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▶ **To cite this version:**

Karima Alim, Amélie Moreau, Arnaud Bruyère, Elodie Jouan, Claire Denizot, et al.. Inhibition of organic cation transporter 3 activity by tyrosine kinase inhibitors. Fundamental & Clinical Pharmacology, 2021, 35 (5), pp.919-929. 10.1111/fcp.12657 . hal-03134613

HAL Id: hal-03134613
https://hal.science/hal-03134613

Submitted on 19 Feb 2021

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Article type : Original Article

Inhibition of organic cation transporter 3 activity by tyrosine kinase inhibitors

Running title: OCT3 inhibition by tyrosine kinases inhibitors

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ABSTRACT

Organic cation transporter (OCT) 3 (*SLC22A3*) is a widely-expressed drug transporter, handling notably metformin and platinum derivatives, as well as endogenous compounds like monoamine neurotransmitters. OCT3 has been shown to be inhibited by a few marketed tyrosine kinase inhibitors (TKIs). The present study was designed to determine whether additional TKIs may interact with OCT3. For this purpose, the effects of 25 TKIs towards OCT3 activity were analyzed using OCT3-overexpressing HEK293 cells. 13/25 TKIs, each used at 10 μM, were found to behave as moderate or strong inhibitors of OCT3 activity, *i.e.*, they decreased OCT3-mediated uptake of the fluorescent dye 4-(4-(dimethylamino)styryl)-N-methylpyridinium iodide by at least 50% or 80%, respectively. This OCT3 inhibition was correlated to some molecular descriptors of TKIs, such as the percentage of H atoms and that of cationic forms at pH = 7.4. It was concentration-dependent, notably for brigatinib, ceritinib and crizotinib, which exhibited low half maximal inhibitory concentration (IC_{50}) values in the 28-106 nM range. Clinical concentrations of these three marketed TKIs, as well as those of pacritinib, were next predicted to inhibit *in vivo* OCT3 activity according to regulatory criteria. Cellular TKI accumulation experiments as well as *trans*-stimulation assays however demonstrated that OCT3 does not transport brigatinib, ceritinib, crizotinib and pacritinib, thus discarding any implication of OCT3 in the pharmacokinetics of these TKIs. Taken together, these data suggest that some TKIs may act as potent inhibitors of OCT3 activity, which may have consequences in terms of drug-drug interactions and toxicity.

Keywords: Drug-drug interaction, molecular descriptors, organic cation transporter, pharmacokinetics, tyrosine kinase inhibitor.

LIST OF ABBREVIATIONS

ALK, anaplastic lymphoma kinase;

ALOGP, Ghose-Crippen octanol-water partition;

DDI, drug-drug interaction;

DiASP, 4-(4-(dimethylamino)styryl)-N-methylpyridiniumiodide;

EGFR, epidermal growth factor receptor;

EMT, extraneuronal monoamine transporter;

FAU, fluorescence arbitrary unit;

FDA, Food and Drug Administration;

Flt3, Fms-like tyrosine kinase 3;

IC_{50}, half maximal inhibitory concentration;

JAK, Janus kinase;

LC-MS/MS, liquid chromatography-tandem mass spectrometry;

MATE, multidrug and toxin extrusion protein;

MLOGP, Moriguchi octanol-water partition coefficient;

MPP^+, 1-methyl-4-phenylpyridinium;

OCT, organic cation transporter; PBS, phosphate-buffered saline;

TKI, tyrosine kinase inhibitor;

TPSA, topological polar surface area.

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INTRODUCTION

Organic cation transporter (OCT) 3 (*SLC22A3*, also known as the extraneuronal monoamine transporter (EMT)), is a membrane drug transporter belonging to the solute carrier (SLC) family [1]. OCT3 handles various drugs, including cisplatin, metformin and quinidine, as well as endogenous compounds like neurotransmitters, histamine and creatinine [2-5]. Many of its substrates are shared with the two other OCTs, *i.e.*, OCT1 and OCT2. OCT3, unlike OCT1 and OCT2, is rather widely expressed [6]. OCT3 is notably found in tissues relevant for pharmacokinetics, such as the liver and kidney [7], where it is localized at the sinusoidal membrane of hepatocytes and the basolateral membrane of renal proximal tubule epithelial cells, respectively [8]. The transporter is also present at the luminal membrane of small intestinal enterocytes, where it contributes to oral absorption of metformin [9]. OCT3 is additionally found in the heart and may consequently play a role in cardiac tissue drug exposure [10], as well as in the placenta, where it facilitates foetal exposure to metformin during pregnancy [11]. Taken together, these data suggest that OCT3 may be a drug transporter to consider according to a pharmacokinetical, pharmacological and/or toxicological point of view, even if it is presently not included in the list of drug transporters that have to be studied during the development of new molecular entities according to regulatory authorities for drug approval in the US and Europe [12, 13].

Various drugs block OCT3 activity and may consequently theoretically cause drug-drug interactions (DDIs) and/or OCT3 inhibition-related adverse effects [8]. In particular, some tyrosine kinase inhibitors (TKIs), like the Bcr-Abl inhibitors imatinib, dasatinib and nilotinib, the epidermal growth factor receptor (EGFR) inhibitors gefitinib and erlotinib, and the multi-kinase inhibitors sunitinib and sorafenib, inhibit *in vitro* OCT3 activity, at concentrations thought to be reached *in vivo* for at least nilotinib, thus supporting a potential clinical relevance of these effects [14]. Whether other TKIs can also behave as inhibitors and/or substrates for OCT3 is however unknown, but likely deserves interest owing to the current development of TKIs as a leading pharmacological class for various therapeutic indications, to the role that transporters play in TKI pharmacokinetics and activity [15, 16] and to the fact that some TKIs like abemaciclib have been clinically shown to increase exposure to the OCT3 substrate metformin [17]. To get insights about

this point in the present study, we have analyzed the interactions of OCT3 with 25 TKIs, targeting various tyrosine kinases and regulatory-approved (n=23) or under clinical development (n=2).

MATERIAL AND METHODS

Chemicals

The 23 marketed TKIs abemaciclib, acalabrutinib, afatinib, alectinib, brigatinib, cabozantinib, ceritinib, crizotinib, dacomitinib, entrectinib, ibrutinib, lapatinib, lenvatinib, lorlatinib, neratinib, nintedanib, osimertinib, ponatinib, regorafenib, ribociclib, ruxolitinib, tofacitinib and vemurafenib as well as itacitinib and pacritinib, 2 TKIs under clinical phase 3 development, were provided by MedChemtronica (Sollentuna, Sweden). These TKIs target various kinases, as indicated in Table 1. Corticosterone and unlabeled 1-methyl-4-phenylpyridinium (MPP^+) were from Sigma-Aldrich (Saint Quentin Fallavier, France) and 4-(4-(dimethylamino)styryl)-N-methylpyridiniumiodide (DiASP) from Thermo Fisher Scientific (Waltham, MA, USA). N-[methyl-^{3}H]-MPP^+ (specific activity = 81.8 mCi/mmol) was purchased from PerkinElmer (Villebon-sur-Yvette, France).

Cell culture

HEK293 cells overexpressing OCT3 (HEK-OCT3 cells) as well as control HEK293-MOCK cells (HEK-CTR cells), obtained by stable transfection [18], were cultured in DMEM medium, supplemented with 10% (vol/vol) fetal calf serum, 1 µg/mL bovine insulin, 1% (vol/vol) non-essential amino acid solution, 800 µg/mL G418, 20 UI/mL penicillin and 20 µg/mL streptomycin.

OCT3 activity inhibition assay

Inhibition of cellular OCT3 activity was investigated using the fluorescent OCT probe DiASP, which is transported into HEK-OCT3 cells in a saturable manner (K_m=24.8 ± 5.1 µM) [19]. Briefly, cells were incubated with 10 µM DiASP for 5 min at 37°C, in the absence or presence of the reference OCT3 inhibitor corticosterone or of TKIs, in the transport assay medium previously described [20]. After washing with phosphate-buffered saline (PBS), intracellular accumulation of the dye was determined by spectrofluorimetry, using a SpectraMax Gemini SX spectrofluorometer

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(Molecular Devices, Sunnyvale, CA, USA); excitation and emission wavelengths were 485 nm and 607 nm, respectively. Data were finally normalized to total protein content, determined by the Bradford's method [21]. They were expressed as fluorescence arbitrary unit (FAU)/mg protein or as percentages of OCT3 activity or of OCT3 activity inhibition according to the equation (A) or (B), respectively:

$$\%\ \text{OCT3 activity} = \frac{([\text{DiASP}_{\text{TKI}}] - [\text{DiASP}_{\text{Corticosterone}}]) \times 100}{([\text{DiASP}_{\text{Control}}] - [\text{DiASP}_{\text{Corticosterone}}])} \quad \text{(A)}$$

$$\%\ \text{OCT3 activity inhibition} = 100\% - \%\ \text{OCT3 activity} \quad \text{(B)}$$

with $[\text{DiASP}_{\text{TKI}}]$ = DiASP concentration in the presence of a defined concentration of TKI, $[\text{DiASP}_{\text{Corticosterone}}]$ = DiASP concentration in the presence of 100 µM corticosterone and $[\text{DiASP}_{\text{Control}}]$ = DiASP concentration in control cells not exposed to TKI or corticosterone.

Half maximal inhibitory concentration (IC_{50}) for TKIs towards OCT3 activity was calculated using Prism 8.4.2 software (GraphPad Software, La Jolla, CA, USA), through nonlinear regression based on the following four-parameter logistic equation:

$$A = \frac{100}{1 + 10^{([\text{I}] - \text{Log(IC50)}) \times \text{Hill slope}}} \quad \text{(C)}$$

with A = percentage of transporter activity for a given concentration of TKI determined as described in equation (A), [I] is the TKI concentration in the medium, and Hill slope is a coefficient describing the steepness of the curve.

Reversibility of OCT3 activity inhibition by TKIs

HEK-OCT3 cells were first incubated in the absence (control) or presence of 10 µM TKIs for 5 min at 37°C. After washing, cells were either used directly for OCT3 activity measurement, performed as described above, or were re-incubated in TKI-free medium for 3 h before OCT3 activity measurement. Data were finally expressed as percentages of OCT3 activity inhibition.

Prediction of *in vivo* inhibition of OCT3 by TKIs

From *in vitro* IC_{50} values, *in vivo* inhibition of OCT3 activity by TKIs was predicted through applying the criteria defined by the US Food and Drug Administration (FDA) guidance on *in vitro* drug interaction studies [12] to OCT3, *i.e.*, a transporter-related DDI can be expected if :

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$$IC50 / I_{max,u} > 0.1 \quad (D)$$

with $I_{max,u}$ = maximum unbound plasma concentration of the TKI.

Trans-stimulation assay

Trans-stimulation assays were performed in HEK-OCT3 cells using the OCT3 substrate MPP^+ [22]; such *trans*-stimulation assays for OCTs are based on the fact that substrates of these transporters can both *cis*-inhibit and *trans*-stimulate them [23]. Briefly, HEK-OCT3 cells were first incubated with 1 mM unlabeled MPP^+ or 30 µM TKI for 30 min at 37 °C. After washing with PBS, cells were next re-incubated with radiolabeled [^{3}H]-MPP^+ (used at 6.0 nM) for 5 min at 37 °C. Intracellular accumulation of radiolabeled MPP^+ was next determined by liquid scintillation counting using a Tri-Carb 4810TR counter (Perkin-Elmer), after addition of 2 ml/sample of the liquid scintillation cocktail Ultima Gold™ (Perkin Elmer). Data were normalized to protein content and finally expressed as percentages of [^{3}H]-MPP^+ accumulation found in control cells not pre-treated by unlabeled MPP^+ or TKIs.

TKI accumulation assay

HEK-CTR and HEK-OCT3 cells were incubated with 10 µM TKI, in the absence or presence of 100 µM corticosterone, for 5 min at 37°C. Cells were then washed twice in ice-cold PBS, lysed in distilled water and acetonitrile-based extraction of cell lysates was performed. TKI quantification was next performed through liquid chromatography-tandem mass spectrometry (LC-MS/MS), based on a high-performance liquid chromatography Aria system (Agilent, Les Ulis, France), equipped with a Poroshell 120 C18 (4.6 × 100 mm) column (Agilent, Les Ulis, France) and coupled to a tandem mass spectrometry TSQ Quantum (Thermo Fisher Scientific, Villebon sur Yvette, France), fitted with an electrospray ionization source (ESI+). Monitored ion transitions were at 466.1 > 372.1 m/z (for acalabrutinib), 584.0 >484.2 m/z (for brigatinib), 558.0>433.0 (for ceritinib), 449.9>260.1 (for crizotinib) and 437.1>316.1 (for pacritinib). Data were finally normalized to protein content.

TKI molecular descriptor generation

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83 molecular descriptors belonging to the blocks "constitutional indices" (n = 47), "charge descriptors" (n = 15) and "molecular properties" (n = 20), were determined using the Dragon 7.0 software (Talete, Milano, Italy) (See http://www.talete.mi.it/products/dragon_molecular_descriptor_list.pdf for a complete list of these descriptors). TKIs, initially expressed in SMILES format, were converted to 3D format using MarvinView 20.19.0 software (ChemAxon, Budapest, Hungary) before processing by Dragon 7.0 software to obtain molecular descriptors, as previously described [19]. An additional charge descriptor, *i.e.*, the percentage of cationic form of TKIs at pH = 7.4, was determined using MarvinSketch 20.19.0 software; the total number of molecular descriptors analyzed for each TKI was therefore 84.

Statistical analysis

Experimental data were routinely expressed as means ± SD of at least three independent assays, each being usually performed in triplicate. Normality distribution of percentages of OCT3 activity inhibition by TKIs was checked by D'Agostino and Pearson omnibus normality test. Data were statistically analyzed through analysis of variance (ANOVA) followed by the Dunnett's or Tukey's post-hoc test. Correlation between molecular descriptor indexes and percentages of OCT3 activity inhibition by TKIs was performed through Pearson correlation. The criterion of significance was $p < 0.05$. All the statistical analyses were done using Prism 8.4.2 software.

RESULTS

Inhibition of *in vitro* OCT3 activity by TKIs

The effects of 10 µM TKIs towards OCT3-mediated transporter of DiASP were investigated in HEK-OCT3 cells. As shown in Table 1, 5/25 TKIs, *i.e.*, alectinib, cabozantinib, itacitinib, lapatinib and vemurafenib, failed to significantly alter OCT3 activity. Among the 20/25 TKIs which inhibited OCT3, some of them (7/25 TKIs), *i.e.*, lorlatinib, neratinib, nintedanib, osimertinib, regoferanib, ruxolitinib and tofacitinib, decreased OCT3 activity by less than 50% and can thus be considered as weak OCT3 inhibitors. Other TKIs (8/25 TKIs), *i.e.,* abemaciclib, afatinib, dacomitinib, entrectinib, ibrutinib, lenvatinib, ponatinib and ribociclib, were found to

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inhibit OCT3 activity by more than 50% but less than 80% (Table 1) and thus appear as moderate inhibitors of the transporter. 5/25 TKIs, *i.e.*, acalabrutinib, brigatinib, ceritinib, crizotinib and pacritinib were found to inhibit OCT3 by more than 80% (Table 1) and are consequently potent inhibitors of OCT3 activity. These five strong OCT3 inhibitors were next found to inhibit OCT3 in a concentration-dependent manner, with IC_{50} values ranging from 28.2 nM (for ceritinib) to 1841 nM (for acalabrutinib) (Figure 1). We next investigated whether TKI-mediated inhibition of OCT3 may be reversible. For this purpose, cells were initially exposed to 10 µM TKI for 5 min, washed and re-incubated in TKI-free medium for 3 h before measurement of OCT3 activity. As indicated in Figure 2, inhibition of OCT3 activity by acalabrutinib was fully suppressed by the 3 h TKI-free post-incubation period, whereas those due to brigatinib or pacritinib were partly reversed. This indicates that the inhibitory effects of these TKIs towards OCT3 were fully (acalabrutinib) or partly (brigatinib and pacritinib) reversible after a 3 h TKI-free post-incubation period. By contrast, inhibition of OCT3-mediated transport by ceritinib and crizotinib was not significantly impaired by a 3 h TKI-free post-incubation when compared to the control condition without a TKI-free post-incubation (Figure 2).

In order to investigate physico-chemical structural requirements for OCT3 inhibition by TKIs, percentages of inhibition of OCT3 activity by the 25 TKIs (each used at 10 µM) were next confronted to values of 84 TKI molecular descriptors, through Pearson correlation analysis. 7/84 molecular descriptors were found to be significantly either positively (n=2) or negatively (n=5) correlated with OCT3 inhibition (Table 2). This notably concerns the percentage of H atoms (H%) and the percentage of cationic forms. The 77/84 molecular descriptors not correlated with OCT3 activity inhibition, including lipophilicity-related parameters such as Moriguchi octanol-water partition coefficient (MLOGP) and Ghose-Crippen octanol-water partition (ALOGP) as well as topological polar surface area (TPSA), are listed in Table S1.

Prediction of *in vivo* OCT3 inhibition by TKIs

The prediction of *in vivo* OCT3 inhibition was performed for TKIs defined above as strong inhibitors, *i.e.*, acalabrutinib, brigatinib, ceritinib, crizotinib and pacritinib. It was done using the criteria defined by the U.S. FDA and based on the ratio $IC_{50}/I_{max,u}$. As indicated in Table 3, brigatinib, ceritinib, crizotinib and pacritinib, unlike acalabrutinib, were predicted to inhibit *in vivo* OCT3 activity at clinically achievable concentrations.

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Transport of TKIs by OCT3

To determine whether TKIs potently interacting with OCT3 activity, *i.e.*, acalabrutinib, brigatinib, ceritinib, crizotinib and pacritinib, may be subtrates for OCT3, *trans*-stimulation assays were performed, because substrates for OCTs, including OCT3, are postulated to *trans*-stimulate OCT activities [22, 23]. As shown in Figure 3, acalabrutinib, brigatinib, ceritinib, crizotinib and pacritinib failed to *trans*-stimulate OCT3-mediated radiolabeled MPP^+ accumulation in HEK-OCT3 cells. By contrast, unlabeled MPP^+ *trans*-stimulated OCT3 activity (by a 4.7-fold-factor) (Figure 3). Intracellular accumulation of TKIs was next determined in HEK-CTR and HEK-OCT3 cells by LC-MS/MS assay, in the absence or presence of the OCT3 inhibitor corticosterone. As shown in Figure 4, accumulations in HEK-CTR and HEK-OCT3 cells were similar for each analyzed TKI and corticosterone failed to reduce TKI concentrations in HEK-OCT3 cells. By contrast, accumulation of the reference OCT3 substrate DiASP was significantly increased in HEK-OCT3 cells when compared to HEK-CTR cells (by a 4.2-fold factor). This enhanced accumulation of DiASP in HEK-OCT3 cells was nevertheless markedly reduced in the presence of corticosterone (Figure 4).

DISCUSSION

The data of the present study fully confirm that TKIs can interact with OCT3 activity. Indeed, only 5/25 TKIs were devoid of any inhibitory effect towards OCT3 activity, whereas 13/25 TKIs were characterized as moderate or strong inhibitors of OCT3 and 7/25 TKIs as weak inhibitors. TKIs acting as moderate and strong inhibitors block more than 50% of OCT3 activity when used at 10 μM, thus supporting the conclusion that their IC_{50} values towards OCT3 are less than 10 μM. Such compounds can therefore be unambiguously added to the list of TKIs blocking OCT3, which already comprises 6 other TKIs, including imatinib, nilotinib and erlotinib [14]. The kinases targeted by these TKIs blocking OCT3 activity are diverse, including the anaplastic lymphoma kinase (ALK) inhibited by brigatinib, ceritinib and crizotinib, the Janus kinase (JAK) 2 and the Fms-like tyrosine kinase 3 (Flt3) inhibited by pacritinib, the kinase Bcr-Abl inhibited by imatinib and nilotinib and the EGFR inhibited by erlotinib. Inhibition of OCT3 activity by TKIs is therefore not linked to the targeting of a specific tyrosine kinase. For some strong inhibitors of OCT3 such

as brigatinib, ceritinib and crizotinib, IC_{50} values are comprised in the 28-106 nM range, whereas IC_{50} values for OCT3 inhibitors are commonly in the 1-100 μM range [18, 24-27]; this illustrates the rather potent *in vitro* inhibitory effect of these TKIs towards OCT3, similar to that of reference OCT3 inhibitors such as corticosterone or decynium 22, for which IC_{50} values are in the 100-300 nM range [28]. Moreover, the inhibition of OCT3 activity by 10 μM ceritinib or 10 μM crizotinib was not impaired by a 3 h TKI-free post-incubation period, suggesting a long-lasting action of these two TKIs. Beyond OCT3, other SLCs handling organic cations such as OCT1, OCT2, multidrug and toxin extrusion protein (MATE) 1 and MATE2-K have been shown to be inhibited by various TKIs [14], thus indicating that transporters of organic cations may represent privileged targets of TKIs. In this context, it is noteworthy that one TKI may inhibit multiple SLCs transporting organic cations. As an example, in addition to OCT3, the transporters OCT1, MATE1 and MATE2-K are inhibited by brigatinib [29], whereas clinically relevant concentrations of crizotinib block OCT1 and OCT2 activities [30], therefore supporting the hypothesis that these TKIs may act as powerful inhibitors of organic cation transport.

Among the five strong OCT3 inhibitors reported in the present study, 4, *i.e.*, the ALK inhibitors brigatinib, ceritinib and crizotinib as well as the JAK2/Flt3 inhibitor pacritinib are predicted to *in vivo* inhibit OCT3 according to the criteria defined by the U.S. FDA. This point may merit consideration in terms of possible DDIs and/or adverse effects. Indeed, even if OCT3 is presently not considered as a priority transporter by drug agencies, it is present at various sites implicated in pharmacokinetics and handles various marketed drugs. It is notably the case for metformin, for which OCT3 is involved in pharmacokinetics and pharmacological response in humans [31-33]. OCT3 may notably be implicated in intestinal absorption of metformin [9], as well as in fetal exposure to this hypoglycemic agent [11]; it may also govern its antiproliferative effects in OCT3-expressing cancer cells [34, 35]. TKIs inhibiting *in vivo* OCT3 activity may therefore be suspected to alter pharmacokinetics and/or pharmacological activity of metformin. Further studies are likely required to investigate these points. The possible inhibition by TKIs of the OCT3-mediated transport of endogenous compounds, like monoamine neurotransmitters, histamine or creatinine, and its clinical consequences may also deserve interest. This likely concerns the placental OCT3, known to be an essential component of foeto-placental homeostasis of serotonin [36].

Various molecular descriptors have been associated with inhibition of SLC transporter activities, notably for OCTs and MATEs [24, 37, 38]. Inhibition of OCT3 by heterocyclic

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aromatic amines is thus positively correlated with percentage of cationic form at pH = 7.4 and percentage of H atoms [19]. Such molecular descriptors are similarly correlated with OCT3 inhibition by TKIs (Table 1), thus supporting the idea that they correspond to robust molecular descriptors to be considered for OCT3 inhibition. Cationic positive fraction at pH = 7.4 has also a high value for OCT1, OCT2 or MATE1 inhibition [24, 37, 38], therefore suggesting that this descriptor is a key-parameter associated with impairment of OCT/MATE activities by drugs. By contrast, parameters such as lipophilicity (LogP) or TPSA are correlated with inhibition of OCT1 and OCT2, but not with blockage of OCT3 by heterocyclic aromatic amines [19] or by TKIs [14] (Table S1). This lack of contribution of lipophilicity to OCT3 inhibition however likely merits complementary investigations because it is challenged by the enhanced inhibitory potency of phenylguanidines with increased lipophilic character towards OCT3 activity [39]. Excessive lipophilicity may nevertheless make it difficult for a TKI to access the substrate binding region of OCT3 in contact with the aqueous phase, as already proposed for OCT1 [14].

Acalabrutinib, brigatinib, ceritinib, crizotinib and pacritinib failed to *trans*-stimulate OCT3 activity, in contrast to the reference substrate MPP^+. Moreover, similar accumulation of these TKIs were found in HEK-CTR and HEK-OCT3 cells, without impairment by the OCT3 inhibitor corticosterone. Taken together, these data suggest that these TKIs are not substrates for OCT3. OCT3 is therefore unlikely to play a major role in their pharmacokinetic properties. In the same way, the TKI imatinib blocks OCT3, but is not handled by this transporter [14, 40]. Such data consequently suggest that TKIs likely interact with OCT3 through *cis*-inhibiting its activity, without being transported.

In summary, various TKIs (13/25) were demonstrated to behave as moderate or strong inhibitors of OCT3. For some of them like brigatinib, ceritinib, crizotinib and pacritinib, realistic clinical concentrations were predicted to *in vivo* inhibit OCT3 activity, which may have consequences in terms of DDIs or toxicity. OCT3 may therefore appear as a drug transporter worthy of interest when considering the use of TKIs.

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ACKNOWLEDGMENTS

A.T. Nies was in part funded by the Robert Bosch Stiftung (Stuttgart, Germany) and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy - EXC 2180 – 390900677.

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LEGENDS TO FIGURES

Figure 1. Concentration-dependent inhibition of OCT3 activity by TKIs.

HEK-OCT3 cells were incubated with 10 µM DiASP for 5 min at 37 °C, in the absence or presence of various concentrations of TKIs (from 1 nM to 30 µM) or of 100 µM corticosterone, used here as a reference OCT3 inhibitor. After washing with PBS, intracellular accumulation of DiASP was determined by spectrofluorimetry and normalized to protein content. Data are expressed as percentages of OCT3 activity found in control cells exposed only to DiASP, arbitrarily set at 100%; they are the means ± SD of at least three independent experiments. TKI IC_{50} values as well as 95 % confidence intervals (in brackets) are indicated on the top of graphs.

Figure 2. Reversibility of TKI-mediated OCT3 activity inhibition.

HEK-OCT3 cells were first incubated in the absence or presence of 10 µM TKIs for 5 min at 37°C. After washing, cells were either used directly for OCT3 activity measurement, performed as described in Materials and Methods, or were re-incubated in TKI-free medium for 3 h before OCT3 activity measurement. Data are expressed as percentages of OCT3 activity inhibition and are the means ± SD of at least three independent experiments. ***, $p<0.05$ when compared to counterparts not subjected to a 3 h TKI-free post-incubation.

Figure 3. *Trans*-stimulation of OCT3 activity by TKIs.

HEK-OCT3 cells were first incubated in the absence (untreated) or presence of 1 mM unlabeled MPP^+ or 30 µM TKIs for 30 min at 37 °C. After washing with PBS, cells were next re-incubated with 6 nM [^{3}H]-MPP^+ for 5 min at 37 °C. Intracellular accumulation of [^{3}H]-MPP^+ was finally determined by scintillation counting. Data were expressed as percentages of [^{3}H]-MPP^+ accumulation found in control untreated cells, arbitrarily set at 100% and indicated by a dashed line on the graph; they are the means ± SD of three independent assays. ***, $p < 0.001$ when compared to untreated cells.

Figure 4. Accumulation of TKIs in HEK-CTR and HEK-OCT3 cells.

HEK-CTR and HEK-OCT3 cells were incubated with 10 µM TKIs or 10 µM DiASP, in the absence or presence of 100 µM corticosterone, for 5 min at 37 °C. After washing with PBS, intracellular accumulations of TKIs and DiASP were determined by LC-MS/MS and spectrofluorimetry, respectively, and normalized to protein content. Data are the means ± SD of

four independent assays. ***, $p<0.001$; NS, not statistically significant. FAU, fluorescence arbitrary unit.

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TKI	Main targeted kinase	% inhibition of OCT3 activity (Mean ± SD)
Abemaciclib	CDK4/6	69.4 ± 7.1 ***
Acalabrutinib	BTK	88.6 ± 6.3 ***
Afatinib	EGFR	76.3 ± 9.3 ***
Alectinib	ALK	11.3 ± 16.7
Brigatinib	ALK	103.3 ± 7.7 ***
Cabozantinib	VEGFR2, c-Met, c-Kit, Axl and Flt3	22.8 ± 29.8
Ceritinib	ALK	99.9 ± 7.9 ***
Crizotinib	ALK	99.8 ± 4.3 ***
Dacomitinib	EGFR	71.4 ± 13.0 ***
Entrectinib	Pan-TRK, ROS1, and ALK	77.4 ± 10.8 ***
Ibrutinib	BTK	75.1 ± 14.2 ***
Itacitinib	JAK1	0.0 ± 10.1
Lapatinib	ErbB-2 and EGFR	1.7 ± 7.3
Lenvatinib	VEGFR1-3, FGFR1-4, PDGFR, Kit, and Ret	67.0 ± 11.3 ***
Lorlatinib	ROS1 and ALK	41.0 ± 3.6 ***
Neratinib	ErbB-2 and EGFR	39.5 ± 7.6 ***
Nintedanib	VEGFR, FGFR and PDGFR	47.6 ± 12.4 ***
Osimertinib	EGFR	48.5 ± 19.8 ***
Pacritinib	JAK2 and Flt3	90.0 ± 5.8 ***
Ponatinib	CDK4/6	79.2 ± 7.1 ***
Regorafenib	VEGFR1/2/3, PDGFRβ, Kit, Ret and Raf-1	29.0 ± 24.9 *
Ribociclib	CDK4/6	74.3 ± 7.3 ***
Ruxolitinib	JAKs	47.6 ± 11.4 ***
Tofacitinib	JAKs	46.2 ± 15.0 ***
Vemurafenib	B-Raf	16.0 ± 3.1

Table 1. Effect of 10 µM TKIs on OCT3 activity in HEK-OCT3 cells.

ALK, anaplastic lymphoma kinase; BTK, Bruton's tyrosine kinase; c-Met, hepatocyte growth factor receptor (HGFR); CDK, cyclin-dependent kinase; EFGR, epidermal growth factor receptor (also known as ErbB-1 or HER1); ErbB-2, human epidermal growth factor receptor-2 (also known as HER2); FGFR, fibroblast growth factor receptor; Flt3, Fms-like tyrosine kinase 3; JAK, Janus kinase ; PDGFR, platelet-derived growth factor;

TRK, tropomyosin receptor kinase (also known as NTRK, neurotrophic tyrosine receptor kinase); VEGFR, vascular endothelial growth factor.

*, $p<0.05$ and ***, $p<0.001$, when compared to control cells not exposed to TKIs.

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Table 2. List of TKI molecular descriptors correlated with OCT3 activity inhibition by 10 μM TKIs.

Molecular descriptor		**Correlation with OCT3 inhibition %**	
Block	**Name**	**Pearson coefficient (r)**	**Significance (p value)**
Constitutional indices (n=47)[a]	Mean atomic van der Waals volume (scaled on Carbon atom) (Mv)	-0.4682	0.018
	mean atomic Sanderson electronegativity (scaled on Carbon atom) (Me)	-0.4255	0.034
	Percentage of H atoms (H%)	0.4886	0.013
	Percentage of C atoms (C%)	-0.4501	0.024
	mean absolute charge (charge polarization) (Qmean)	-0.4135	0.040
Charge descriptors (n=16)	percentage of cationic forms at pH=7.4	0.4946	0.012
	Unsaturation index (Ui)	-0.4184	0.037
Molecular properties (n=20)			

[a]n = total number of molecular descriptors/block included in the study; these molecular descriptors were given by Dragon 7.0 software, excepted the % of cationic form at pH = 7.4 for the block "Charges descriptors", which was determined using MarvinSketch 20.19.0 software.

Table 3: Prediction of *in vivo* OCT3 inhibition by TKIs.

Drug	**C_{max}**	**F_u**	**$I_{max,u}$** (nM)	**IC_{50}** (nM)	**Ratio $I_{max,u}/IC_{50}$**	**Predicted *in vivo* inhibition**	**Reference**
Acalabrutinib	1209 nM	0.025	30.2	1841.0	0.02	No	[41]
Brigatinib	2482 nM	0.340	844.0	41.0	20.59	Yes	[29]
Ceritinib	1800 nM	0.028	50.4	28.2	1.79	Yes	[42]
Crizotinib	1061 nM	0.093	98.7	106.1	0.93	Yes	[43]
Pacritinib	22500 nM	0.011	247.5	854.9	0.26	Yes	[44]

C_{max}, maximum serum concentration; F_u, unbound fraction; $I_{max,\,u}$, maximum unbound serum concentration.

An *in vivo* inhibition can be predicted if the ratio $I_{max,u}/IC_{50} > 0.1$ according to the US FDA [12].

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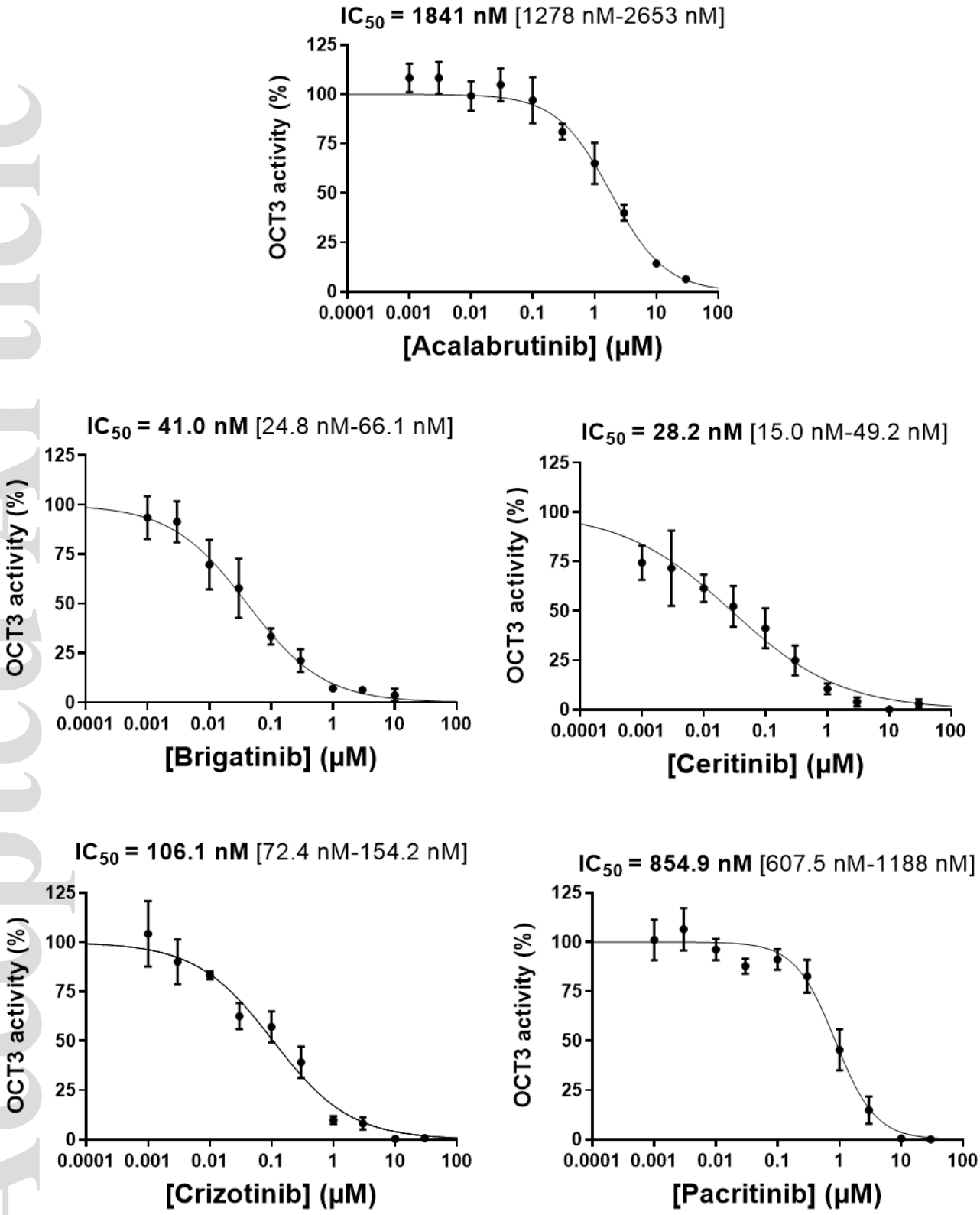


Figure 1

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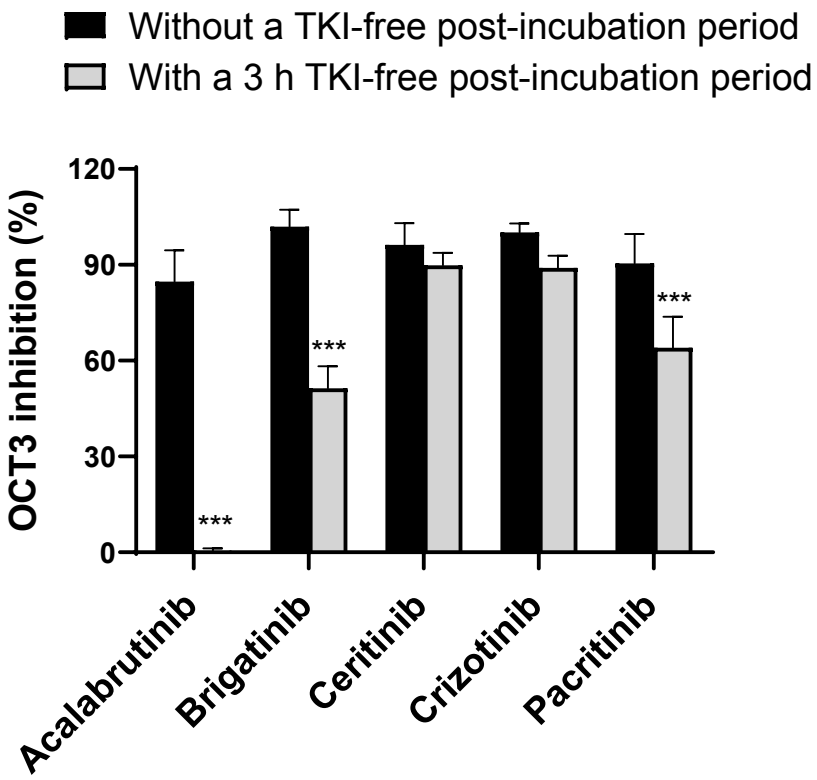


Figure 2

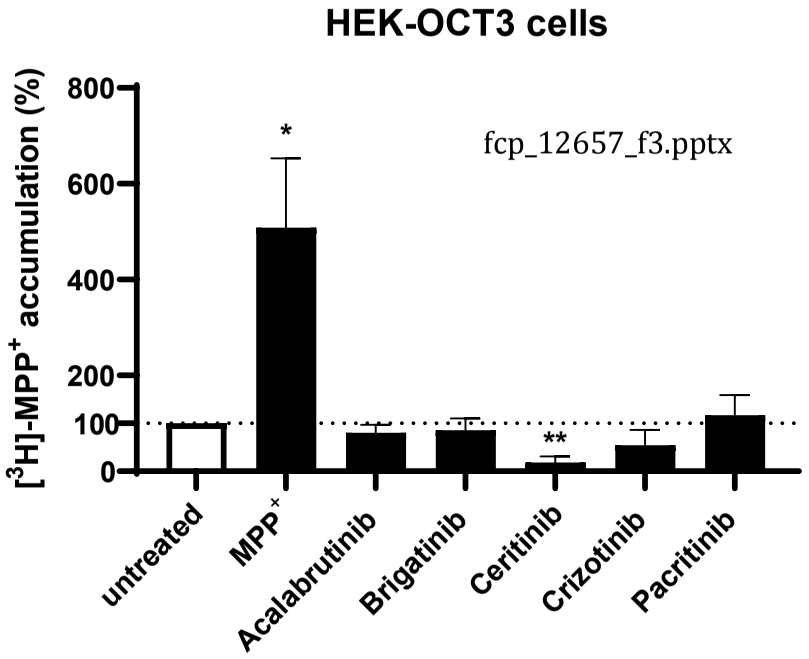


Figure 3

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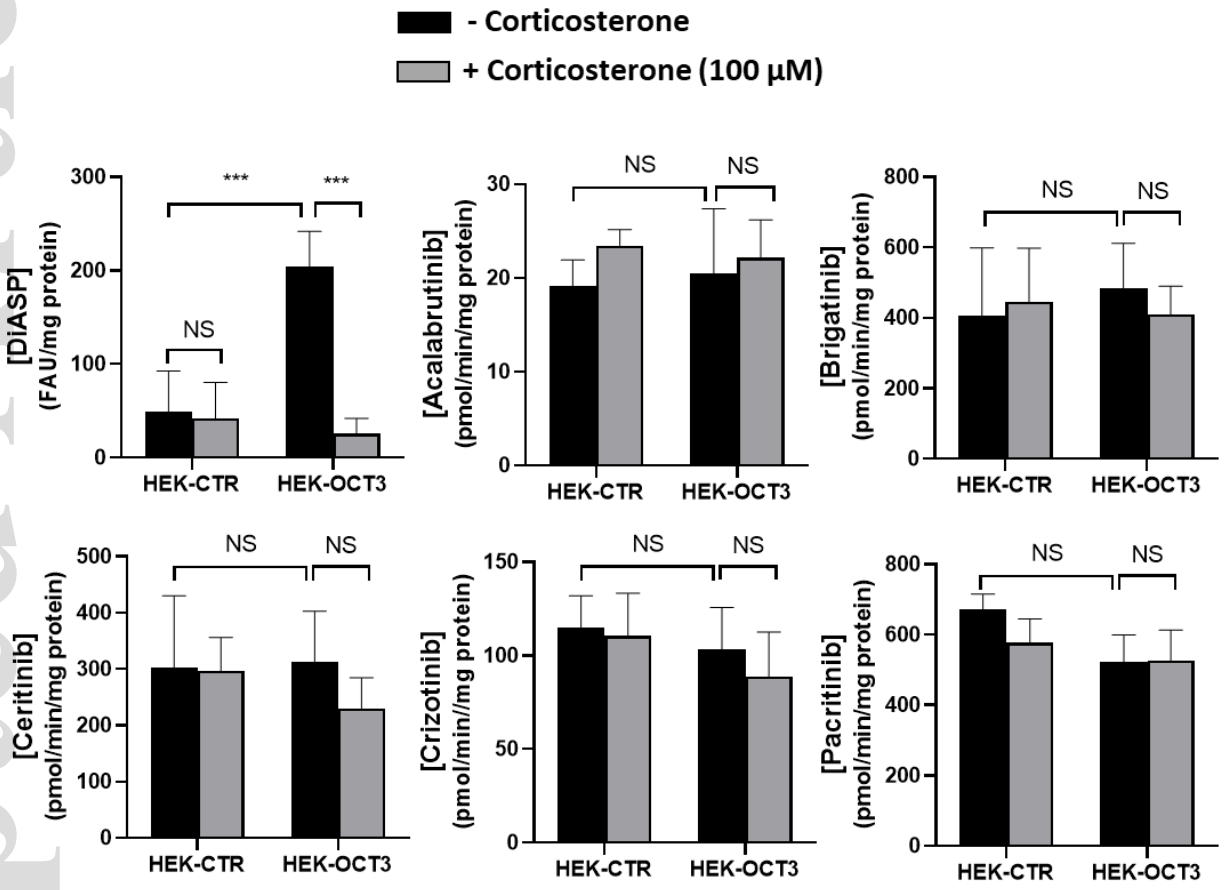


Figure 4