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REVIEW ARTICLE

Structure–activity relationship of polyamine conjugates for uptake via polyamine transport system

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Abstract

High toxicity of anticancer drugs led to development of targeted drug delivery directly to the specific organs. Polyamine transport system (PTS) of mammalian cells is one of the targets for a cell-selective drug delivery of polyamine–drug conjugates into specific organs. Even without having a 3D structure for mammalian PTS, synthesis of polyamine derivatives and evaluation of their cytotoxic effects are potential practical approaches to find optimal polyamine moieties to be transported from the PTSs. Chinese hamster ovary (CHO) and its mutant cell line (CHO-MG) are two important cells for evaluation of polyamine transportation by polyamine transporters (PAT). If a polyamine conjugate ligand demonstrates a high IC_{50} ratio on CHO-MG/CHO cells, this indicates a high selectivity of such compound toward PAT. This study discussed the structural requirements (charge, linker, vector, cargo) of polyamine conjugates in order to be transported into the cells by the mean of PTS.

Keywords Polyamine conjugates · Polyamine transport system (PTS) · Membrane transporter · Chinese hamster ovary (CHO) · CHO-MG

Introduction

The polyamines (PAs), including spermine, spermidine, and putrescine, are an essential class of metabolites with flexible aliphatic carbon chains. Although PAs have simple structure, yet they are essential for eukaryotic cells growth [1–3]. Spermine was discovered by Leuwenhoek in 1678 in seminal fluid [4, 5]. These polycations are bonded to anions by reversible ionic interactions to DNA, RNA, proteins, phospholipids, and mediate molecular functions such as maintenance of chromatin conformation, regulation of specific gene expression, regulation of ion-channel, the stability of membrane, and free-radical scavenging [6–10]. During the last decades, the metabolism of polyamines is studied as a potential target for cancer treatment.

The first rate-limiting step in polyamine biosynthesis is production of putrescine, which is tightly regulated by ornithine decarboxylase (ODC), a phosphate-dependent decarboxylase. It has a short half-life and is controlled at several steps including transcription, post-transcriptional processing, and changes in translational efficiency and altered stability of the protein [11–13] ODC is inhibited by 2-difluoromethylornithine (DMFO) that decrease cellular ODC enzyme activity [14]. Another rate-limiting enzyme in polyamine synthesis can be S-adenosylmethionine decarboxylase (AdoMetDC) that produces the aminopropyl donor via the aminopropyl transferases spermidine synthase and spermine synthase, respectively for the synthesis of both spermidine and spermine [15–17].

Moreover, the intracellular amounts of polyamine are adjusted through the activity of two enzymes involved in polyamine catabolism. Spermidine/spermine N1-acetyltransferase (SSAT) is the first catabolic enzyme of PAs which transfer an acetyl group to the N position of spermine or spermidine, from an acetyl-coenzyme A molecule, and produce N1-acetylspermine or N1-acetylspermidine, respectively [18, 19]. The acetylated polyamines then can either be transferred outside the cell by diamine exporter (DAX) [20]or be used as a substrate for flavin-dependent polyamine oxidase (APAO), in which it converts the N1-acetylspermine to spermidine and N1-acetylspermidine to putrescine [21, 22].

Polyamine transport system is more studied in prokaryotic system rather than eukaryotic systems. In eukaryotic cells, this

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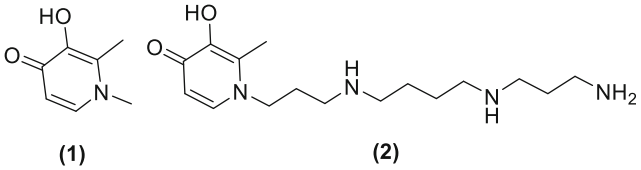
transport system plays an essential role in maintaining intracellular polyamine homeostasis via a known energy-dependent and carrier-mediated polyamine uptake mechanism. The first model was described by Poulin et al. and addressed the structural characterization of mammalian polyamine transport system [23]. They suggested that polyamines are first entered into the cell by a membrane carrier followed by localization of PAs in polyamine-sequestering vesicles (PSVs) through a mechanism that requires a directed pH gradient and proton exchange. The second model was described by Belting et al. (1999, 2003), suggesting that before being transported to the cell, spermine is binds to heparan sulfate groups of GPC1 heparan sulfate on the cell surface, and when it is inside the cell, the spermine is released by nitric oxide synthase-2 (NOS2) activity through NO-oxidation-mediated process [24, 25].

Chemotherapeutic compounds are highly toxic, and serious side effects are inevitable consequences of treatment with these compounds; thus, specific targeting and delivery of therapeutic molecules into a certain organ, tissue, or cell have been progressively studied as alternative treatment approaches, particularly for cancer. On the one hand, previous reports demonstrated that in tumor cells, polyamine metabolisms, including biosynthesis and uptake, are particularly amplified, and the efficiency of the polyamine transport system (PTS) is significantly increased [26]. This particular characteristic of tumoral cells allows for cell-selective drug delivery; in other words, targeted delivery of polyamine–drug conjugates into specific cell types is a possibility [27].

On the other hand, the PTS system in mammalian cells is poorly understood [11] and the majority of the studies have been addressing the therapeutic effects of homologous derivatives of polyamines. The effective factors in designing PAs derivatives are (i) increasing the length of polyamine backbone, (ii) the atom type responsible for the attachment of polyamine to the drug, and (iii) the physiochemical properties of the drugs.

Chinese hamster ovary (CHO) with a mutant cell line (CHO-MG) is an important mean for detection and screening the selective conjugate delivery by polyamine transporters (PAT) [28]. For example, MGBG methylglyoxal bis-(guanylhydrazone) is a PA-conjugated drug and its transportation is PTA-mediated. It has a high toxicity for CHO, while CHO-MG cell line is much more resistant to this drug [29]. Therefore, determination and comparison of the half maximal inhibitory concentration (IC_{50}) of such drugs (PAs with PTS-mediated transport systems) using these two CHO lines can be used to estimate the delivery rate of the drugs by PAT [30]. In other words, to obtain polyamine conjugate ligands with high selectivity toward the PTS system, achieving a high (CHO-MG/CHO) IC_{50} ratio in in vitro studies is a must [31].

This review study aimed at addressing important structural factors for PTS transportation of polyamine-derivative drug conjugates, including charge, linker, and vector length cargo effects.



(1) (2)

Fig. 1 The structure of deferiprone (compound **1**) and hydroxypyridinone–polyamine conjugate (compound **2**)

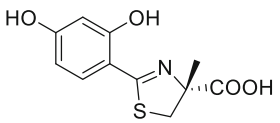
Structure–activity research of drug–polyamine conjugates

Charge effects

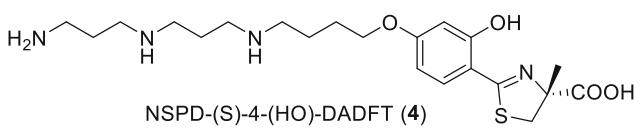
Iron chelators and their chemical charge are of critical importance for transportation of PAs [32–34]. Ligand donors in iron chelators, e.g., carboxylates, are highly polar and often ionizable. The transporter tendency is largely toward binding to neutral or positively charged ligands rather than negatively charged ones.

Bergeron et al. [35] synthesized 1-(12-amino-4,9-diazadodecyl)-2-methyl-3-hydroxy-4(1H)-pyridinone (Fig. 1, compound **2**), a bidentate hydroxypyridinone–polyamine conjugate, and evaluated its IC_{50} against L1210 murine leukemia cell line in vitro at a concentration of 0.2 M and reported that compared to the parent ligand (Fig. 1, compound **1**), the conjugated drug demonstrated > 230 folds increase in its effect.

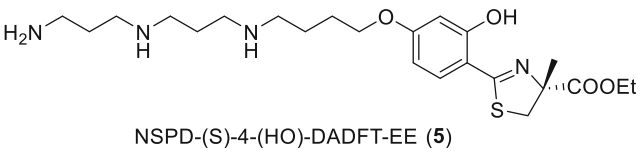
Bergeron et al. synthesized polyamine conjugates using neutral chelator (L1 conjugate) [35] with a PAT-mediated transportation mechanism. (S)-2-(2,4-Dihydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic acid [(S)-4′-(HO)-



(S)-4-(HO)-DADFT (**3**)

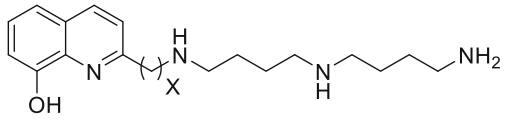


NSPD-(S)-4-(HO)-DADFT (**4**)



NSPD-(S)-4-(HO)-DADFT-EE (**5**)

Fig. 2 The structure of iron chelator (compound **3**) and polyamine chelator conjugate (compounds **4** and **5**)



X: (CH2)y ;
Y=0, HQ-44(**6**)
Y=1, HQ-44(**7**)
Y=2, HQ-44(**8**)

X:
C=O,HQCO-44(**9**)
C=S,HQCS-44(**10**)

Fig. 3 The structure of a new generation of iron chelators designed based on 8-hydroxyquinoline (8-HQ) scaffold which are linked to linear polyamine

DADFT] (Fig. 2, compound **3**) is an active oral analogue of desferrithiocin (DFT). DFT itself is a neutral iron chelator isolated from *Streptomyces* antibiotics [36].

Later, Bergeron et al. (2005, 2010) [37, 38] synthesized (S)-2-(2,4-Dihydroxyphenyl)-4,5-dihydro-4-methyl-4-thiazolecarboxylic acid [(S)-4′-(HO)-DADFT] (Fig. 2, compound **4**) that was attached to norspermidine (NSPD) at the C-4′-oxygen through a butyl group and its ethyl ester analogue (NSPD-(S)-4′-(HO)-DADFT-EE) (Fig. 2, compound **5**). The IC_{50} value (IC_{50} = 40 μM) of acid conjugates (**4**) was more than the ester analogue (**5**) (IC_{50} = 1.5 μM); thus, antiproliferative effects of (**4**) are less than the ester analogue. Moreover, as the name suggests, the (S)-4′-(HO)-DADFT acid analogue (Fig. 2, compound **4**) has an acidic functional group at its chelator fragment, which has negatively charge at physiological pH (i.e., carboxylates). This negative charge is hindrance for transformation by PAT and is not likely to be taken up by PAT cells.

Linker effects

Corcé et al. synthesized a new generation of iron chelators, quilamines, which are based on 8-hydroxyquinoline (8-HQ) scaffold linked to linear polyamine vectors [31]. The synthesized quilamines were differed in length (HQ0-44 (**6**), HQ1-44 (**7**), HQ2-44 (Fig. 3, compound **8**)) and/or the chemical nature of the spacer compound, i.e., HQCO-44 (Fig. 3, compound **9**) and HQCS-44 (Fig. 3, compound **10**)) (Fig. 3). The difference between the added ligands of compounds **9** and **10** is their heteroatom containing either amide or thioamide functional group, respectively. The IC_{50} ratio of these compounds against CHO-MG/CHO was assessed, which as mentioned above, is a mean to measure the selectivity for PTS. According to the IC_{50} ratio, those ligands differ in the length of the spacer and showed different IC_{50} ratio values (different selectivity toward PTS) and higher antiproliferative activities (IC_{50} values between 0.4 and 1.8 μM) while ligands differ in the chemical nature of the spacer (**9**, **10**) were less efficient (IC_{50} values 35.25 μM), with lower IC_{50} ratio values.

Corcé et al. design [31] was based on a structural constraints model of the PTS proposed by Phanstiel and Delcros (Fig. 4) [39–41], in which they indicated that the charge and size of polyamines are critical parameters for synthesis of PTS-targeting compounds.

According to this model, insertion of carbonyl and thiocarbonyl groups between iron chelator and polyamine chain resulted in decreased affinity of quilamines **9** and **10** toward PTS, in a sense that the (thio)amide group inhibited protonation of the nitrogen at physiological pH, omitting the electrostatic interaction required for recognition and attachment to the PTS (Fig. 5).

The quilamine compounds which were wary based on their length (**6**, **7**, **8**) have three nitrogen atoms which can form three positive charges at physiological pH, an important feature that requires for higher affinity toward PTS and transportation by this system [39, 40], and as a result of higher PTS selectivity, these compounds have higher IC_{50} ratio as well. The length of the spacer between N1 nitrogen and the chelator plays an essential role in PAT selectivity as well. Corcé et al. suggested that the presence of a hydrophobic pocket within the recognition site of the PTS favors the affinity (Fig. 6) [31]. Moreover, compared to quilamine compounds **6** and **7**, increasing the size of the spacer in compound **8** led to a better

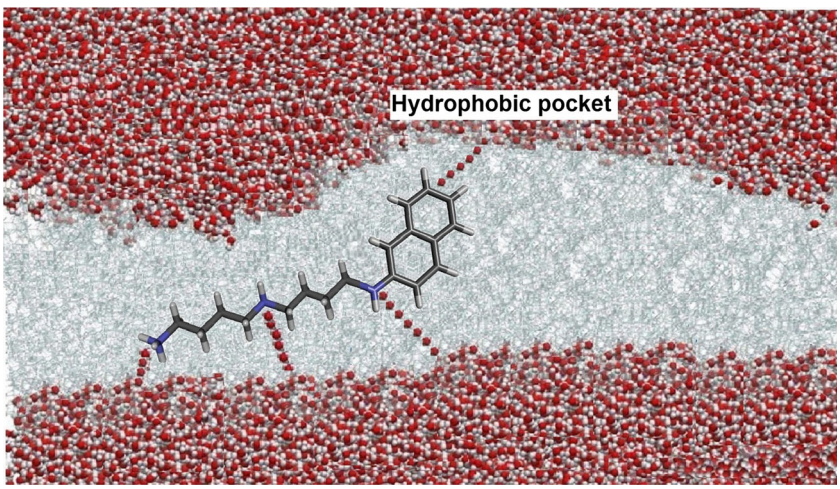


Fig. 4 A model of the structural constraints of the PTS proposed by Phanstiel [41] and the transporter pathway which was modified according to Padariya et al. [42]

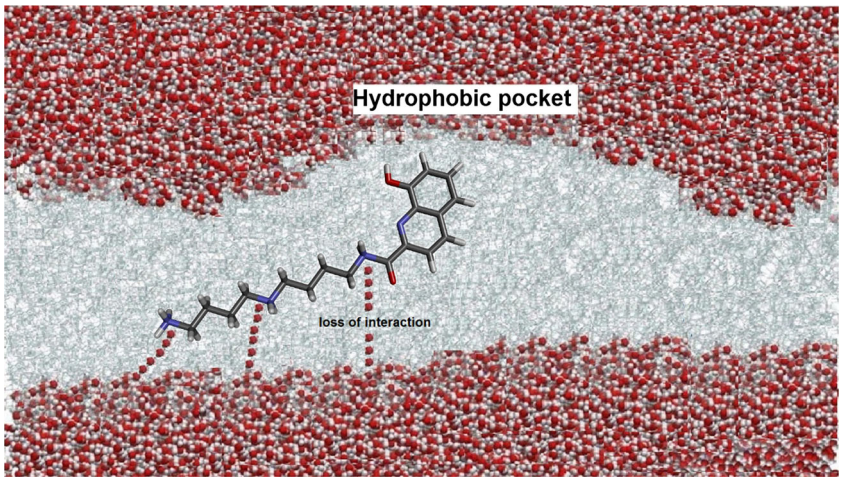


Fig. 5 A hypothetical model, based on Phanstiel and Delcros [41], to explain selectivity loss of quilamine compounds **9** and **10** toward PTS

fit the chelator moiety inside the hydrophobic cavity, resulting a better hydrophobic interaction, in other words a stronger affinity/selectivity toward PTS (Fig. 6).

An interesting finding was the higher IC_{50} ratio of quilamine **6** than that of quilamine **7**. Corcé et al. suggested that in the case of N1 nitrogen, which is cationic at the physiological pH, its positive charge is distributed between the N1 nitrogen itself and the nitrogen of the heteroaromatic ring. Therefore, this delocalization resulted in a higher electrostatic interaction and as a result an increased PTS selectivity (Fig. 7).

The linker effects were studied using two groups of compounds. Phanstiel et al. [41] studied the first group in which have 1-naphthyl moiety. Some derivatives had a methylene (compound **10**) or ethylene analogue (compound **11**) linkers which showed a high selectivity toward PAT with an IC_{50} ratios > 164, but the propylene analogue (compound **12**) showed no PAT selectivity (IC_{50} ratios: no activity) (Fig. 8).

The second group was studied by Breitbeil et al. in which the polyamine was conjugated with anthracenyl moiety (Fig. 9). Increasing the linker length from methylene (compound **13**) with a CHO IC_{50} of 0.45 μM to ethylene analogue (compound **14**) with a CHO IC_{50} of 9.8 μM and propylene (compound **15**) with a CHO IC_{50} of 130.1 μM) changed the IC_{50} ratio, in which their CHO-MG/CHO IC_{50} ratios were calculated at 148, 3.4, and 1, respectively [43]. Increasing the length of the linkers leads to formation of other conformations or molecular shapes, which moved the bulky aryl group out of the hydrophobic pocket, which itself resulted in a decreased PAT selectivity. In other words, there is a significant decrease in drug uptake via the PAT when the linker length was increased.

Cargo effects

Phanstiel et al. [44] synthesized several N1-substituted polyamines containing various cargo units (Fig. 10) including benzyl (compound **16**), naphthyl (compound **17**), anthracenyl (compound **18**), and pyrenyl (compound **19**). PAT selectivity of these compounds was studied by evaluating their IC_{50} against CHO-MG/CHO. In comparison with compound **17** with a (CHO-MG/CHO) IC_{50} ratio more than 164, the

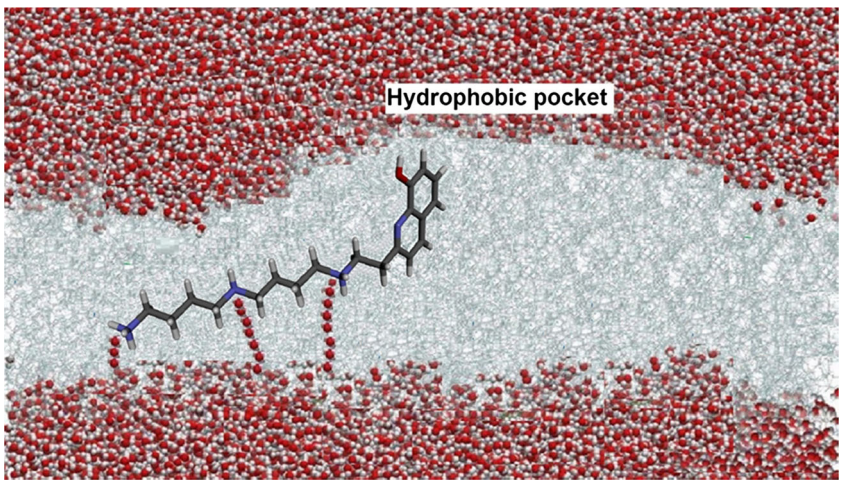


Fig. 6 The hypothetical model of the structure–selectivity relationship between compound **8** and PTS according to Phanstiel and Delcros model [41]

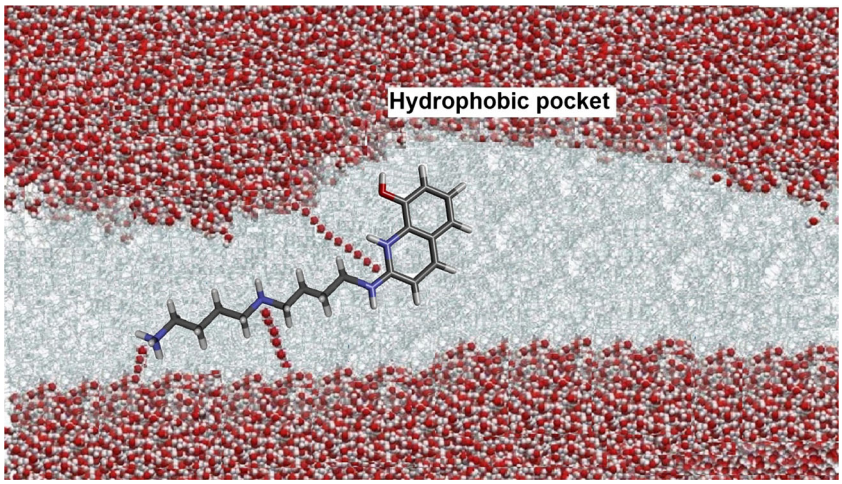


Fig. 7 The hypothetical model of the structure–selectivity relationship between compound **6** and PTS according to Phanstiel and Delcros model [41]

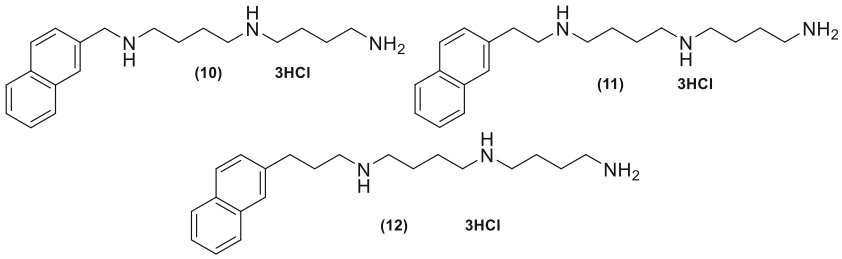


Fig. 8 The structure of polyamine–(naphthyl moiety) conjugates with different linkers

reported IC_{50} ratio for compound **16** was not significant. It is suggested that the bulky N-aryl substituent increased the binding affinity toward PAT, and as a result, the cytotoxicity of the polyamine conjugates in vitro. However, on the other hand, increasing the size of the arenyl unit from that of compound **17** to a more hydrophobic and bulkier cargo unit such as the ones in compounds **18** and **19** resulted in lower selectivity for the polyamine transporter. The respective CHO-MG/CHO IC_{50} ratios for compounds **18** and **19** conjugates were 148 and 34, respectively. Therefore, the hydrophobicity and the size of the arenyl unit are significant influencing parameters for the PAT selectivity profile of conjugates, in a sense that the polyamines conjugated with a compound that its cargos have moderate hydrophobicity and size (e.g., naphthyl) demonstrated higher (CHO-MG/CHO) IC_{50} ratios.

Phanstiel et al. [45] synthesized several mono, di, and trisubstituted arylene–polyamine conjugates (Fig. 11) and investigated their PAT-mediated transportation yield inside the cell. They synthesized three disubstituted compounds (anthryl, naphthyl, benzyl) which demonstrated the highest reported CHO-MG/CHO IC_{50} ratios among all the related studies with a value of > 2222, > 833, and 677, respectively. It is suggested that these disubstituted derivatives have an extremely high selectivity toward PAT of the CHO cells. There is a direct correlation between the size of arylene groups of the disubstituted derivatives and the related PAT selectivity. On the one hand, increasing the size of anthracenyl units resulted in a higher affinity/selectivity toward PAT. In contrast, in monosubstituted increasing, the size of the aryl unit resulted in a lower affinity/selectivity toward PAT [45]. The selectivity of the trisubstituted compound (**25**) was not determined because CHO and CHO-MG IC_{50} were > 500 and IC_{50} ratio was not applicable.

One of the most serious issues arising from using polyamine-based derivatives is the metabolism by polyamine oxidase (PAO), in which by degrading the compound it

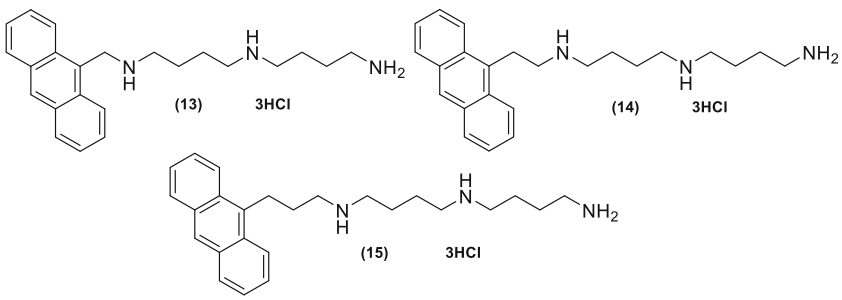


Fig. 9 The structure of polyamine–(anthracenyl moiety) conjugates with different linkers

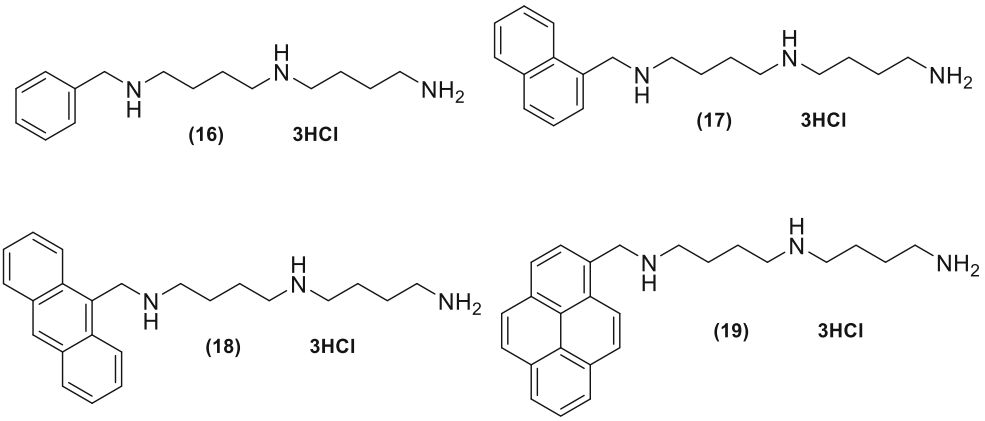


Fig. 10 The structure of polyamine analogue with a cargo consist of different arenyl units

significantly reduces the PTS selectivity of a polyamine–drug conjugates. To tackle this issue in a cell culture, the aminoguanidine (as a known inhibitor of PAO) is added to the medium and prevents the degradation of such compounds. An alternative approach to handle PAO issue is N-methylation of primary amines [46].

Phanstiel et al. [47] combined N-methylation method with the synthesis of disubstituted derivatives to obtain superior, more metabolically stable PTS ligands. The primary experiment was performed with and without aminoguanidine (AG) and using nonmethylated analogues, in which the absence of AG resulted in a dramatic decrease in PTS selectivity, for instance, CHO-MG/CHO IC_{50} ratio of compound **26** > 3571 and 2.1, with and without AG, respectively. Absence of AG additive resulted in a significantly lower PTS selectivity for compounds **28** and **30**, while with AG, they resulted in IC_{50} ratio of > 4645 and 727, respectively.

The second experiment was performed with and without aminoguanidine (AG) and using methylated compounds (**27**, **29**, **31**). Using N-methylation strategy resulted in a higher metabolic stability in which the CHO-MG/CHO IC_{50} ratio with AG for compound **27** was > 1204, while without the AG, it was measured at > 1190. Ultimately, the disubstituted platforms, which enhanced PTS targeting, and the N-methylation strategies, which increase the metabolic stability, are two effective approaches in designing compounds with desirable affinity/selectivity toward the PTS system (Fig. 12).

Vector lengths

In several studies, N1-substituted tri- and tetraamines with different tether lengths (number of CH2 spacer units) between nitrogen centers were synthesized (Fig. 13) [39, 40, 48]. Biological evaluation of different vectors (triamines and tetraamine motif) using CHO cell lines revealed that all polyamine vectors do not have the same selectivity, and only a few of the compounds demonstrated a desirable PAT selectivity characteristics. A comparison between compound **41** and **44** (Fig. 13) suggests that using compound **44** with longer tether leads to a significant increase in polyamine uptake with a CHO-MG/CHO IC_{50} ratio of 148. However, using all tetraamine conjugates resulted in a dramatic decrease in PAT selectivity ($0.7 < IC_{50}$ ratio < 3.1).

By taking into account the IC_{50} ratio and the vector design, triamine derivatives (compounds **41**–**46**) showed a significantly higher PTS selectivity compared to the tetraamine conjugates (compounds **32**–**40**). It is reported that 4,4-triamine motif (homospermidine) was the optimal chemical structure to increase the PTS-dependent cell entry.

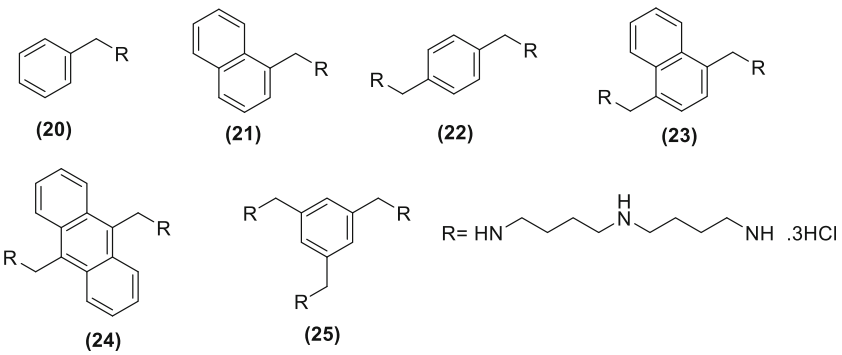


Fig. 11 The structure of mono, di, and trisubstituted derivatives of arylene-polyamine analogue

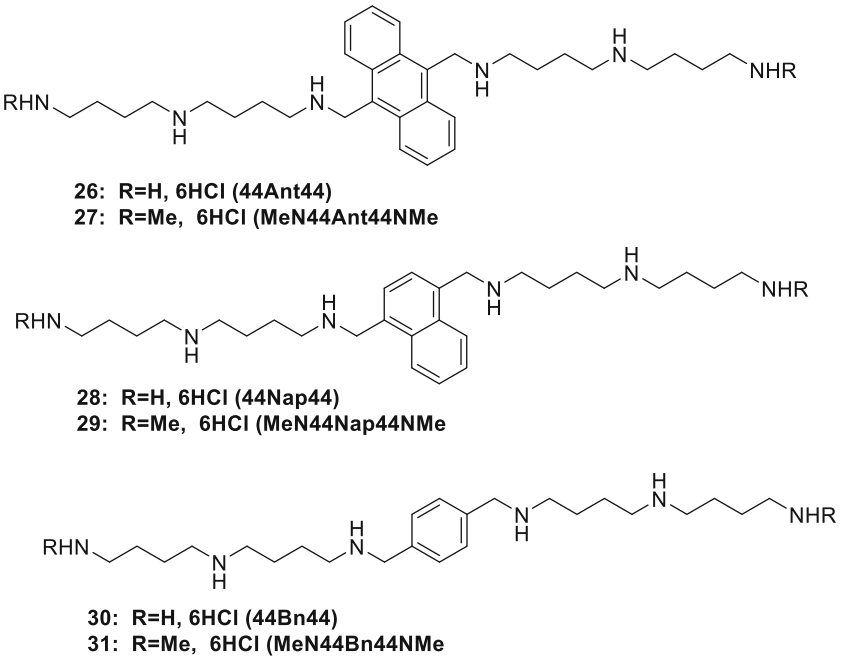


Fig. 12 The structure of disubstituted derivatives of polyamine analogue with different moieties

Gaboriau et al. [49] developed new polyaminoquinoline iron chelators with different polyamine vectors (Fig. 14). In spite of different cellular targets of these quilamines and polyamine–anthracene conjugates (compounds **41**, **42**, and **50**) (iron depletion vs. DNA intercalation), there was a good agreement between two conjugates. Based on the IC_{50} of these compounds, triamine analogues resulted in higher PTS selectivity, but it was decreased in the case of 4,4,4-tetraamine. The observed IC_{50} for compounds **52** and **47** was 249 and 38 while for **55** and **51** it was 21 and 18, respectively. These findings suggest that quilamines with a 4,4 polyamine chain (compounds **52** and **47**) shows higher PTS selectivity than those of 3,3 chains (compounds **51** and **55**). Moreover, the polyamine conjugates bearing 3,4 chains (**54**, **48**, **50**; with IC_{50} ratio of 2, 4, and 4, respectively) were wither weakly transported or were not transported by PTS.

Phanstiel et al. reported that the substitution degree at the N1 position in the polyamine vectors (Fig. 15) is a critical factor in the selective delivery of polyamines via the PAT [50]. The N1 anthracenylmethylhomospermidine (compound

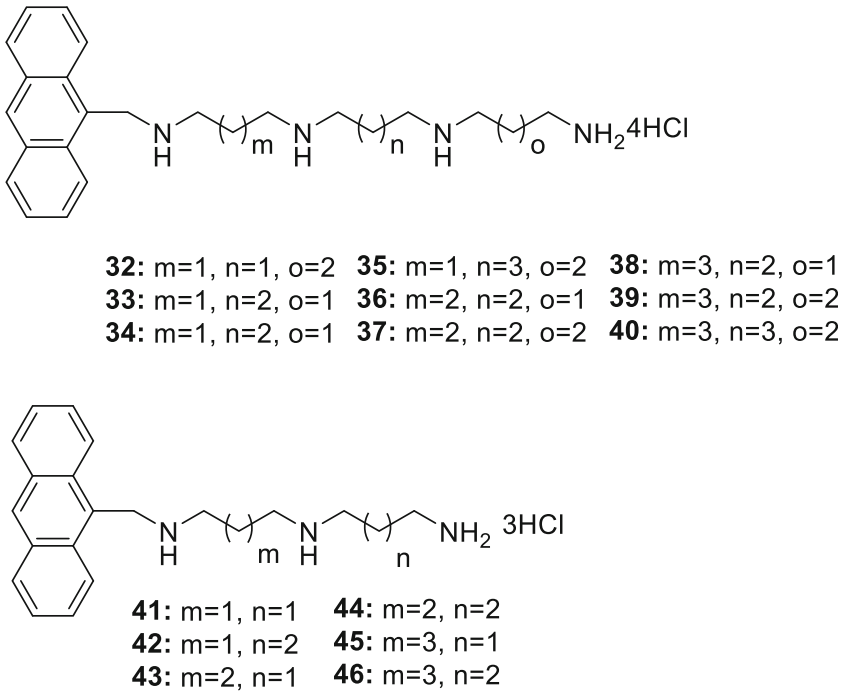


Fig. 13 The structure of anthracenyl–polyamine conjugates with different tether lengths

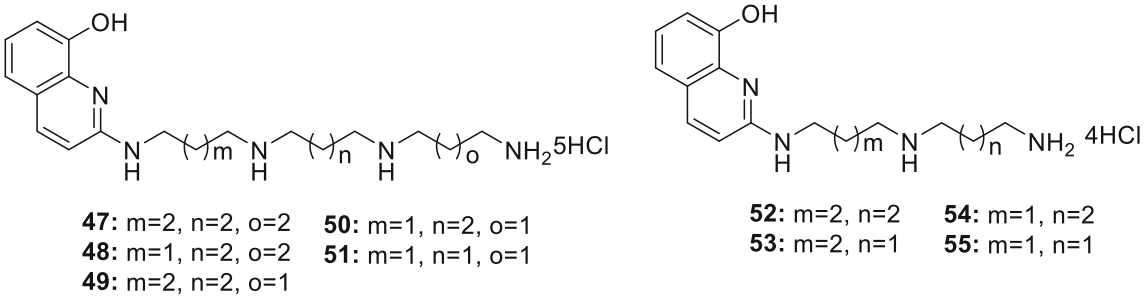


Fig. 14 The structure of polyaminoquinoline iron chelators with different tether lengths

57) which contains a secondary nitrogen at the N1 position showed a CHO-MG/CHO IC_{50} ratio of 148, and compound **56** with a tertiary nitrogen at the N1 position showed a CHO-MG/CHO IC_{50} ratio of 1. Moreover, PAT selectivity test of the 3°-amine-containing derivatives at the N1 position of dihydroMotu (3,3) and dihydroMotu (4,4) showed a CHO-MG/CHO IC_{50} ratio of 1; in other words, these compounds were not PAT-selective. Therefore, compounds containing an N1 tertiary substitution pattern are not selective for PAT.

Conclusion

Although the molecular structure of mammalian PTS is poorly understood, the structure–activity relationships of polyamine-like molecules which are transported by this system have been an interesting field of research particularly for the PTS targeting anticancer drug delivery treatment approaches. With regard to the unclear 3D structure of the PTS, indirect approaches were developed and have been used to study the transport of polyamine conjugates using the PTS in mammalian cells, in which using CHO-MG and CHO cell, two PTS-deficient cell lines with different susceptibilities toward PTS-targeted drugs, is one of the most important developed techniques in this regard. The CHO-MG cell line was particularly selected due to its resistance against MGBG methylglyoxal bis-(guanylhydrazone); thus, while polyamine conjugate drugs with high selectivity toward the PTS can impose highly toxic effects on the CHO cells, it demonstrates lower toxicity on CHO-MG cells. This is an important aspect for two reasons, first, compare to native cells, the PTS-deficient cells have lower polyamines uptakes, and second, the CHO-MG/CHO IC_{50} ratio determination in these two CHO lines provided a relative delivery yield using PAT. Moreover, a high IC_{50} ratio is an important factor for screening of highly selective PTS polyamine conjugate ligands. In this review, we discussed the structure–delivery relationship of polyamines for PTS targeting with regard to the effects of their charge, linker, vector length, and cargo.

In terms of charge, compared to negatively charged conjugates, the neutral or positively charged ligands are much favorable to bind with the PTS. Moreover, increasing the length of the linkers resulted in decreased drug uptake by PAT. Furthermore, with regard to the vector length of the conjugates, 4,4-triamine motif (homospermidine) was found to be the optimal chemical structure with an increased cell entry using PAT. Finally, the cargo effect was evaluated using hydrophobicity and the size of arenyl unit, and it is shown that cargos with moderate hydrophobicity and size have a higher uptake ratio by PAT. In conclusion, to design a successful PTS-targeting polyamine-conjugated drug, many parameters need to be considered.

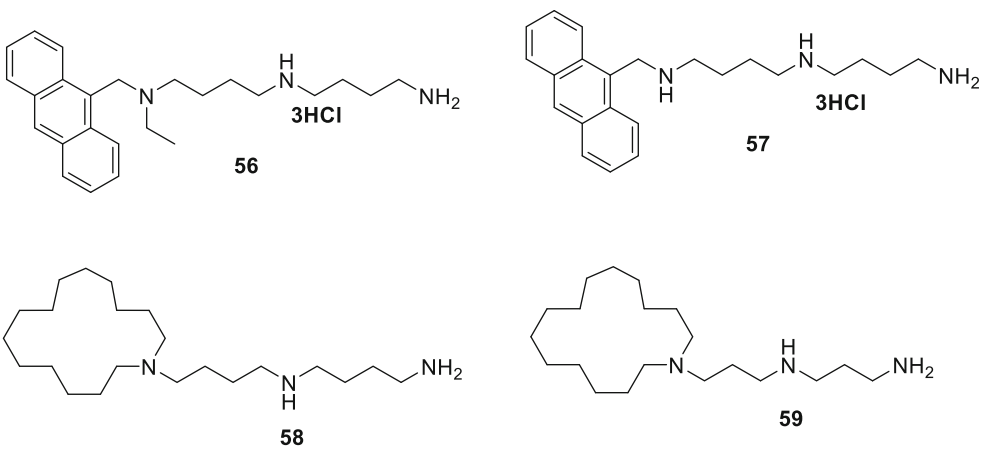


Fig. 15 The structure of polyamine conjugate with substitution at the N1 position of the polyamine vectors

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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