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FULL PAPER

The Evaluation of L-Tryptophan Derivatives as Inhibitors of the L-Type Amino Acid Transporter LAT1 (SLC7A5)

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Abstract: A series of derivatives of the substrate amino acid L-tryptophan have been investigated for inhibition of the L-type amino acid transporter LAT1 (SLC7A5), which is an emerging target in anticancer drug discovery. Of the four isomeric 4-, 5-, 6-, or 7-benzyloxy-L-tryptophans, the 5-substituted derivative was the most potent, with an IC$_{50}$ of 19 μM for inhibition of [^{3}H]-L-leucine uptake into HT-29 human colon carcinoma cells. The replacement of the carboxy group in 5-benzyloxy-L-tryptophan by a bioisosteric tetrazole moiety led to a complete loss in potency. Likewise, the corresponding tetrazolide derived from L-tryptophan itself was found to be neither a substrate nor an inhibitor of the transporter. Increasing the steric bulk at the 5-position, while reasonably well tolerated in some cases, did not result in an improvement in potency. At the same time, none of these derivatives was found to be substrates of LAT1-mediated transport.

Introduction

LAT1 (SLC7A5) is an L-type amino acid transporter that effects sodium-independent transfer of branched or aromatic neutral amino acids across the cell membrane from the exterior of cells into the cytoplasm.[1-4] As for all L-type transporters, LAT1 functions as an obligate antiporter, with amino acid import being strictly coupled to export of other amino acids, mostly glutamine.[5,6] In cells, LAT1 is covalently linked, via a disulfide bond at the extracellular side of the membrane, to the heavy chain of the 4F2 cell-surface antigen (4F2hc, SLC3A2) and it has been suggested that heterodimerization is functionally essential;[2-4] however, LAT1-mediated transport into proteoliposomes has also been reported to occur independent of heterodimerization.[6]

In contrast to the closely related LAT2 (SLC7A8), which is found at the plasma membrane of virtually all cell types,[7-10] LAT1 is specifically expressed in endothelial cells of tissue barriers, such as the blood brain barrier, the placenta, and the spleen[2,4,11,12] In addition, and most important for the work described here, LAT1 is

also highly upregulated in various types of human cancers,[13-17] thus suggesting that LAT1-mediated amino acid transport is critically involved in the supply of essential amino acids to rapidly growing tumor cells. As a consequence, LAT1 has emerged as an attractive target for new, selective anticancer drugs.

The most advanced LAT1 inhibitor reported to date is the triiodothyronine (T3)-derived amino acid **JPH203** (also previously known as **KYT-353**) (Fig.1), which has recently been advanced to Phase II clinical studies in Japan (UMIN000034080) in patients with advanced biliary tract cancers.[18,19] Furthermore, **OKY 034**, an allosteric binder of LAT1, is currently evaluated in a Phase I/IIa clinical study (UMIN000036395) in advanced pancreatic cancer patients.[20] The structure of this compound has not been publicly disclosed.
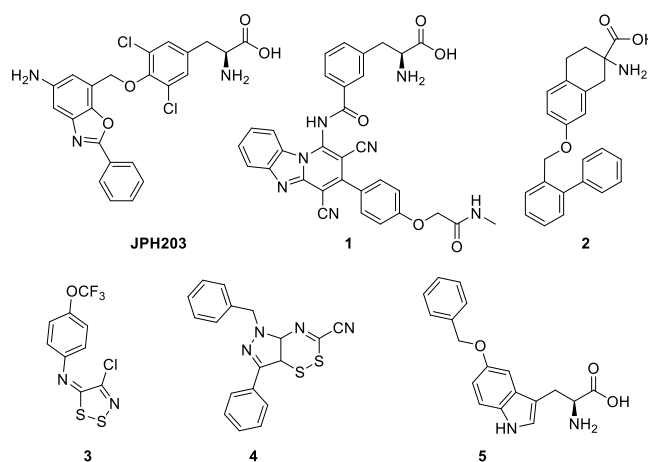
Figure 1. Structures of LAT1 inhibitors reported in the literature.

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JPH203 potently reduces LAT1-mediated [^{14}C]-L-leucine uptake into human HT-29 colon carcinoma cells (IC$_{50}$ = 0.06 μM) and it inhibits tumor cell growth *in vitro* with single digit μM IC$_{50}$ values.[18] The compound has also been shown to retard tumor growth in mouse models of colon cancer (human HT-29 xenograft)[18] and in a genetically engineered mouse model of anaplastic thyroid carcinoma (ATC).[21] A number of tyrosine derivatives related to **JPH203** have also been described;[22] however, these compounds appear to be less potent LAT1 blockers than **JPH203**. In addition to tyrosine derivatives modeled after the thyroid hormone T3, SAR studies by Thomas and co-workers have identified *meta*-substituted phenylalanines as a promising chemotype for the development of new LAT1 inhibitors (with an IC$_{50}$ value of 6.9 μM for the best compound in the series).[23] This work also demonstrated that the replacement of the carboxy group in phenylalanine or tyrosine (or their derivatives) by a tetrazole or acylsulfonamide carboxylate isostere resulted in a loss of either inhibitory activity against or substrate properties for LAT1.[24] In contrast, some level of LAT1-mediated transport was retained for amino acid esters and hydroxamic acids, thus confirming previous data by Kanai and co-workers (on esters).[25] These observations both confirm and extend previous findings that had highlighted the importance of both a free carboxy group and a primary amino group for transport by LAT1, in addition to a hydrophobic side chain moiety for full substrate capacity.[26]

The *meta*-substituted phenylalanine derivative **1** (Fig. 2) has been reported by Huttunen and co-workers as a slowly reversible LAT1 inhibitor with favorable pharmacokinetic properties (IC$_{50}$ = 18.2 μM).[27] Most recently, our own laboratory has shown that the constrained L-*meta*-tyrosine derivative **2** and two related structures inhibit transport of [^{3}H]-L-leucine into HT-29 cells with IC$_{50}$ values between 100 and 250 nM.[28] These compounds are related to previously investigated conformationally constrained melphalan analogs.[29,30] intriguingly, the cryo-EM structures of complexes of these inhibitors with LAT1-4F2hc showed the transport domain to be present in an outward-open conformation.[28] This contrasts with the inward-open conformation that has been reported for the unliganded transporter[31,32] and for its complex with the non-specific, low potency LAT1 inhibitor 2-aminobicyclo-[2.2.1]heptane-2-carboxylic acid (BCH).[31]

Only few potent inhibitors of LAT1 have been reported so far that are not derived from L-phenylalanine / L-tyrosine as structural templates. This includes dithiazole- and dithiazine-based structures such as **3** and **4**, respectively, which are the only potent, non-amino acid-based inhibitors that have been reported so far, with IC$_{50}$ values in the double digit or even sub-μM range).[33]

Most important for the work discussed here is a recent report by Ecker and co-workers, who identified 5-benzyloxy-L-tryptophan (**5**) as a potential LAT1 inhibitor in a virtual screening campaign based on a LAT1 homology model.[34] The predicted activity of **5** was subsequently confirmed experimentally in a transport assay with LAT1-containing proteoliposomes, where **5** inhibited the [^{3}H]-His$_{ex}$/His$_{int}$ antiport activity of LAT1 with an IC$_{50}$ of 1.48 μM.

As will be detailed below, we have independently discovered the LAT1-inhibitory activity **5** in our own laboratory, as part of a broader effort directed at the exploration of L-tryptophan as an alternative starting point for the development of new LAT1 inhibitors. In this contribution, we describe the synthesis of a series of derivatives of **5** and the assessment of their LAT1-inhibitory activity. While this work has not yet yielded an L-tryptophan-based LAT1 inhibitor more potent than **5**, it has provided important initial insights into the SAR around this lead structure.

Results and Discussion

Benzyloxy-L-tryptophans. As an initial step in our exploration of L-tryptophan derivatives as potential LAT1 inhibitors, we assessed the ability of the four isomeric benzyloxy-tryptophans **5**, **5a**, **5b**, and **5c** (Schemes 1 and 2) to inhibit [^{3}H]-L-Leu uptake into HT-29 human colon carcinoma cells, which are known to express high levels of LAT1.[18,35] In analogy to the situation with **JPH203** and related derivatives, where the addition of steric bulk to the L-tyrosine side chain (in addition to 3,3'-dichlorination) results in the conversion of a substrate amino acid into a potent inhibitor,[18,22] we hoped that this approach would also be applicable to L-tryptophan. While the steric bulk of a benzyloxy group is obviously not comparable with that of the (2-phenylbenzo[d]oxazol-7-yl)methoxy substituent in **JPH203**, we expected that the benzyloxy modification would at least produce a weak inhibitory effect that could then be enhanced by further modification.

The synthesis of **5** was accomplished in five steps from commercial 5-hydroxy-L-tryptophan (**6**) *via* known intermediate **8**[36] (Scheme 1).
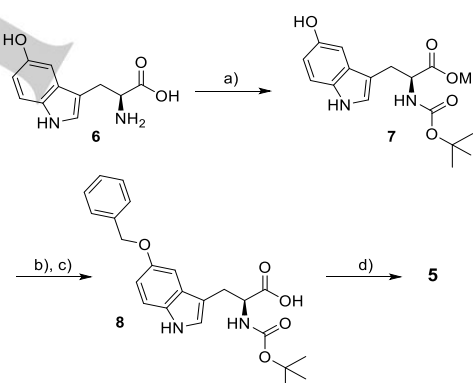
Scheme 1. *Reagents and conditions*: a) SOCl$_2$, MeOH, 0 °C to RT, 18 h, then Boc$_2$O, DIEA, CH$_2$Cl$_2$, RT, 4 h, 97% (2 steps); b) benzyl bromide, Cs$_2$CO$_3$, acetone, RT, 18 h, 65%; c) LiOH•H$_2$O, THF/H$_2$O, 5 °C, 18 h, 99%; d) 4N HCl/dioxane, RT, 1 h, 83%. **5** was isolated as the hydrochloride salt.

Thus, conversion of **6** into the corresponding methyl ester by treatment with SOCl$_2$/MeOH followed by Nα-BOC protection gave **7**, which was alkylated with benzyl bromide. Ester saponification and BOC-cleavage with HCl then furnished the desired **5** as the hydrochloride salt in 50% overall yield for the 5-step sequence from **6**.

Tryptophan derivatives **5a**, **5b**, and **5c** were prepared from the corresponding hydroxy indoles as exemplified in Scheme 2 for 6-benzyloxy-tryptophan (**5b**). The key step of these syntheses consists in the nucleophilic opening of the commercially available Cbz-protected aziridine **14** with a TBS-protected hydroxy indole, such as **10b**[37] in Scheme 2;[38,39] in the presence of Yb(OTf)$_3$ as a Lewis acid, the fully protected tryptophan derivative **11b** was obtained in 60% yield; yields were somewhat lower for the

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corresponding reactions with 4- and 7-TBSoxy indole, respectively.

A three-step sequence comprising OH-deprotection, amine deprotection and amine re-protection then afforded **12b** in 60% overall yield from **11b**. Alkylation of **12b** with benzyl bromide followed by methyl ester cleavage with LiOH and subsequent BOC-cleavage with 4 N HCl/dioxane finally furnished **5b** (45% yield based on **12b**).
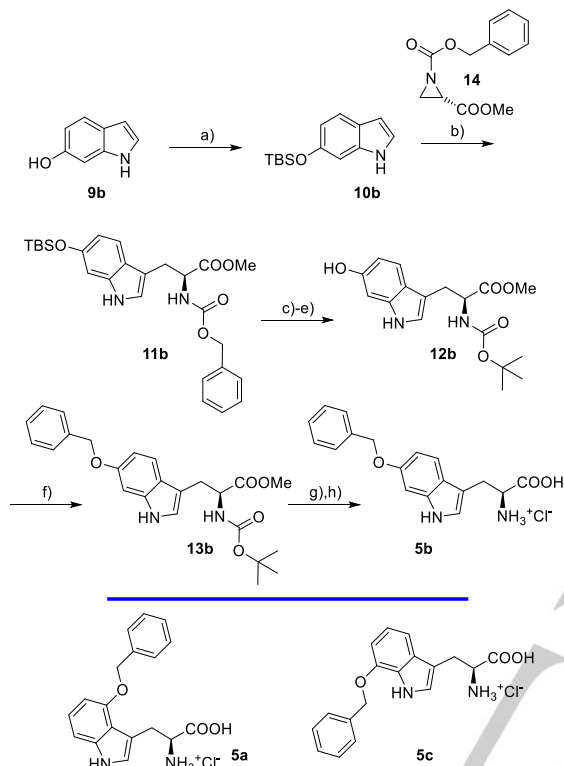
Scheme 2. *Reagents and conditions*: a) TBSCl, imidazole, DMF, RT, 18 h, 75%; b) **14**, Yb(OTf)₃, CH₂Cl₂, RT, 18 h, 60%; c) TBAF, THF, RT, 1 h, 89%; d) H₂, Pd/C, MeOH, RT, 18 h, 87%; e) Boc₂O, TEA, CH₂Cl₂, 0 °C to RT, 18 h, 78%; f) benzyl bromide, Cs₂CO₃, acetone, RT, 18 h, 76%; g) LiOH·H₂O, THF/H₂O, 5 °C, 18 h, 99%; h) 4N HCl/dioxane, RT, 1 h, 62%. Tryptophan derivatives **5a-5c** were isolated as hydrochloride salts.

Table 1. Inhibition of [³H]-L-Leu uptake into HT-29 cells by benzyloxy-L-tryptophan derivatives **5**, **5a**, **5b**, and **5c**.[a]

Compound	IC₅₀ [μM][b]
5	18.8 (7.3 – 48.3)
5a	> 100
5b	> 100
5c	> 100
JPH203	0.14 (0.09 - 1.8)

[a] For experimental details see the Experimental Section. [b] Numbers represent mean values from at least three independent experiments each carried out in triplicate. Numbers in parentheses indicate 95% confidence intervals.

as 5-(2-fluoroethoxy)-L-tryptophan, which has been demonstrated to exhibit LAT1 substrate properties and has been successfully employed for PET imaging of tumors in mice (as its [¹⁸F]-derivative).[40, 41] Our findings also suggest that the 5-position of L-tryptophan is not a suitable drug attachment site for prodrugs meant to exploit LAT1 transport for selective cell uptake, contrary to the conclusions presented in ref.[42]
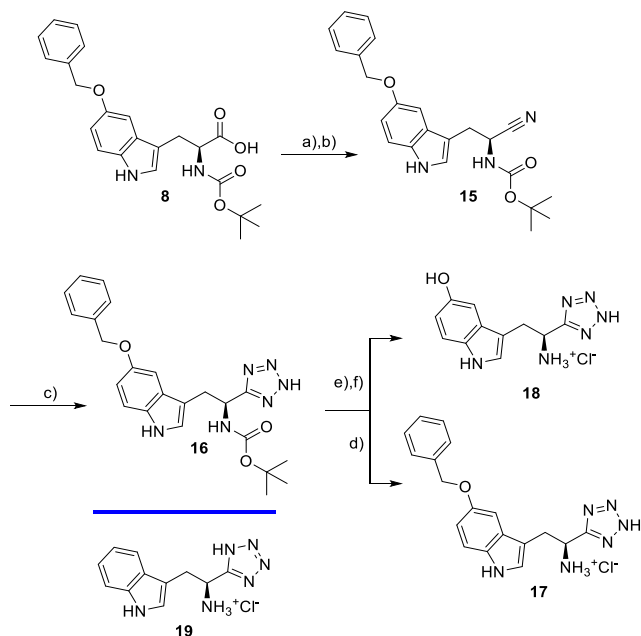
In light of the findings above, only compound **5** was pursued as a lead scaffold for further SAR studies. In a first step, we assessed the replacement of the carboxy group in **5** by a tetrazole moiety as a well-established carboxylate isostere.[43] As discussed in the Introduction, the incorporation of various carboxylate isosteres, including the tetrazole moiety, into phenylalanine derivatives completely abolished inhibitory activity and substrate characteristics.[24] However, we felt that it was still important to determine the effect of an isosteric carboxylate replacement in compound **5** independently, rather than relying on extrapolating conclusions from a different scaffold.

As outlined in Scheme 3, the synthesis of the respective tetrazolide **17** proceeded through nitrile **15**, which was obtained from carboxylic acid **8** in 59% overall yield by activation as the mixed anhydride with ethyl carbonic acid, amide bond formation, and then dehydration of the amide with trifluoroacetic anhydride. Nitrile **15** underwent Huisgen [2+3] cycloaddition with sodium azide to furnish **16** in excellent yield (93%); the latter was deprotected with 4 N HCl/dioxane to give the desired tetrazolide **17** in 33% overall yield from **15**. In addition to **17**, intermediate **16** was also converted into tetrazolide **18** by benzyl ether cleavage prior to BOC-removal.

None of these analogs showed any inhibition of [³H]-L-leucine transport into HT-29 cells or was amenable to LAT1 transport (based on the lack of compound-induced [³H]-L-leucine efflux from pre-loaded cells) at concentrations up to 50 μM. To put these findings into context, we also prepared compound **19** (from L-tryptophan, in analogy to the synthesis of **17** from **8**) (Scheme 3), in order to establish the baseline effect for a tetrazolide with an unmodified tryptophan side chain. Like **17** and **18**, tetrazolide **19** was neither an inhibitor of nor a substrate for LAT1-mediated transport. In contrast, L-Trp itself elicited clear [³H]-L-leucine efflux from preloaded HT-29 cells (Fig. S6).

Having established the 5-position on the indole ring as the seemingly most favorable site for the attachment of space-filling substituents on tryptophan-based LAT1 inhibitors, we next investigated if sterically more demanding groups than a benzyloxy

As illustrated by the data summarized in Table 1, out of the four benzyloxy-substituted L-tryptophan derivatives **5**, **5a**, **5b**, and **5c**, only 5-benzyloxy-L-tryptophan (**5**) was found to clearly inhibit LAT1-mediated [³H]-L-leucine transport into H-29 cells at concentrations below 100 μM (IC₅₀ of 19 μM) (Fig. S1). As alluded to in the Introduction, the inhibition of LAT1-mediated [³H]-L-leucine transport by **5** was in line with previous observations by Ecker and co-workers on the effects of this compound on [³H]-L-histidine transport into LAT1 containing proteoliposomes.[29] 5-benzyloxy-L-tryptophan (**5**) was ca. 25-fold less potent in our [³H]-L-leucine uptake assay than the clinical compound **JPH203**; while this difference is significant, it still made **5** a feasible starting point for optimization.

None of the different benzyloxy-L-tryptophan derivatives induced efflux of [³H]-L-leucine from preloaded HT-29 cells at concentrations up to 100 μM, thus indicating that they are not LAT1 substrates (Fig. S1). This is contrary to L-tryptophan derivatives with smaller substituents on the indole moiety, such

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Scheme 3. *Reagents and conditions*: a) ethyl chloroformate, NMM, THF, −10 °C, 20 min, then 7N NH₃/MeOH −10 °C to RT, 1 h, 82%; b) TFAA, pyridine, THF, −10 °C to RT, 2 h, 77%; c) NaN₃, TEA, acetic acid, toluene, reflux, 93%; d) 4N HCl/dioxane, RT, 1 h, 79%; e) Pd/C, H₂, MeOH, RT, 6 h, 91%; f) 4N HCl/dioxane, RT, 1 h, 66%. All final products were isolated as hydrochloride salts.

substituent could lead to enhanced potency over **5**. To this end, we prepared a series of derivatives of **5**, each carrying an additional aromatic group at the 2-, 3-, or 4-position of the benzyl moiety.

As exemplified in Scheme 4 for the *ortho*-substituted series, these compounds were obtained through Suzuki cross couplings with iodide **20a** as the key step; the latter was obtained from Nα-BOC-protected methyl ester **7** by alkylation with *o*-iodobenzyl bromide (**23a**).The Suzuki couplings proceeded in good to excellent yields (84%-95%) and the coupling products were converted into the desired L-tryptophan derivatives by ester saponification with LiOH (77%-98%) and BOC-removal with 3N HCl/CPME (50%-89%). The materials were thus obtained as hydrochloride salts and were used as such in the biological tests. Deprotection of **20a** gave the *o*-iodo substituted derivative of **5**, i. e. **21a**, in 75% overall yield.

As can be seen from the data summarized in Table 2, further extension of the benzyloxy group in **5** resulted in a modest potency increase for an *ortho* thien-3-yl (**22ae**, IC₅₀ 5.5 μM) or an *ortho* phenyl (**22a**, IC₅₀ 8 μM) substituent, although the value for **22ae** has to be interpreted with some care, given the shape of the dose-response curve (see the SI). Several compounds exhibited IC₅₀ values between 10 and 20 μM, which makes them equally active transport inhibitors as the parent compound **5**. No clear pattern is discernible from the inhibition data with regard to the most favorable attachment site for an additional aromatic ring on the benzyloxy group; in fact, the SAR across all derivatives investigated is relatively flat and does not provide any clear guidance on how to improve the activity of **5** by modification of the benzyloxy group. Independent of this, it is interesting to note that, like **5**, none of the substituted analogs **21** or **22** was found to be a substrate for LAT1-mediated transport in our assay configuration. These findings re-confirm the above conclusion that the 5-position

of L-tryptophan is not a suitable drug attachment site for prodrugs meant to exploit LAT1 transport for selective cell uptake.
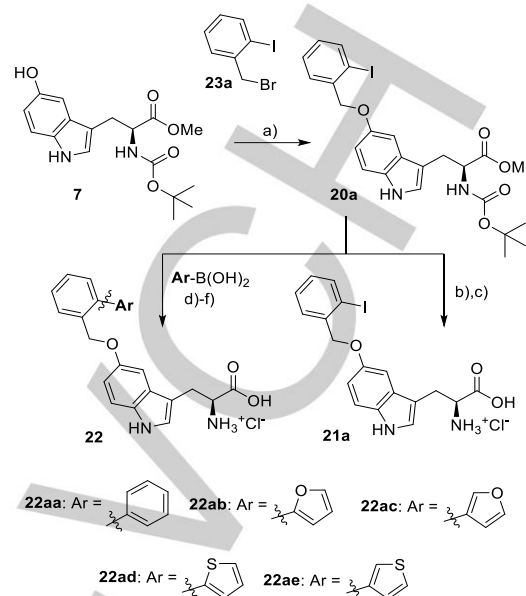
Scheme 4. *Reagents and conditions*: a) **23a**, Cs₂CO₃, acetone, RT, 15 h, 82%; b) LiOH•H₂O, THF/H₂O, 0 °C to RT, 2 h, 94%; c) 3N HCl/CPME, RT, 3 h, 80%; d) ArB(OH)₂, Pd(OAc)₂, PPh₃, K₂CO₃, DME/H₂O, 90 °C (microwave), 15 min.; e) LiOH•H₂O, THF/H₂O, 0 °C to RT, 2-18 h; f) 3N HCl/CPME, RT, 1 h, 61% (**22aa**, 3 steps); 78% (**22ab**, 3 steps); 49% (**22ac**, 3 steps); 76% (**22ad**, 3 steps); 66% (**22ae**, 3 steps). All final products were obtained as hydrochloride salts.

In addition to the L-tryptophan derivatives carrying a mono-arylated benzyloxy group at the 5-position of the indole moiety, we have also investigated L-tryptophan derivative **33**, where the 5-O-benzyl group in **5** was modified such as to arrive at the same bulky 5-O-substituent that is attached to the phenolic hydroxy group of the dichlorotyrosine core in **JPH203**.

As illustrated in Scheme 5, this compound was prepared from partially protected 5-hydroxy tryptophan **30** by alkylation with bromide **29** in the presence of Cs₂CO₃, followed by reduction of the nitro group with SnCl₂ and subsequent concomitant cleavage of the trifluoroacetamide and methyl ester moieties with LiOH. The synthesis of the crucial bromide intermediate **29** proceeded through alcohol **28**; the latter was prepared following a procedure that has been reported in the patent literature[44] in the context of the synthesis of **JPH203**, departing from commercially available nitrophenol **24**. The latter was reduced by hydrogenation over Pd/C to the corresponding aniline **25** in 92% yield. Benzoylation of **25** followed by *para*-selective nitration with a mixture of nitric acid and acetic acid and treatment of the resulting nitro arene **26** with polyphosphoric acid trimethylsilyl ester in refluxing chlorobenzene furnished the desired benzoxazole **27** in 73% overall yield from **24**. Reduction of the ester moiety with lithium aluminum hydride followed by Appel reaction of the ensuing alcohol **28** finally provided bromide **29** in 44% yield for the two-step sequence from **27**.

Assessment of **33** in the [³H]-L-leucine transport assay revealed an IC₅₀ value for inhibition of [³H]-L-leucine transport of 18 μM. The activity of **33** is thus similar to the activity of several of the mono-arylated 5-benzyloxy-L-tryptophan derivatives **22**. Overall, the results obtained for compounds **21**, **22**, and **33** suggest that

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space exists around the 5-benzyloxy substituent of LAT1-bound **5** that can be filled with additional substituents without abrogating transport inhibition. At the same time, none of the expanded benzyl moieties investigated here has led to substantially enhanced LAT1-inhibitory activity over the parent compound **5**, indicating a lack of favorable hydrophobic interactions between the extension modules with the protein.

Conclusions

We have assessed a series of new L-tryptophan derivatives with bulky substituents on the benzene part of the indole ring system as potential inhibitors of the L-type amino acid transporter LAT1 (SLC7A5). Based on our initial results with benzyloxy-tryptophans **5**, **5a**, **5b**, and **5c**, the 5-position of the indole moiety appeared to be the most promising site for the attachment of space filling substituents, in order to convert the substrate amino acid L-tryptophan into a transport inhibitor. However, attempts to improve the LAT1-inhibitory activity of 5-benzyloxy-tryptophan (**5**) through substitution of the benzyl group with different aryl moieties were unsuccessful, including the attachment of the (2-phenylbenzo[d]oxazol-7-yl)methoxy group that is part of the structure of **JPH203**. In the best cases, the activity of the parent compound **5** was essentially retained; in general, within the group of compounds investigated here, the SAR is relatively flat and the observed activity differences should not be overinterpreted. Clearly, additional SAR work will be required to fully explore the potential of 5-substituted L-tryptophans as LAT1 inhibitors. Obviously, such studies will be facilitated by the structural information that has become available for complexes between LAT1 and different types of inhibitors.

While it remains to be seen if potent LAT1 inhibitors can eventually be derived from L-tryptophan, our data do show that (i) the replacement of the carboxylate group in L-tryptophan (derivatives) by an isosteric tetrazole moiety abrogates recognition by the transporter, as has been demonstrated previously for phenylalanine/tyrosine; and that (ii) the attachment of a bulky substituent to the 5-position of the indole ring system leads to a loss of substrate properties (which was complete within the accuracy limits of our assay system at the concentrations tested). The latter finding suggests that the 5-position on L-tryptophan is not a suitable attachment point for drug moieties in the construction of LAT1-directed prodrugs, contrary to previous suggestions.

In summary, we have presented the first systematic study on L-tryptophan derivatives as potential LAT1 inhibitors, based on the hypothesis that the attachment of bulky groups to the indole side chain will confer inhibitory properties on this substrate amino acid. Work along these lines is continuing in our laboratories, in order to determine if this concept could eventually lead to more potent LAT1 blockers as potential anticancer drug candidates.

Table 2. Inhibition of [³H]-Leu uptake into HT-29 cells by substituted benzyloxy-L-tryptophans **21** and **22**.[a]
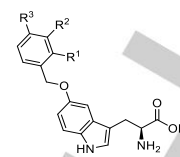
| Compound	R¹	R²	R³	IC₅₀ [μM][b]
21a	I	H	H	17 (10 - 28)
22aa	phenyl	H	H	8.0 (5.4 - 12)
22ab	furan-2-yl	H	H	32 (13 - 78)
22ac	furan-3-yl	H	H	16 (11 - 22)
22ad	thien-2-yl	H	H	12 (6.1 - 24)
22ae	thien-3-yl	H	H	5.5 (3.6 - 8.4)
21b	H	I	H	16 (6.5 - 38)
22ba	H	phenyl	H	24 (16 - 37)
22bb	H	furan-2-yl	H	20 (8.5 - 49)
22bc	H	furan-3-yl	H	40 (26 - 63)
22bd	H	thien-2-yl	H	13 (5.1 - 31)
22be	H	thien-3-yl	H	19 (15 - 24)
21c	H	H	I	23 (6.7 - 77)
22ca	H	H	phenyl	19 (13 - 28)[b]
22cb	H	H	furan-2-yl	31 (15 - 64)
22cc	H	H	furan-3-yl	26 (15 - 43)
22cd	H	H	thien-2-yl	20 (8.9 - 40)[b]
22ce	H	H	thien-3-yl	19 (11 - 32)

[a] For experimental details see the Experimental Section. [b] Numbers represent mean values from at least three independent experiments each carried out in triplicate. Numbers in parentheses indicate 95% confidence intervals. [b] These numbers are EC₅₀ rather than IC₅₀ values (see the SI for the corresponding dose-response curves).

Experimental Section

Chemistry. For general information and experimental details on the synthesis of tryptophan derivatives **5a-c**, **17-19**, **21a**, **22ab-ae**, **21b**, **22ba-22be**, **21c**, **22ca-22ce**, and **33** see the SI.

(*S*)-2-(5-(Benzyloxy)-1*H*-indol-3-yl)-1-carboxyethan-1-amine · HCl (5): Acid **8** (50 mg, 0.12 mmol, 1.0 eq.) was dissolved in HCl in dioxane (4 N, 2.0 mL,). The mixture was stirred at RT for 1 h. The crude mixture was concentrated under reduced pressure gave amino acid hydrochloride **1** as a white/grey powder (35 mg, 83%). ¹H NMR (400 MHz, DMSO-*d₆*) δ (ppm) = 10.93 (d, *J* = 2.5 Hz, 1H), 8.33 (s, 3H), 7.51 – 7.45 (m, 2H), 7.43 – 7.36

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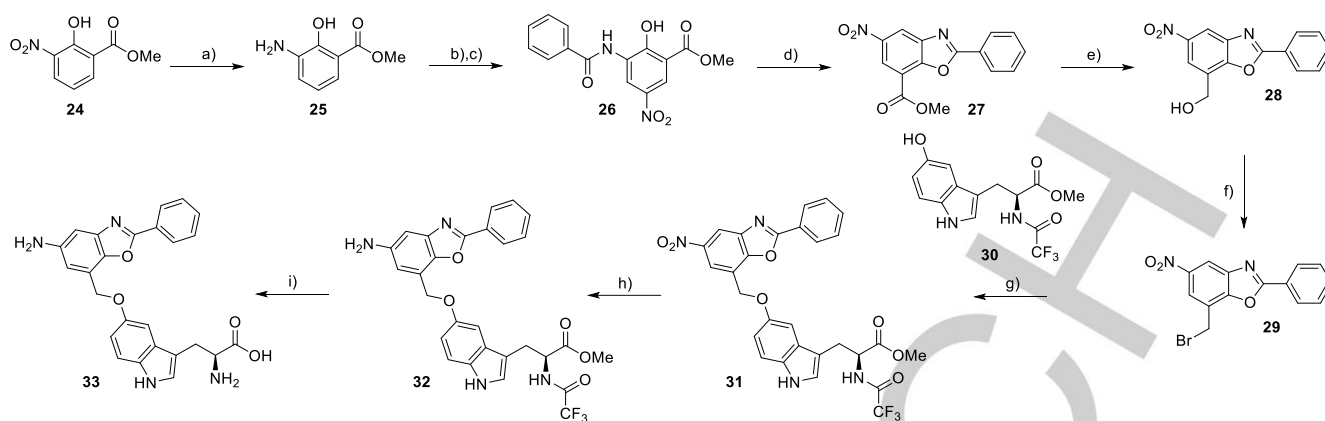
Scheme 5. *Reagents and conditions*: a) H_2, Pd/C, MeOH, RT, 18 h, 92%; b) BzCl, *N,N*-DMA, THF, 0 °C, 1 h, 99%; c) HNO_3, AcOH, RT, 2 h, 83%; d) polyphosphoric acid TMS ester, chlorobenzene, reflux, 3 h, 97%; e) $LiAlH_4$, THF, 0 °C to RT, 3 h, 52%; f) CBr_4, PPh_3, CH_2Cl_2, 0 °C to RT, 18 h, 84%; g) **30**, Cs_2CO_3, acetone, RT, 18 h, 54%; h) $SnCl_2$•$2H_2O$, EtOAc, reflux, 18 h, 78%; i) $LiOH$•H_2O, THF/H_2O, 5 °C, 18 h then 1N HCl, 12%.

(m, 2H), 7.35 – 7.30 (m, 1H), 7.27 (s, 1H), 7.26 (d, *J* = 8.7 Hz, 1H), 7.20 (d, *J* = 2.4 Hz, 1H), 6.81 (dd, *J* = 8.7, 2.4 Hz, 1H), 5.10 (s, 2H), 4.07 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.73 – 3.64 (m, 1H), 3.50 – 3.44 (m, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ (ppm) = 170.8, 152.3, 137.7, 131.5, 128.4, 127.7, 127.6, 127.4, 125.7, 112.1, 111.8, 106.5, 101.9, 69.8, 52.6, 26.1. HRMS (ESI) calculated for $C_{18}H_{19}N_2O_3$ [M+H]$^+$ 311.1390, found 311.1395.

6-((*tert*-Butyldimethylsilyl)oxy)-1*H*-indole (10b): 1*H*-Indol-6-ol (**9b**) (5.15 g, 38.7 mmol, 1.0 eq.) was dissolved in dry DMF (25 mL). TBDMSCl (7.00 g, 46.4 mmol, 1.2 eq.) and imidazole (6.58 g, 96.7 mmol, 2.5 eq.) were then added to the medium. The all mixture was stirred at RT for 18 h. Finally, EtOAc was added to the mixture and the organic phase was washed two times with $NaHCO_3$ saturated solution and two times with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (hexane/EtOAc 8:2) afforded TBS-ether**10b** as a white solid (7.15 g, 75%). ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) = 7.96 (s, 1H), 7.47 (ddd, *J* = 8.5, 0.7, 0.7 Hz, 1H), 7.09 (dd, *J* = 3.2, 2.3 Hz, 1H), 6.86 (ddd, *J* = 2.2, 0.7, 0.7 Hz, 1H), 6.72 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.48 (ddd, *J* = 3.1, 2.0, 0.9 Hz, 1H), 1.03 (s, 9H), 0.22 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ (ppm) = 151.8, 136.7, 123.3, 122.8, 121.0, 114.6, 102.5, 101.8, 25.9, 18.4, −4.2.

Methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-(6-((*tert*-butyl-dimethyl-silyl)oxy)-1*H*-indol-3-yl)propanoate (11b): 1-Benzyl 2-methyl (*S*)-aziridine-1,2-dicarboxylate (**14**) (1.78 g, 7.57 mmol, 1.0 eq.) was dissolved in dry CH_2Cl_2 (30 mL). TBS-ether **10b** (3.74 g, 15.1 mmol, 2.0 eq.) and Yb(OTf)$_3$ (4.69 g, 7.57 mmol, 1.0 eq.) were then added to the medium. The all mixture was stirred at RT for 18 h. Finally, CH_2Cl_2 was added to the mixture and the organic phase was washed two times with a $NaHCO_3$ saturated solution and two times with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (hexane/EtOAc 8:2) afforded ester **11b** as a yellowish oil (2.2 g, 60%). ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) = 7.87 (s, 1H), 7.40 – 7.27 (m, 6H), 6.85 (d, *J* = 2.3 Hz, 1H), 6.79 (d, *J* = 2.1 Hz, 1H), 6.66 (dd, *J* = 8.6, 2.1 Hz, 1H), 5.32 (d, *J* = 8.3 Hz, 1H), 5.13 (d, *J* = 12.2 Hz, 1H), 5.08 (d, *J* = 12.3 Hz, 1H), 4.69 (ddd, *J* = 8.0, 5.4, 5.4 Hz, 1H), 3.67 (s, 3H), 3.26 (d, *J* = 5.5 Hz, 2H), 1.00 (s, 9H), 0.19 (s, 6H). ^{13}C NMR (101 MHz, CDCl$_3$) δ (ppm) = 172.5, 155.9, 152.1, 137.1, 136.5, 128.6, 128.3, 128.3, 122.6, 121.8, 119.0, 114.4, 110.0, 101.9, 67.1, 54.6, 52.5, 28.2, 25.9, 18.4, −4.2.

Methyl (*S*)-2-(((benzyloxy)carbonyl)amino)-3-(6-hydroxy-1*H*-indol-3-yl)propanoate (11bA): Ester **11b** (2.20 g, 4.56 mmol, 1.0 eq.) was dissolved in THF (50 mL). Then TBAF trihydrate (2.16 g, 6.84 mmol, 1.5 eq.) was then added to the medium. The all mixture was stirred at RT for 1 h. Finally, water was added to the mixture and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried with $MgSO_4$, filtered, and concentrated under reduced

pressure. Purification by flash column chromatography (hexane/EtOAc 6:4) afforded the free phenol **11bA** as a white solid (1.49 g, 89%). ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) = ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.88 (s, 1H), 7.39 – 7.28 (m, 6H), 6.80 (d, *J* = 2.6 Hz, 1H), 6.74 (d, *J* = 2.2 Hz, 1H), 6.63 (dd, *J* = 8.5, 2.2 Hz, 1H), 5.33 (d, *J* = 8.3 Hz, 1H), 5.20 (s, 1H), 5.12 (d, *J* = 12.3 Hz, 1H), 5.07 (d, *J* = 12.3 Hz, 1H), 4.70 (ddd, *J* = 8.1, 5.7, 5.7 Hz, 1H), 3.68 (s, 3H), 3.25 (d, *J* = 5.5 Hz, 2H). ^{13}C NMR (101 MHz, CDCl$_3$) δ (ppm) = 172.7, 156.0, 152.4, 137.2, 128.7, 128.3, 128.3, 122.2, 121.8, 119.4, 110.0, 97.1, 67.1, 54.6, 52.6, 28.2.

Methyl (*S*)-2-amino-3-(6-hydroxy-1*H*-indol-3-yl)propanoate (11bB): Ester **11bA** (1.49 g, 4.05 mmol, 1.0 eq.) and Pd/C (103 mg) were dissolved in MeOH (50 mL). H_2 was directly bubbled in the solution. The all mixture was stirred at RT for 18 h. The reaction was then filtered through a pad of celite and washed two times with an excess of CH_2Cl_2. Purification by flash column chromatography (CH_2Cl_2/MeOH 9:1) afforded amine **11bB** as a yellowish solid (820 mg, 87%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm) = 10.43 (s, 1H), 8.84 (s, 1H), 7.23 (d, *J* = 8.5 Hz, 1H), 6.87 (d, *J* = 2.2 Hz, 1H), 6.68 (d, *J* = 1.8 Hz, 1H), 6.50 (dd, *J* = 8.5, 2.1 Hz, 1H), 3.58 (dd, *J* = 6.3, 6.3 Hz, 1H), 3.55 (s, 3H), 2.94 (ddd, *J* = 14.1, 6.2, 0.8 Hz, 1H), 2.85 (ddd, *J* = 14.2, 6.5, 0.8 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ (ppm) = 175.6, 152.9, 137.2, 121.4, 120.9, 118.6, 109.7, 108.9, 96.4, 55.1, 51.3, 30.9.

Methyl (*S*)-2-((*tert*-butoxycarbonyl)amino)-3-(6-hydroxy-1*H*-indol-3-yl)propanoate (12b): Amino ester **11bB** (820 mg, 3.50 mmol, 1.0 eq.) was dissolved in dry CH_2Cl_2 (15 mL) and MeOH (5 mL) was added for solubility issues. At 0 °C, dry TEA (0.71 mL, 5.3 mmol, 1.5 eq.) and Boc$_2$O (840 mg, 3.85 mmol, 1.1 eq.) were added and the mixture was stirred at RT for 18 h. Finally, water was added to the mixture and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (CH_2Cl_2/MeOH 95:5) afforded the BOC-protected amino ester **12b** as a brownish solid (913 mg, 78%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm) = 10.43 (s, 1H), 8.85 (s, 1H), 7.23 (d, *J* = 8.4 Hz, 1H), 7.12 (d, *J* = 7.7 Hz, 1H), 6.91 (d, *J* = 2.2 Hz, 1H), 6.69 (d, *J* = 2.1 Hz, 1H), 6.51 (dd, *J* = 8.5, 2.1 Hz, 1H), 4.23 – 4.11 (m, 1H), 3.59 (s, 3H), 3.02 (dd, *J* = 14.6, 5.3 Hz, 1H), 2.92 (dd, *J* = 14.6, 8.9 Hz, 1H), 1.34 (s, 9H). ^{13}C NMR (101 MHz, DMSO-*d6*) δ (ppm) = 173.0, 155.3, 153.0, 137.2, 121.5, 120.6, 118.3, 109.6, 109.0, 96.5, 78.2, 54.6, 51.7, 28.2, 27.0.

Methyl (*S*)-3-(6-(benzyloxy)-1*H*-indol-3-yl)-2-((*tert*-butoxycarbonyl)amino)propanoate (13b): Amino ester **12b** (200 mg, 0.598 mmol, 1.0 eq.), benzyl bromide (0.085 mL, 0.72 mmol, 1.2 eq.) and Cs$_2$CO$_3$ (0.253 g, 7.78 mmol, 1.3 eq.) were dissolved in dry acetone (3.0 mL). The all mixture was stirred at RT for 18 h. The reaction was quenched by adding water and

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CH$_2$Cl$_2$ and then the layers were separated. The combined organic layers were washed with water, dried with MgSO$_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (hexane/EtOAc 6:4) afforded benzyl-ether **13b** as a yellowish semi-solid (192 mg, 76%). ^{1}H NMR (400 MHz, CDCl$_3$) δ (ppm) = 7.98 (s, 1H), 7.48 – 7.36 (m, 5H), 7.35 – 7.29 (m, 1H), 6.90 – 6.84 (m, 3H), 5.09 (s, 2H), 5.06 (s, 1H), 4.62 (ddd, J = 6.1, 5.7, 5.7 Hz, 1H), 3.67 (s, 3H), 3.24 (d, J = 5.4 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (101 MHz, CDCl$_3$) δ (ppm) = 172.9, 155.9, 155.4, 137.5, 136.9, 128.7, 128.0, 127.6, 122.5, 121.7, 119.6, 110.6, 110.4, 96.1, 80.0, 70.7, 54.3, 52.4, 28.5, 28.2.

(*S*)-3-(6-(Benzyloxy)-1*H*-indol-3-yl)-2-((*tert*-butoxycarbonyl)amino) propanoic acid (13bA): Ester **13b** (180 mg, 0.424 mmol, 1.0 eq.) and LiOH monohydrate (27 mg, 0.64 mmol, 1.5 eq.) were suspended in THF/H$_2$O (2:1, 6.0 mL). Then the all mixture was stirred at 5 °C for 18 h. The pH was adjusted to 2-3 with HCl (1 N) and the mixture was concentrated under reduced pressure. Purification by flash column chromatography (CH$_2$Cl$_2$/MeOH 8:2) afforded acid **13bA** as a yellowish solid (173 mg, 99%). ^{1}H NMR (400 MHz, DMSO-*d$_6$*) δ (ppm) = 10.61 (s, 1H), 7.48 – 7.42 (m, 2H), 7.43 – 7.35 (m, 3H), 7.35 – 7.27 (m, 1H), 6.96 (d, J = 2.2 Hz, 1H), 6.88 (d, J = 2.3 Hz, 1H), 6.68 (dd, J = 8.6, 2.3 Hz, 1H), 6.43 (s, 1H), 5.09 (s, 2H), 4.03 (d, J = 6.6 Hz, 1H), 3.39 – 3.33 (m, 1H), 2.95 (dd, J = 14.5, 7.7 Hz, 1H), 1.33 (s, 9H). ^{13}C NMR (101 MHz, DMSO-*d$_6$*) δ (ppm) = 174.5, 155.0, 154.3, 137.7, 136.5, 128.4, 127.6, 127.4, 122.5, 122.2, 119.0, 110.8, 108.9, 95.8, 77.6, 69.5, 55.3, 28.3, 27.2. HRMS (ESI) calculated for C$_{23}$H$_{26}$N$_2$NaO$_5$ [M+Na]$^+$ 433.1734, found 433.1746.

(*S*)-2-(6-(Benzyloxy)-1*H*-indol-3-yl)-1-carboxyethan-1-amine · HCl (5b): Acid **13bA** (50 mg, 0.12 mmol, 1.0 eq.) was dissolved in HCl in dioxane (4 N, 2.0 mL). The mixture was stirred at RT for 1 h. The crude mixture was concentrated under reduced pressure. Purification by flash column chromatography (CHCl$_3$/MeOH/H$_2$O 65:44:12) afforded amino acid hydrochloride **5b** as a yellowish powder (26 mg, 62%). ^{1}H NMR (500 MHz, DMF-*d$_7$* with 5% TFA-*d*) δ (ppm) = 7.59 (d, J = 8.6 Hz, 1H), 7.57 – 7.50 (m, 2H), 7.47 – 7.39 (m, 2H), 7.39 – 7.32 (m, 1H), 7.28 (d, J = 1.7 Hz, 1H), 7.12 (dd, J = 1.8, 1.2 Hz, 1H), 6.84 (dd, J = 8.6, 1.8, 1.8 Hz, 1H), 5.18 (s, 2H), 4.52 (s, 1H), 3.54 (dd, J = 15.1, 5.0 Hz, 1H), 3.43 (dd, J = 15.1, 7.6 Hz, 1H). ^{13}C NMR (126 MHz, DMF-*d$_7$* with 5% TFA-*d*) δ (ppm) = 171.7, 156.2, 138.9, 138.4, 129.2, 128.5, 128.4, 124.9, 122.8, 119.8, 110.5, 108.0, 96.9, 70.7, 54.4, 27.2. HRMS (ESI) calculated for C$_{18}$H$_{17}$N$_2$O$_3$ [M−H]$^−$ 309.1245, found 309.1242.

Transport experiments. HT-29 cells were purchased from ATCC (cat. no. HTB-38) and cultured in Modified McCoy's5A medium (Gibco, cat. no. 26600–080). Media of all cell lines were supplemented with 10% FBS (Gibco, cat. no.10270), 100 U/ml penicillin (Sigma-Aldrich, cat. no. P0781), 0.1 mg/ml streptomycin (Sigma-Aldrich, cat. no. P0781). All cell lines were kept up to 50 passages or 6 months, whichever limit was reached first.

[^{3}H]-L-Leucine uptake assay: Cells were seeded at 60% confluency in a 96-well plate using complete culture medium and cultured until confluent. Cells were washed three times with 37 °C pre-warmed Na$^+$-free Hank's balanced salt solution (HBBS) containing 125 mM choline-Cl, 25 mM HEPES, 4.8 mM KCl, 1.2 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$, 1.3 mM CaCl$_2$ and 5.6 mM glucose (pH 7.4) and further incubated in the same buffer at 37 °C for 7 min. L-leucine uptake was measured for 3 min at 37 °C in the same buffer containing 30 μM [^{3}H]-L-leucine (60 Ci/mmol) and different concentrations of test compounds. Uptake was terminated by removing the solution followed by three washings with ice-cold Na$^+$-free HBBS. Cells were lysed and mixed with Microscint20 (Perkin-Elmer Life Sciences). The radioactivity was measured with a scintillation counter (TopCount NXT, Perkin-Elmer Life Sciences). In this assay system, the IC$_{50}$ value for inhibition of [^{3}H]-L-leucine uptake in HT-29 cells by **JPH203** was between 100 nM and 200 nM. (See also ref.[21]).

[^{3}H]-L-Leucine efflux assay: The same protocol as for the uptake was used with the following differences after the initial washing- and starvation-step. Cells were preloaded for 5 min at 37 °C in the Na$^+$-free HBBS

containing 30 μM [^{3}H]-L-leucine (60 Ci/mmol). After washing three times with Na+-free HBBS (4 °C), efflux of radioactivity was induced by incubation in the presence or absence of indicated concentrations of test compounds for 1.5 min at 37 °C. The medium was then collected and its radioactivity was counted. The cells were washed three times with ice-cold Na$^+$-free HBBS. Cells were lysed and mixed with Microscint20 (Perkin-Elmer Life Sciences). The radioactivity was measured with a scintillation counter (TopCount NXT, Perkin-Elmer Life Sciences). The [^{3}H]-L-leucine efflux values were expressed as percentage radioactivity (radioactivity of medium)/(radioactivity of the medium + radioactivity of the cells)). **JPH203** did not induce leucine efflux in this system up to a concentration of 100 μM (the highest concentration tested).

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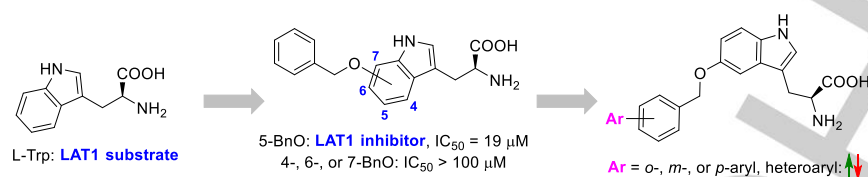
The attachment of a benzyloxy group to the 5-position of the indole system of the LAT1 (SLC7A5) substrate amino acid L-Trp produced a moderately potent inhibitor of LAT1-mediated leucine transport in HT-29 cells, while no inhibition of LAT1 was observed for 4-, 6-, or 7-benzyloxy-L-Trp at concentrations below 100 μM. None of these benzyloxy-L-Trp's was found to be a transport substrate. The extension of the benzyloxy group in 5-benzyloxy-L-Trp with aryl or heteroaryl moieties in most cases did not result in significant changes in LAT1 inhibitory activity.

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