REVIEW ARTICLE

LAT1: A POTENTIAL CEREBROVASCULAR TARGET TO BREACH BBB

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ABSTRACT

Central nervous system (CNS) is always an area of thrust where continuous efforts are required to achieve targeted drug delivery. The blood brain barrier (BBB) is the chief interfering element in the development of effective neurotherapeutics and efficient drug delivery to the CNS. Large neutral amino acid (LAT1) is the one which is the most promising gateway and belongs to the carrier-mediated transporters (CMTs), which is also called as Solute Carrier Family 7 Member 5 (SLC7A5). LAT1 is a sodium- and pH-independent transporter, which not only supplies essential amino acids to cells but also plays an important role in the facilitated transport of thyroid hormones, pharmaceuticals and metabolites into the brain by breaching the BBB. Levodopa, melphalan, gabapentin, brexpiprazole, valbenazine and α-methyldopa are already known drugs which cross the BBB via LAT1 mediated transport; therefore, the LAT1 is thought to be a potential target for piercing the BBB. This review is a combined effort to shed light on the pharmaceutical importance of this transporter and how this can be exploited further as a mediator for drugs to cross the BBB.

Keywords: LAT1, BBB, Carrier-mediated transport, GLUT1, MCT1, CAT1, ENT 1-2, CNT1-2 and choline

INTRODUCTION

BBB and its highly complicated and selective nature is always a point of discussion among researchers. It is known that the epithelial-like tight junctions within the brain capillary endothelium make the BBB so judgemental and impervious. It is very crucial to thoroughly understand the importance of BBB and to troubleshoot the limitations in CNS therapies[1]. A rough estimation of the number of drugs that do not cross the BBB is around ≥ 98 % among the small molecular drugs and almost all of large molecular drugs[2-4]. All this is due to tight intercellular junctions, restricting penetration of all the molecules which do not come under the specific molecular weight which is comparatively feasible to cross BBB[5-8].

Therefore, in reality, the BBB is actually defending the CNS against toxin exposure, regulating ion homeostasis and controlling immune-CNS communication[9].

It has been found that substances cross the BBB through many pathways (Fig. 1)[10]. Transmembrane diffusion (passive diffusion), carrier mediated transport (influx and efflux), and transcytosis are the most common pathways.

The emphasis of this review is the CMT systems, which consist of highly stereo specific pore-based transporters, and more precisely there are substantial structural requirements for transporter affinity. This gives an opportunity to the researchers to either couple the

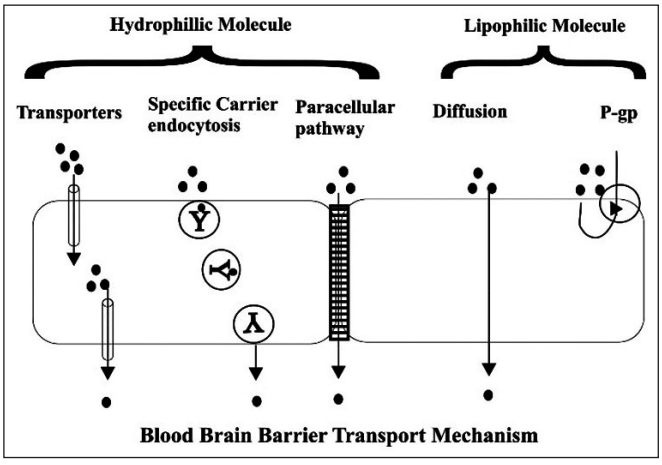

Fig. 1: BBB transport mechanism

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drugs (which are generally not transported across the BBB), with other molecules that undergoes CMT across the BBB and via breakable bond. Another approach is to modify the structure of the active drug with the help of medicinal chemistry, which acts as pseudo-nutrient for the transport system and thus facilitates transport, across the BBB via one of the CMT systems (Fig. 2).

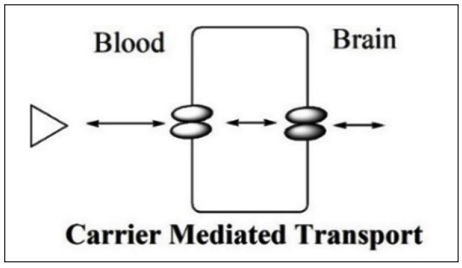

Fig. 2: Carrier mediated transport mechanism across the blood- brain barrier (BBB)

Carrier-mediated transport is a rapidly acting system and is available abundantly at the BBB[11]. More than 20 transporters have been identified in the BBB which are situated in cerebral capillaries (Fig. 3), (Table I)[4].

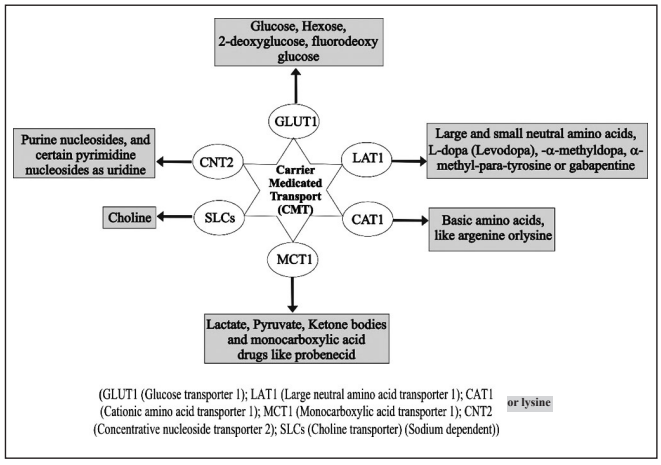

Fig. 3: CMT system with transporters and their respective endogenous molecules to be transported

L-type amino acid transporter (LAT1)

There are several carriers known for the influx through BBB. However, the transporter for LAT1, which is also called as Solute Carrier Family 7 (SLC7), displays several properties which can be well exploited and this what makes it suitable as a brain drug delivery vector[24,25].

It is already a known fact that being a major nutrient transporter protein, LAT1 is responsible for the penetration of large neutral, aromatic or branched amino acids (Phe, Trp, Leu, Met, He, Tyr, His, Val, and GIn) from extracellular fluids into the cells. Due to its high affinity, it is normally saturated with amino acids as a group at normal plasma concentration[26, 27]. The order of transport of branched amino acids is favourably in the following order: Phe > Trp > Leu > Ile > Met > His > Tyr > Val (Fig.4)[28, 29].

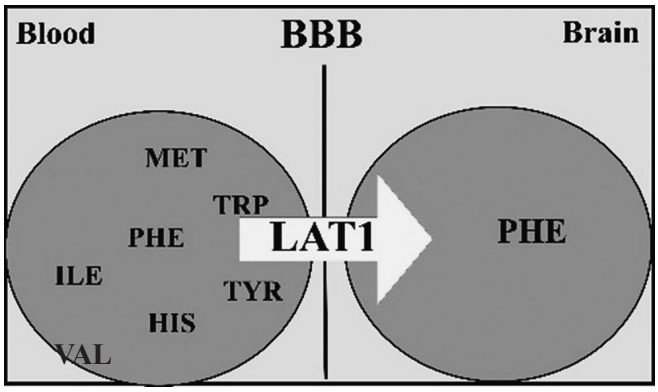

Fig. 4: Mechanism of action of LAT 1 transporter

Brain drug delivery via LAT1

Apart from large neutral amino acids, human LAT1 can also bind to glutamine and asparagine with comparably lower affinity. Tissue (brain, tumors and placenta) localization of LAT1 shows its importance and essentiality for the supply of amino acids into growing cells. Apart from acting as a carrier for the transport of the proteins and nutrients, the LAT1 also displays numerous other properties which are listed below, that makes it an appropriate agent to assist as a drug carrier into the brain:

- LAT1 has both a large maximal transport capacity and appreciable binding affinity, resulting in rapid rates of BBB exchange with half-times of less than 15 min for high-affinity substrates[26,30].
- The structural requirements for substrate binding to LAT1 are not very strict[31,32].
- A transient disruption in the brain supply of essential amino acids will not evoke any irreversible brain damage.

Moreover, LAT1 is an antiporter and as an exchanger it also transports intracellular amino acids (e.g. glutamine) in stoichiometry across the cell membrane. It has been assumed that the intracellular substrate concentration is the controlling factor for the transport rate[33,34]. In early 2000s, it was recognized that LAT1 could not only carry amino acids, but also some drugs as well[31, 35].

One of the best examples is levodopa (L-dopa) (1), which also acts as “pseudo-nutrient”, as the drug is hydrophilic and also vulnerable to enzymatic attack in the epithelial cells and therefore, exhibits negligible brain

Table I: Types of transporters available at BBB

Type	Abbreviation	Primary transport	Secondary transport	Ref.
l-type amino acid transporter	LAT1	Phenylalanine carrier	10 other large neutral amino acids and to a lesser extent small neutral amino acid.	12, 13
Glucose transporter type 1	GLUT1	Glucose carrier	Hexoses, including mannose, galactose, deoxyglucose, or 3-0-methyl glucose.	14, 15
Monocarboxylate lactate transporter	MCT1	Lactate carrier	Monocarboxylic acids (pyruvate, and the ketone bodies, acetoacetate, and b-hydroxybutryyrate).	16, 17
Cationic amino acid transporter	CAT1	Arginine carrier	Cationic amino acids (lysine, ornithine).	18, 19
Concentrative nucleoside transporter type 2	CNT2	Adenosine transporter	Purine nucleosides (guanosine, inosine), pyrimidine nucleoside, uridine.	20, 21
Choline transporter and sodium-coupled glucose transporters	(ChT) (SGLTs),	Choline and glucose transporters		22, 23

uptake[36]. As soon the drug is transported, by the action of aromatic amino acid decarboxylase, it is decarboxylated into dopamine thereby, enabling effective drug therapy[36, 37].

Other examples are melphalan (2) and gabapentin (3) which are also substrates for LAT1 and were found to cross the BBB through receptor-mediated endocytosis[38,39]. This is due to their branched chemical structures which consist of an amino group, a carboxyl group, and a hydrophobic side chain which eventually mimic the large amino acids, such as tyrosine (4).

Many of the newer CNS agents which have been approved by the FDA include cariprazine (5) and brexpiprazole (6) for schizophrenia[40], daclizumab and ocrelizumab for multiple sclerosis[41], edaravone (7) for amyotrophic lateral sclerosis[42] and valbenazine (8) for tardive dyskinesia[43]. Cerebrovascular LAT1 recognizes a wide range of amino acid-based drugs[44-46] including baclofen (9) (an antispasmodic)[47], α-methyldopa (10) (a centrally acting, antihypertensive)[48] and pragabiline (11) (anticonvulsant drug)[49]. There are several other examples of CNS active drugs which were found to be having LAT1 affinity and are using this to cross the BBB.

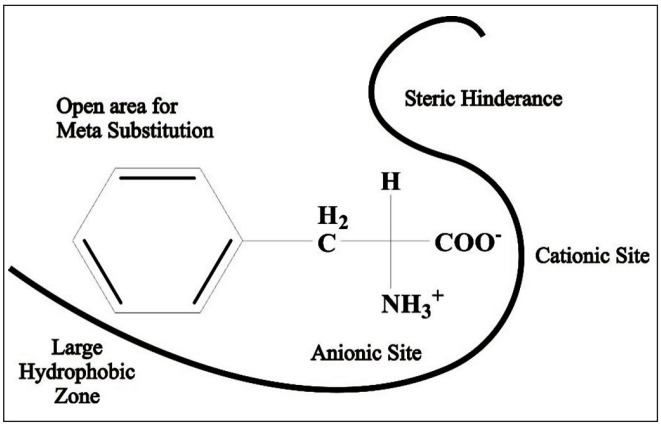

Fig. 5: Model of the binding site of the BBB LAT1 in relation to the amino acid phenylalanine

Basic pharmacophoric requirement for LAT1 affinity

Fig. 5 shows the model of the binding site of the cerebrovascular LAT1 transporter. It is already proven that for a substrate to have affinity for LAT1, it must contain an unsubstituted, free carboxyl group, an unsubstituted α-primary amino group, either a H or CH_3 on the α -carbon and a neutral, uncharged side chain with hydrophobic bulk.

The transporter prefers L-leucine and L-phenylalanine type L-amino acids with large, hydrophobic side chains. Uchino et al. in 2002 were the first who proposed the prototype showing the substrate binding spots of LAT1. They emphasized on the importance of presence of both positive and negative charges at the α-C of the ligand for substrate transport. They also added that the binding site contains specific recognition sites that presumably mediate the binding of charged head groups of amino acids and side chains through electronic and hydrophobic residues, respectively[31]. In the year 2005, a similar model for the binding of the cerebrovascular LAT 1 was proposed by Smith[22].

However, a molecule with a high calculated log P value which can access itself in the binding site may also act as a blocker; such examples are melphalan (2), triiodothyronine (12) and thyroxine (13)[31].

A pharmacophore-based binding hypothesis was proposed by Ylikangas et al. in 2013 with a 3D pharmacophore model (ligand-based) (Fig. 6), which was generated after thorough analysis of a set of 28 LAT1 substrates. The proposed pharmacophore model

also emphasized on the presence of site viz. hydrogen bond acceptor, hydrogen bond donor, negative charge and aromatic ring[32].

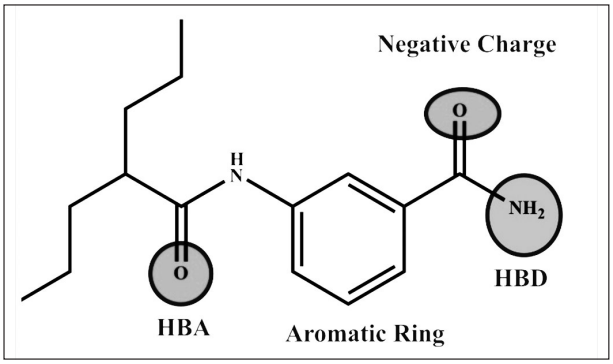

Fig. 6: 3D Pharmacophore model of LAT1 substrate. It is consisting of four pharmacophoric features hydrogen bond acceptor, hydrogen bond donor, negative charge and aromatic ring

SAR analysis of substrates was done in order to develop a pharmacophore model. This model shows the essentiality of negatively charged groups and hydrogen bond donor features for good affinity. Even slight modification of either of those or full removal results in decrease in affinity or loss of the affinity for LAT1. In comparison to the aromatic feature, lipophilicity has given more emphasis and was considered as a superior feature to increase ligand affinity[32].

The above concept was later on supported by Jarkko Rautio et al. In their study, isoleucine–quinidine ester prodrug (14) was synthesized. However, this compound has no free α-carboxyl group. After evaluation *in situ* in rat brain and the human breast cancer cell line, the compound showed no affinity, neither for LAT1 nor for LAT2. They concluded that compounds with substituted α-carboxyl group loses the affinity for binding with LAT1[50].

Another study in support of the proposed theory was done by Mikko Gynther[51] et al. They conjugated ketoprofen (15) with l-lysine in order to access LAT1. The uptake of the ketoprofen L-Lysine conjugate (16) was studied by *in situ* rat brain perfusion technique. In order to analyse the hypothesis, Uchino et al. proposed that compounds (17) and (18) had little effect on LAT1-mediated uptake, while compounds (19) and (20), both of which lack α-carboxyl groups, and compound (21) failed to inhibit LAT1-mediated transport suggesting that both α-amino and α-carboxyl groups are essential for activity, which supports the hypothesis[31,52].

Few other observations with different compounds which were carrying similar features which are essential for a compound to be LAT1 substrate show that these are in agreement with the hypothesis. These observations are as follows Compound (22) is a substrate of LAT1 (not a conventional amino acid). The failure of compound (23) to act as LAT1 ligand was assumed to be due to possible interference with the substrate binding site and intramolecular hydrogen bonding interaction between the α-carboxyl group by the β-hydroxyl group resulting in loss of electron density on the carboxyl group[53,54].

So far, four LATs have been identified, LAT1 (SLC7A5) was identified as the first LAT by two groups in 1998[28, 29]. The other members of the family are LAT2, LAT3 and LAT4; of these, LAT2 displays 50 % amino acid sequence homology with LAT1[55,56].

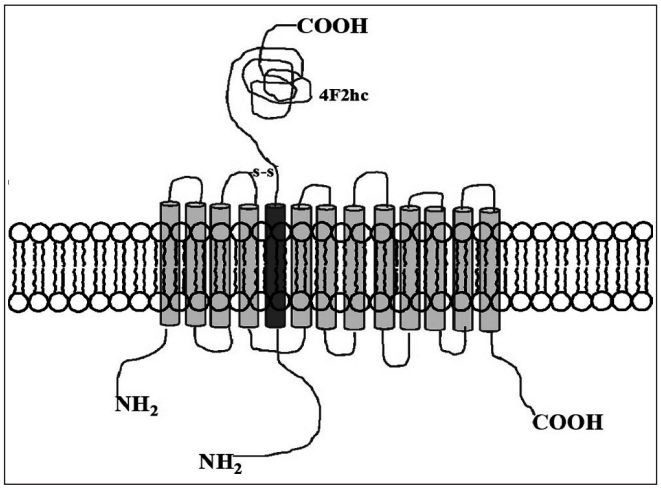

Fig. 7: Structure of LAT1

Both LAT1 and LAT2 are composed of 12 transmembrane domains, these domains are actually used to form the pathway for their substrates (Fig. 7). These domains are associated with the heavy glycoprotein subunit 4F2hc (SLC3A2) by sulphur bond[34]. Although 4F2hc does not seem to have a function to directly transfer the substrates, it makes the localization of its partner LATs more stable at the plasma membrane[57-59].

Therapeutic application of LAT1 substrates

Design of prodrug targeting LAT1 is potential strategy which involves creating a drug conjugated to an amino acid and this is found to enhance brain penetration of various drugs in rats[51,60,61]. All the substrates of LAT1 are shown in Figs. 8,9,10.

It is correctly said that the identification of novel LAT1 substrates, e.g. gabapentin (22), paroxetine (24), clomipramine (25), leucine (26), duloxetine (27) and fluphenazine (28), may focus on the mechanism through which drugs enter the brain and this can actually enlighten

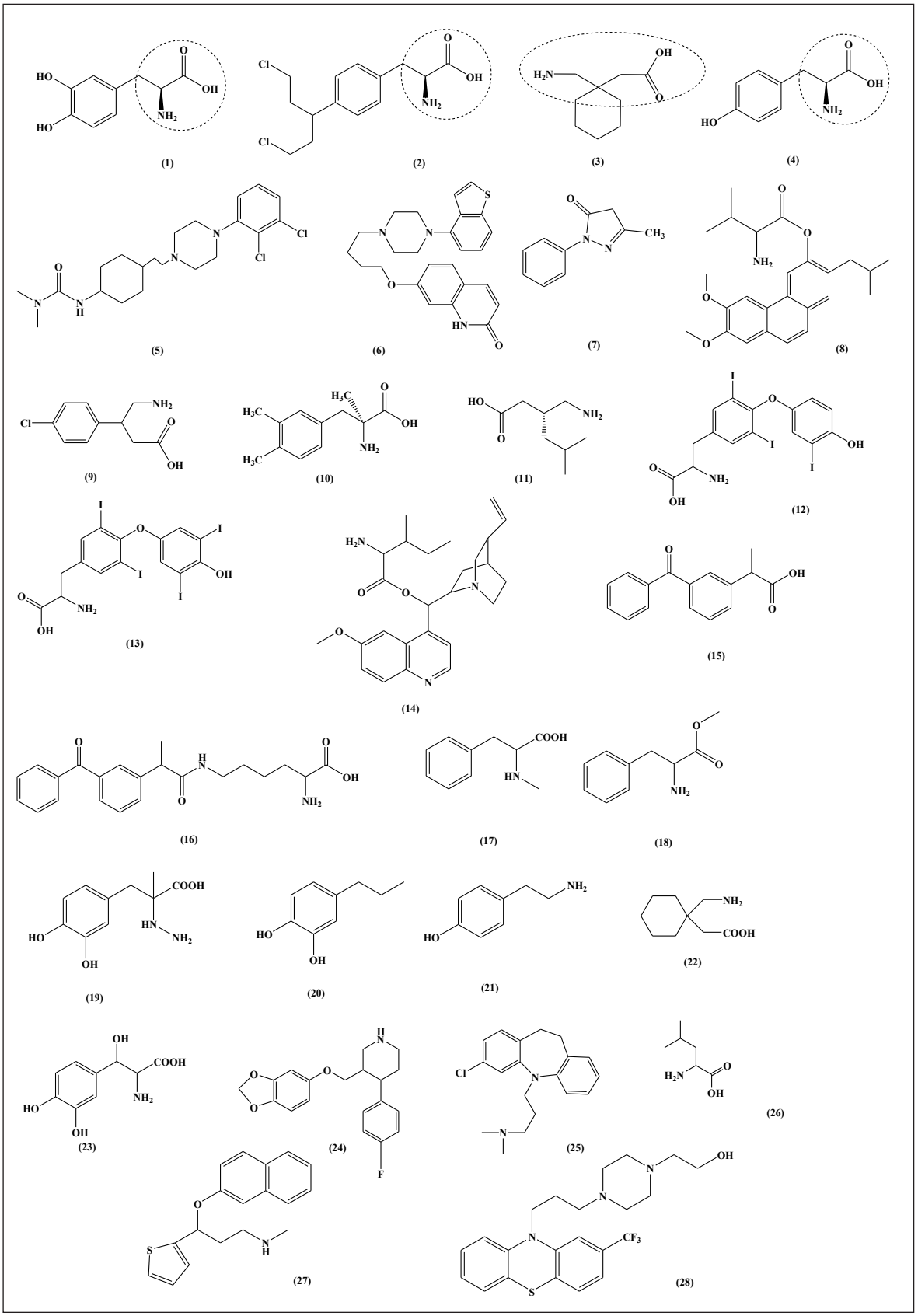

Fig. 8: Structure of LAT1 substrates-A

the path for researchers to develop novel therapeutics that can utilize LAT1 as a drug delivery platform.

Earlier, in the 1950s, anticancer activity of few compounds (alkylating phenylalanine mustards 2–4) as a substrate of LAT1 were studied. The LAT1 substrate phenylalanine (29) and the cytotoxic bis(2-chloroethyl) amino group were conjugated together. The cytotoxic activity is in the order ortho (30) > meta (31) > para (32) [62-64]. Moreover L-form is more active than D-form[65].

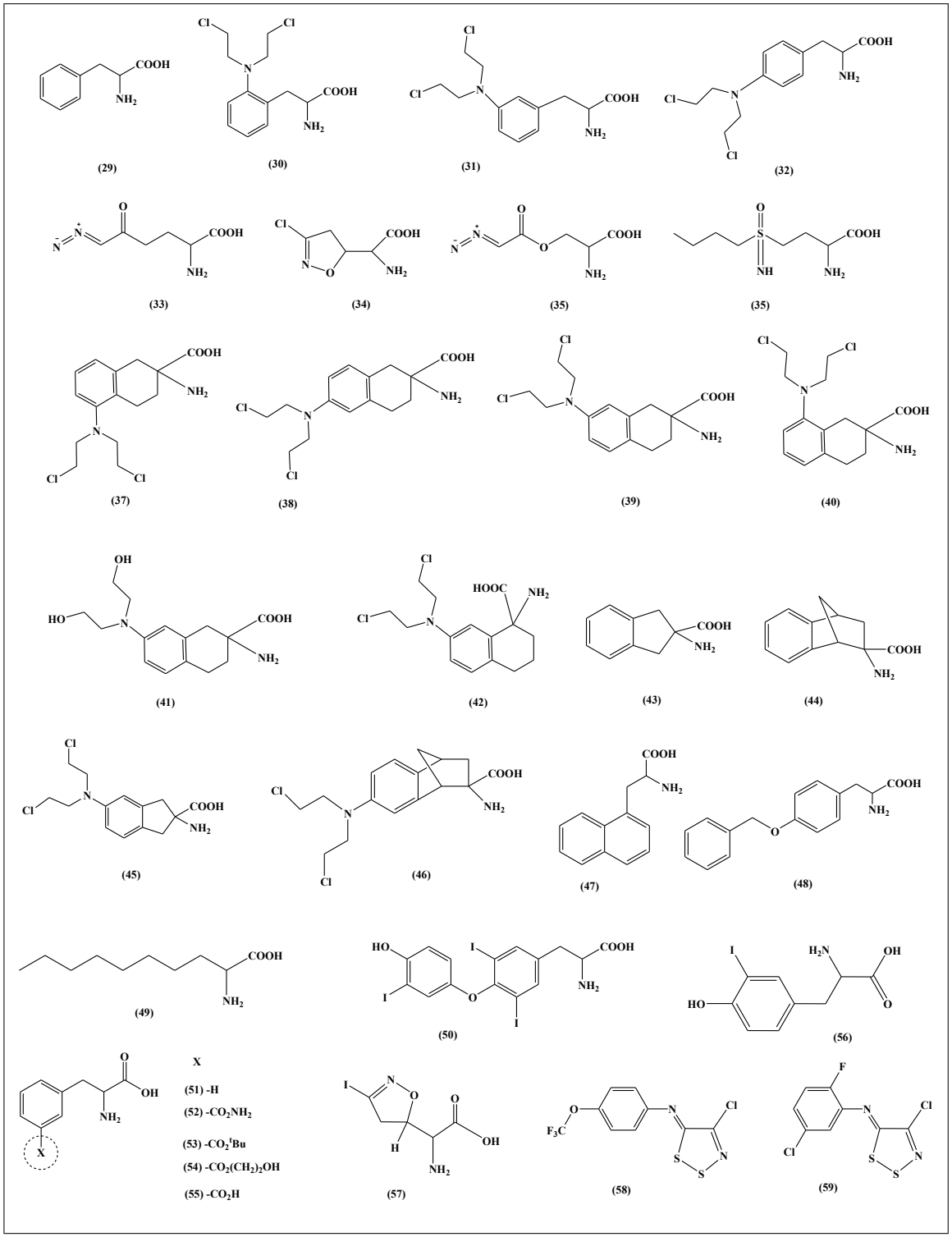

Fig. 9: Structure of LAT1 substrates-B

Takada and co-workers in 1991 screened five anticancer agents, compounds (32, 33, 34, 35, 36)[44]. None of them are found to be stronger than compound (29). In the year 1983 a series of cyclic amino acids for tumour cells were tested by Vistica et al.[66]. They concluded that compound (6) is a potent competitive inhibitor of LAT which was later utilized to obtain derivatives (37-40) by selective introduction of the bis (2-chloroethyl) amino group. Among the obtained one's, compound (39) was found to be an extremely

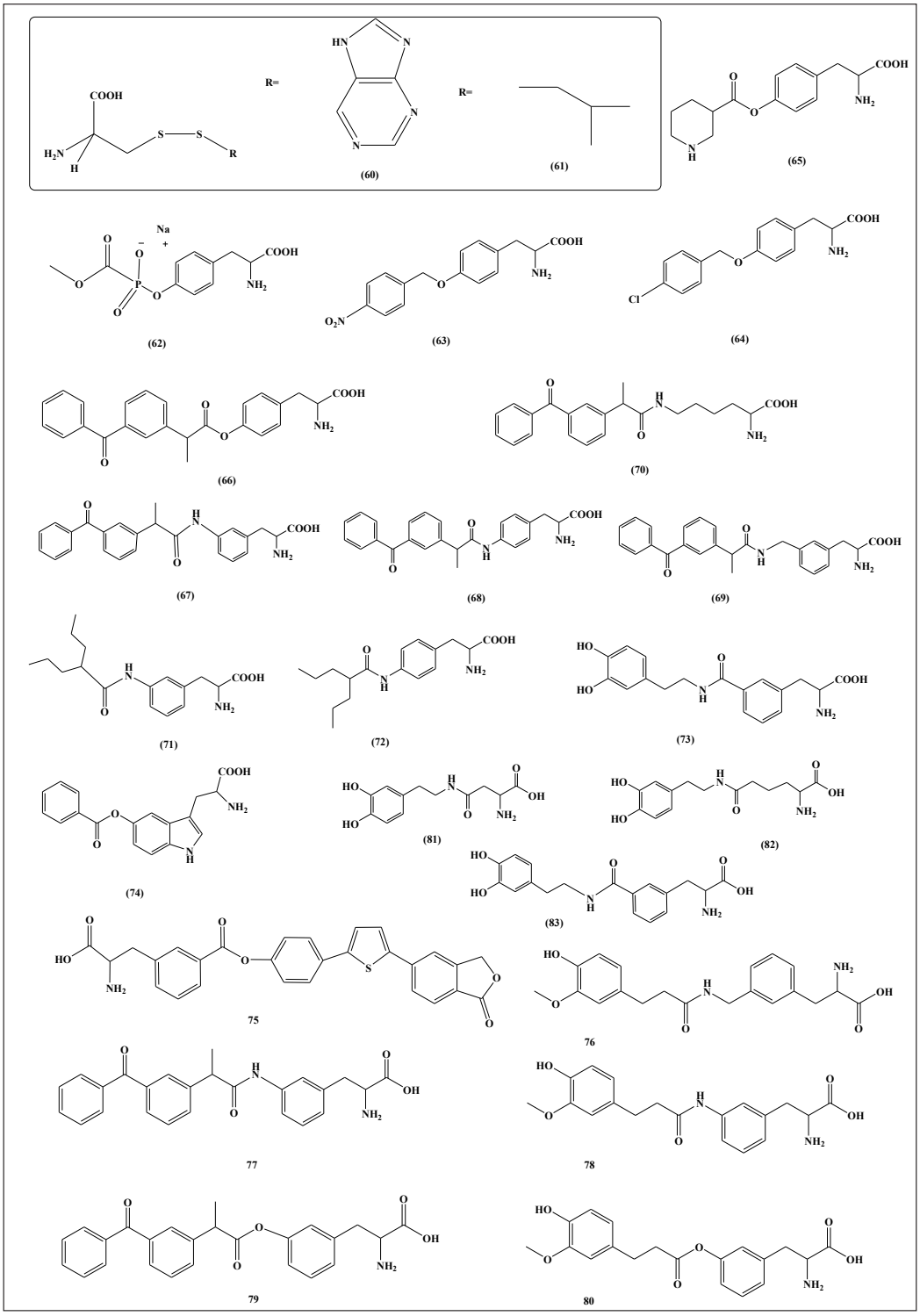

Fig. 10: Structure of LAT1 substrates-C

potent competitive inhibitor of LAT1 when compared to its prototype melphalan (2)[30, 67].

Takada et al., have reported that compound (41) was found to be approximately 10 times lesser in affinity than compound (39) (*in situ* rat brain perfusion technique). Moreover, by the transfer of the amino acid group in compound (39) from C-2 to C-1 in compound (42), the LAT1 affinity was reduced thrice[67]. In continuation to this, Matharu et al. also assessed the LAT1 affinities of compounds (43-46) which are more rigid analogs, but none of them are higher in affinity[68].

Miscellaneous compounds were experimented to access the acceptability of side chain size in LAT1 and out of them, compounds (47-49) were found to exhibit highest affinity. Another compound (50) was accessed for its affinity for LAT1 and it was concluded that the proper binding at the site was as a strong contributing factor for the high affinity[69].

To give a direction to design substituted analogues of phenylalanine and histidine, Huan-Chieh Chien and co-workers developed a structural model of LAT1 (51-55) along with SAR evaluation for both enantiomers (cis-inhibition and trans-stimulation) to determine effectiveness and uptake rate. Their study shows that although enantiomers exhibit different binding modes, the rate of LAT1 transport was non-stereoselective[69].

Another work by Lahoutte et al. has identified 3-iodo-L-tyrosine (56) as a potent LAT-1 inhibitor. It is a thyroid hormone derivative used to treat hormone deficiencies and as a radioactive agent. It reduces proliferation of T98G glioblastoma cells, possibly by starving these cells for nutrients supplied by LAT1[70].

Trans-stimulation and cell-proliferation experiments indicated that acivicin is likely a LAT-1 substrate. Acivicin (57) is an antitumor agent and its target is glutamine-dependent amido-transferases which are used in the biosynthesis of purines and pyrimidines[61,71,72].

In order to synthesize powerful and protracted inhibitor of the LAT1 transporter which can serve as a target for drug design, Lara Napolitanoa synthesized compounds based on dithiazole and dithiazine scaffold and a dose-response analysis was done for fifty-nine compounds. Two of them (58, 59) exhibited IC50 lower than 1 μM as reported from inhibition kinetics experiments performed on them[73].

To attain high affinity recognition for the specific LAT1, Dennis M. Killian and co-workers conjugated L-cysteine (L-Cys) as a carrier via a disulfide bond to either 6-mercaptopurine (60) or 2-methyl-1-propanethiol (IBM) (61) to form brain-targeted drug delivery systems (BTDS)[60].

LAT1-Mediated prodrug delivery

Phosphonoformate (PFA) is an antiviral agent which is unable to cross the BBB due to its hydrophilic nature. Phosphonoformate L-tyrosine conjugate (62) was synthesized by Walker et al.[74]. In porcine brain micro vessel endothelial cells, the conjugate was found to inhibit the transport of L-[3*H*]-tyrosine. In another study by Balakrishnan, A. et al., *p*-nitro- and *p*-chlorobenzyl ether conjugates of L-tyrosine (63) and (64) inhibited the transport of L-[3*H*]-tyrosine in a rabbit corneal cell line[75].

Another drug, nipecotic acid, which is again having poor tendency to cross the BBB, was conjugated with tyrosine and it was experimentally proven that the tyrosine nipecotic acid derivative (65) is transported into the brain via LAT1[76] by genetically seizure-prone strain (DBA/2) of mice. Transport of ketoprofen conjugate with L-tyrosine (by phenolic hydroxyl group) (66) via LAT1 has been demonstrated by using the *in situ* rat brain perfusion method by Gynther et al.[77].

In continuation to the series of the work, Puris et al.[78] designed three prodrugs by the attachment of ketoprofen with phenylalanine (meta- and para-position) (67-69) with significantly inhibition of the cellular uptake of L-[14C]-leucine. In another study done by Schmidt, L.H. et al., they also reported the LAT1-mediated brain uptake of L-lysine conjugate of ketoprofen (ketoprofen–L-lysine) (70) by *in situ* rat brain perfusion method[65]. Peura et al. reported that *meta* substituted phenylalanine derivative of valproic acid (71) bind to LAT1 with a higher affinity as compared to *para* derivative (72)[79]. They also reported the same results with dopamine derivative (meta) of phenylalanine (73)[80]. In a study based on CoMFA model, Ylikangas et al., reported that the benzoic acid prodrug (74) of L-tryptophan (substituted at the 5-position) showed 99 % L-leucine uptake inhibition[81].

Johanna Huttunen and co-workers designed six prodrugs (75-80) with LAT1 affinity for their increased cellular uptake in comparison to their parent drugs[82]. Three new CNS-targeted amino acid prodrugs of dopamine with an aim to enhance its brain uptake have been designed as potential substrates of LAT1. With reference to previous study, *meta* substitutes of phenylalanine viz. valproic acid and dopamine (71, 72), three amino acid pro-moieties, aspartic acid, 2-aminoapidic acid and meta-substituted carboxylic acid (81-83) analogue of phenylalanine were studied as LAT1-targeted pro-moieties[80].

CONCLUSION

LAT1 is not only important for CNS but it is also overexpressed in many types of human pathophysiological functions. Due to the ability of LAT1 to recognize two classes of different substrates i.e. essential amino acids and hormones (main physiological substrates) and some drugs (non-physiological substrates), this two-way feature signifies LAT1 as an intersection point in cell life. This can be supported by the occurrence of different kind of pathologies and alterations of function/expression of LAT1. However, the little knowledge about this protein contrasts its distinguished position.

A lot of information has been gathered by the combined approach of bioinformatics, *in vitro* and *ex vivo* experimental approaches and the results have given a strong input regarding the pharmacology of the protein, which leads to the identification of various substrates able to interact with LAT1 with important outcomes in human health. The findings collectively derived from different works have also opened new perspectives for translational medicine.

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