based on this, it can be concluded that propoxazepam is not expected to be a CYP2C8 inhibitor in vivo.

This is also evidenced by the data on IC_{50} and IC_{50} shifts from our experiments. According to the general standard [35], $IC_{50} < 1$ μM suggests a strong inhibitory effect, $1 \text{ μM} < IC_{50} < 10$ μM suggests a medium inhibitory effect, and $IC_{50} > 10$ μM (as well as propoxazepam) suggests a weak inhibitory effect. Using the IC_{50} shift assay [36], it is possible to distinguish between a reversible and an irreversible inhibition. A fold shift of greater than 1.5 is considered significant, and the compound is classed as a metabolism-dependent inhibitor. In our study, this value was 0.89 (considering the absence of a statistically significant difference equal to 1.0).

To sum up, propoxazepam would be predicted to cause clinically relevant drug interactions with co-administrated CYP2C8 substrates at an unbound plasma C_{max} concentrations of ≥0.0206 μM (about 84 ng/mL). A 30-min pre-incubation of propoxazepam with microsomes and NADPH before substrate addition did not result in a notable change in these values, which suggests that the inhibition mechanism was rather metabolism-dependent than reversible directly. According to our pharmacokinetics data, at least after a single oral administration, these concentrations are not reachable. For multiple course administration, additional studies are needed.

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ОЦІНКА IN VITRO ОБОРОТНОЇ ТА ЗАЛЕЖНОЇ ВІД МЕТАБОЛІЗМУ ІНГІБУЮЧОЇ ДІЇ ПРОПОКСАЗЕПАМУ НА АКТИВНІСТЬ CYP2C8

Стан питання. Взаємодія між лікарськими засобами (DDI) є особливо важливою для онкології через складні схеми лікування хворих на рак. Ці хворі часто потребують застосування кількох лікарських засобів, як власне протиракових, так і тих, що необхідні для зменшення побічних ефектів лікування. Оцінка потенційних DDI через інгібування ензимів CYP є критично важливою в розробці нових лікарських засобів. **Метою** цього дослідження є оцінка впливу пропоксазепаму на активність CYP2C8 in vitro, використовуючи реакцію N-дегідроксидації амодіаквіну в мікросомах печінки людини, та прогнозування ймовірності DDI через зменшення активності CYP. **Матеріали та методи.** Реакція N-дегідроксидації амодіаквіну використовувалася як маркер активності CYP2C8. Позитивними контролями були монтелукаст (1 μM) для оборотного інгібування та гемфіброзил О-глюкуронід (40 μM) для метаболізм-залежного інгібування. Пропоксазепам тестувався в умовах як оборотного, так і метаболізм-залежного інгібування: додавався разом із субстратом або попередньо інкубувався з мікросомами та NADPH. Формування метаболітів визначалося кількісно методом LC-MS/MS у режимі MRM з ESI. **Результати.** Пропоксазепам інгібував активність CYP2C8 у концентраційно-залежний спосіб з IC_{50} значеннями 20.5 ± 2.2 μM для оборотного інгібування та 23.1 ± 3.2 μM для метаболізм-залежного інгібування. Позитивні контролі, монтелукаст та гемфіброзил О-глюкуронід, показали очікуване інгібування (відповідно 4.4% та 12.2% контролю). Пропоксазепам показав низьке зв'язування з мікросомальними білками за експериментальних умов. **Висновок** На основі використаних індикаторів (K_i, IC_{50}, зсув IC_{50} та співвідношення $[I]/K_i$), пропоксазепам не очікується як значний інгібітор CYP2C8 in vitro.

Ключові слова: рак, пропоксазепам, CYP2C8, монтелукаст, гемфіброзил О-глюкуронід, оборотне інгібування, метаболізм-залежне інгібування, прогнозування DDI.