

The New Permeability Pathways: Targets and Selective Routes for the Development of New Antimalarial Agents

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Abstract: The malaria parasite, *Plasmodium falciparum*, spends part of its complex life cycle within the red blood cells of a human host. During this time, the parasite alters the permeability of the red blood cell's plasma membrane to allow the uptake of nutrients, the removal of "waste" and volume and ion regulation of the infected cell. The increased permeability is due to the induction of new permeability pathways (NPP), which are obvious chemotherapeutic antimalarial targets and/or selective routes for drugs, which target the internal parasite. This review covers our present understanding of the NPP, the methods used to screen for putative inhibitors of the NPP, the current repertoire of NPP inhibitors and the problems that need to be addressed to realise the potential of the NPP as antimalarial targets. In addition, the review will cover the use of the NPP as specific drug delivery routes.

Keywords: Malaria, Channel, Anion, Antimalarial.

1. INTRODUCTION

As part of its life cycle, the malaria parasite, *Plasmodium falciparum*, invades the red blood cells (RBCs) of its human host. In so doing, the parasite passes through the host plasma membrane and, at the same time, surrounds its own plasma membrane with a second membrane called the parasitophorous vacuole membrane (PVM). Within the RBC, *P. falciparum* takes approximately 48 hours to mature, divide and release up to 32 new parasites (at a metabolic rate far in excess of that of the host RBC).

Approximately 15 hours into this asexual reproductive phase, the permeability of the host RBC's plasma membrane increases to a range of relatively low molecular weight solutes including sugars, amino acids, nucleosides and inorganic ions. This occurs due to the induction of new permeability pathways (NPP) by the internal parasite. It has yet to be clarified to which compartment the NPP lead. A simple sequential model of solute trafficking, as suggested by Desai [1], would predict that the NPP lead to the RBC cytosol but other models have been hypothesised including the localisation of the NPP to points of contact between the host plasma membrane and the, so called, tubovesicular membrane (a network of tubular membranes extending from the PVM) [2]. The latter would predict that the NPP lead to the compartment between the PVM and the parasite plasma membrane. The evidence behind these models and more contentious models are discussed in greater detail by Kirk [3].

1.1. Why Induce the NPP?

Four reasons have been postulated for why the parasite induces the NPP. Firstly, the parasite has an essential requirement for the presence of several nutrients in the

external medium for normal growth [4]. These must first cross the RBC plasma membrane before they are accessible to the parasite. In the case of glucose (the parasite's primary energy source), the native glucose transporter (GLUT1) is capable of supplying the parasite's requirements [5]. However, there is no native transport pathway for the vitamin, pantothenate (required by the parasite for the production of co-enzyme A). As the NPP are permeable to pantothenate [6], they are thought to aid nutrient uptake.

Secondly, to produce energy (in the form of ATP), the parasite utilises glycolysis primarily. The major by-product of this process is lactate and this is potentially toxic to the parasite if left to accumulate. Besides a small degree of diffusion of the protonated form, the RBC has two native transport pathways for the removal of lactate (the anion exchanger, Band 3, and the monocarboxylate transporter). However, it has been calculated that these pathways are not capable of clearing parasite-derived lactate and so a third route (i.e. the NPP) is required for metabolite removal [7, 8].

Thirdly, the parasite obtains some of the amino acids it requires for protein production from digestion of the host RBC's cytosol (predominantly haemoglobin). Only a relatively small proportion of the amino acids produced by this process are used by the parasite [9]. If left to accumulate, an amino acid concentration gradient would form, which would draw water into the infected cell and swell it (ultimately lysing the cell before the parasite has divided). The NPP have, therefore, been proposed to aid amino acid release and provide the parasite with a mechanism for regulating the volume of the infected RBC [10].

Finally, a RBC maintains a low Na⁺ concentration within its cytosol compared with the external milieu. This inward Na⁺ gradient can be used to facilitate the transport of solutes, against their own concentration gradients, across the cell membrane (known as secondary active transport due to the need for a primary active transport process to produce the

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Na⁺ gradient in the first place). The parasite also maintains a low cytosolic Na⁺ concentration so, upon entry to the low Na⁺ environment of the RBC cytosol, loses the Na⁺ gradient across its own plasma membrane. However, the NPP are permeable to Na⁺ [11] to such an extent that the Na⁺ concentration within the RBC rises to a level similar to outside. The benefit of this ion movement to the parasite is an inward Na⁺ gradient across its own plasma membrane, which Na⁺-dependent parasite plasma membrane transporters (e.g. a Na⁺/H⁺ exchanger [12]) can use.

In the light of these proposed functions, the NPP are thought to be essential for parasite survival and, thus, excellent chemotherapeutic targets for the development of new antimalarial agents [1, 13-15].

1.2. Properties and Nature of the NPP

The functional and pharmacological properties of the NPP have been characterised in great detail over the past 25 years (reviewed by Kirk [3]). The NPP are predominantly anion-selective channels [16]. Although showing a preference for anions, the NPP also allow the transport of both electroneutral (e.g. glucose) and cationic (e.g. Na⁺ and K⁺) solutes at significant, albeit reduced, rates. In addition, the NPP have a preference for small hydrophobic organic solutes over large hydrophilic ones and do not discriminate between enantiomeric forms of a solute. Finally, several compounds that have previously been shown to inhibit anion transport pathways also inhibit the NPP.

At present, neither the molecular natures of the NPP nor the exact number of pathways involved in the formation of the NPP are known. In addition, it is not known whether the NPP are inactive, native RBC plasma membrane transport pathways, which are up-regulated and/or modified by the parasite to induce them, or parasite-derived and inserted into the RBC plasma membrane. All of these topics are hotly debated currently (reviewed by Staines *et al.* [17]).

2. HIGH THROUGHPUT SCREENING FOR INHIBITORS OF THE NPP

In addition to the disease and/or target, high throughput screening (HTS) should include consideration of topics such as the assay(s), compounds, automation and data management. Since combinatorial and/or parallel synthesis chemistry can potentially produce a large number of compounds in a relatively short time period, it is essential that biological testing be carried out quickly and automatically.

Combinatorial chemistry has started to make an impact on antimalarial drug discovery and, as such, development of efficient HTS protocols should be an integral part of antimalarial drug discovery [18]. Recently, HTS parasite assays have been developed in which compounds are automatically tested in solution using 96-well microtiter plates. Testing can also be performed directly on solid phase beads if the library of compounds has been generated via solid phase organic synthesis (this would be appropriate for inhibitors of the NPP as target sites may well be located on the extracellular surface of the infected RBC). On-bead screening has been applied to some parasite targets [18].

However, in order to meet the needs of combinatorial chemistry and attendant large numbers of compounds for antimalarial drug discovery, one of the present challenges is to increase the number of wells and concomitant development of methods such as fluorescence (discussed below) and chemiluminescence, which will allow the simultaneous identification of active wells. Further miniaturization using closed systems also presents another challenge.

Previous reports involving screening for inhibitors of the NPP rely on protocols, with low throughput rates. However, the various techniques used (radio-tracer, haemolysis, fluorescence and electrophysiological methodologies (their use for the characterisation of transport is reviewed by Kirk [3])) have plenty of scope for increasing throughput rates as specific NPP inhibitor libraries are derived. However, as noted in section 4, our current understanding of the NPP limits some of the screening potential of these techniques, presently.

2.1. Transport Inhibition Assays

Until recently, radio-tracer techniques were the preferred choice of laboratory researchers to study transport via the NPP in malaria-infected RBCs. Radio-tracer experiments measure the movement of solutes of interest, which are radio-isotopes (e.g. ²²Na⁺) or are radio-labelled (e.g. with ¹⁴C). This allows a direct measurement of transport rates into (influx) and out of (efflux) cells and, as such, allows detailed transport characterisation. There are several solutes that permeate via the NPP, which make excellent markers to test for the inhibitory characteristics of putative NPP blockers, using HTS radio-tracer techniques.

The most commonly used radio-labelled solute in reports containing NPP inhibition data is ¹⁴C labelled choline (a quaternary ammonium compound, which is used by the parasite in the formation of lipid membranes). This organic cation has a relatively low rate of transport via the NPP, which can be measured in minutes rather than seconds (reducing timing errors) and has only one native transport pathway in the RBC plasma membrane, which is a carrier-type pathway [19]. A high concentration of unlabelled choline (1 mM) can be used to out-compete any radio-labelled choline for the carrier's binding sites. As the transport of [¹⁴C]choline *via* the NPP is unaffected by this level of unlabelled choline [20], under this condition only transport via the NPP is measured.

Besides having to define solute transport rates and ways to inhibit alternative transport routes, there are several problems with radio-tracer techniques, which might limit their use for HTS for inhibitors of the NPP. These include the safety issues, which surround the use of radio-isotopes, the cost of the labelled solutes and the large quantities of purified malaria-infected RBCs required. In addition, influx assays require multiple treatment steps to wash cells free of extracellular radiolabel, followed by cell lysis (to release the internal radiolabel) and deproteinisation (to remove haemoglobin, which quenches isotope counting). These steps, if performed incorrectly, will produce large errors. For efflux assays, which require less processing steps (as only the supernatant is required for transport measurement), there

are two particular problems. Firstly, as the NPP are constitutively open, care must be taken to limit loss of loaded radiolabel prior to the start of an assay. Secondly, it is important to note any degree of cell lysis during an assay as even a small amount of cell lysis can add significant levels of radiolabel to the supernatant, which would produce sizable overestimates of NPP activity.

Fluorescence based assays offer another way to screen putative NPP inhibitor libraries. A number of ion sensing dyes have been developed, which include several for Na^+ , K^+ and Cl^- (all ions which have been shown to permeate via the NPP). The use of ion sensing dyes has been discussed in detail by Desai [1] and will not be covered here. However, an alternative is to use fluorescent solutes, which are known to permeate via the NPP, to measure the effect of inhibitors on NPP activity. Previous reports [21-23] show that NDB-aurine can be used to study the NPP with both influx and efflux fluorescence assays. These types of assays would have many of the pros and cons of radio-tracer assays (as discussed above) but with the advantages of not having to deal with radio-isotopes and being able to measure release (in the case of efflux experiments) instantaneously.

Over the last four years, there has been a major increase in the use of the electrophysiological technique of patch-clamp to study NPP activity. While allowing highly detailed characterisation of transport pathways (with the ability to characterise single transport proteins), the technique is not in general use for HTS due to its complex

nature and expensive equipment. However, this is starting to change as new technology is developed. This technique is unlikely to be used to screen inhibitor libraries for the NPP in the near future but Desai [1] has covered its potential.

The final method, which can be used for HTS of NPP inhibitor libraries, is the haemolysis technique. During haemolysis assays, malaria-infected RBCs are suspended in iso-osmotic solutions of solutes of interest. Transport via the NPP can be related to the speed at which infected cells swell and lyse in these solutions. This can be determined by measuring, amongst other things, haemoglobin release or, as reported recently, light scattering [24]. The solute of choice for this type of assay is usually sorbitol. Sorbitol does not enter uninfected RBCs, which are stable for relatively long periods when suspended in iso-osmotic solutions of sorbitol, and lyses infected cells over a period of approximately 15 minutes (with a half-time for haemolysis of approximately 5 minutes).

The technique is the least quantitative of those discussed here as it measures cell lysis due to solute transport rather than the solute transport directly and, thus, its major disadvantage is that it is not as accurate as the other techniques (see Kirk [3]). However, as an assay for HTS, it has several main advantages over the other techniques. The assays require few infected cells (as the technique is very sensitive), do not require the purification of infected cells from culture (which is normally performed for radio-tracer experiments) and are relatively cheap and easy.

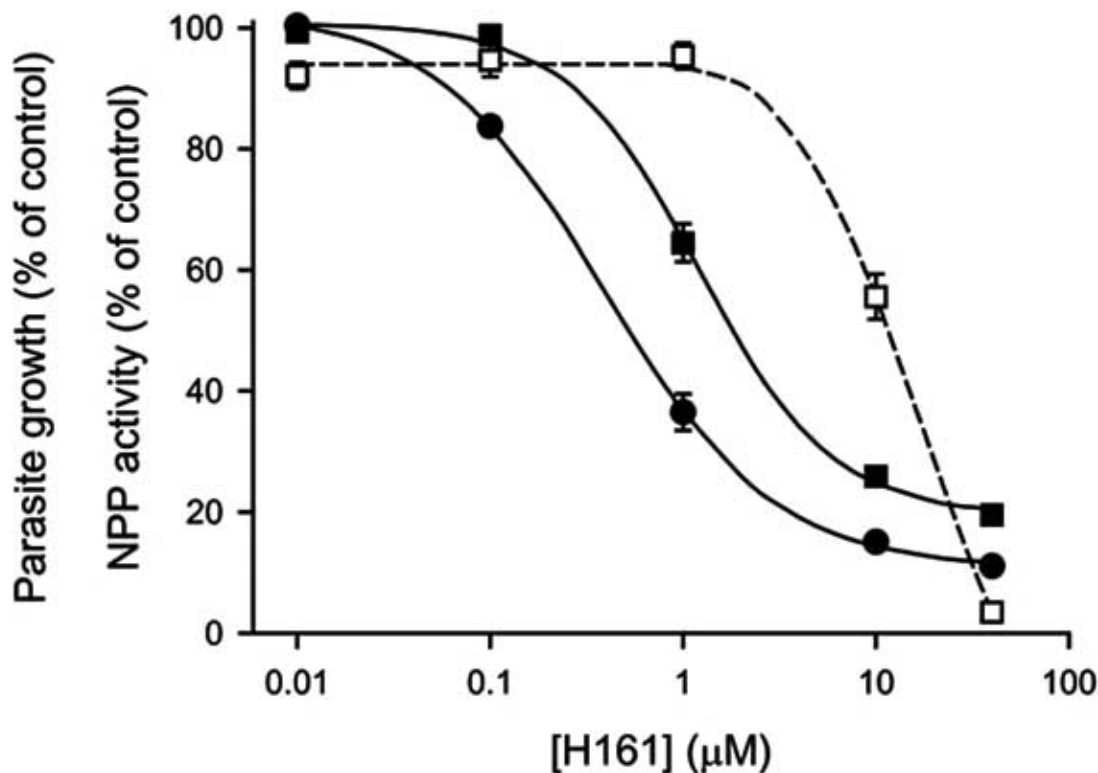


Fig. (1). Dose-response curves for the effect of H161 on the activity of the NPP (solid lines) and on parasite growth (dashed lines) in *P. falciparum*-infected human RBCs in the absence (circles) and presence (squares) of 8.5% v/v human serum.

The influx of [^{14}C]choline was used as a marker of NPP activity and [^3H] hypoxanthine incorporation was used as a marker of parasite growth. NPP activity and parasite growth are expressed as a percentage of that measured in the absence of H161. The data are averaged from three experiments, each on RBCs from a different donor, and are shown as the mean \pm S.E.M. (where not shown the errors lie within the symbols). Modified from [31] with permission from Elsevier.

2.2. Growth Inhibition Assays

An important test for any inhibitors, which are identified to block NPP activity, is to test whether they impair parasite growth and, if they do, how the growth inhibition profile compares with the NPP inhibition profile (see Fig. 1 for an example). It is, therefore, worth mentioning the techniques that are used to measure parasite growth in culture conditions.

It is possible (although very labour intensive) to test the effect of inhibitors by comparing the number of viable parasites left in cultures after a suitable length of time in the absence and presence of the blocker of interest, using Geimsa

stained blood smears. However, the standard screening technique uses the incorporation of [³H]hypoxanthine as a marker for parasite growth [25]. Basically, immature parasitised RBC cultures are grown in the presence of [³H]hypoxanthine for > 24 hours in 96-well plates. The lysed contents of each well is then passed over a glassfibre sheet to trap incorporated [³H]hypoxanthine and the sheets counted on a flat-bed scintillation counter (if parasites fail to grow in the presence of a compound, incorporated [³H]hypoxanthine is reduced compared with controls).

This assay, which uses a radio-labelled compound, has some of the shortfalls of radio-tracer transport assays.

Table 1. Inhibitors of the NPP in *P. falciparum*-Infected Human RBCs

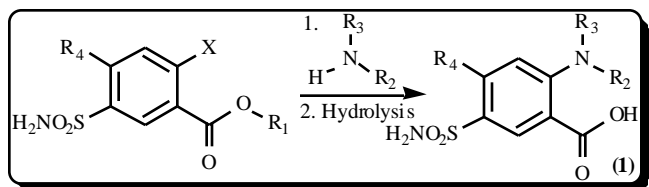
Inhibitor	Structure	IC ₅₀ (μM)	Ref.
Furosemide		1 - 5	[6, 16, 52]
Furosemide derivatives		0.04 - 9	[31]
Glibenclamide		11	[53]
Meglitinide		52	[53]
Niflumate		20	[16]
NPPB		0.1 - 0.8	[16, 30]
NPPB derivatives		0.1 - 11	[30]
Phloridzin		3 - 17	[29, 52, 54]
Phloridzin derivatives		2 - 140	[29]
Piperine		3 - 50	[52, 55, 56]

However, new HTS growth assays have recently been developed [26-28], which utilise fluorescent DNA markers. As human RBCs do not contain nuclei (and thus DNA), these assays quantify the amount of parasite DNA present in a culture (the lower the quantity of DNA compared with controls, the lower the parasite growth).

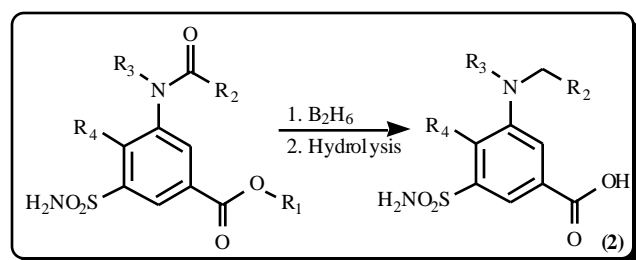
3. INHIBITORS OF THE NPP

A number of inhibitors of the NPP have been reported (Table 1). For some of these inhibitors, small derivative libraries have been created in attempts to identify more potent blockers [29-31]. The most potent blockers of NPP activity are based on furosemide and 5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB) [30, 31]. A number of these inhibitors have IC_{50} values (i.e. the concentration of inhibitor that reduces NPP activity by 50%) in the submicromolar range.

The most recent report presents data for a set of furosemide derivatives [31]. These compounds (synthesised by Aventis Pharma Deutschland GmbH (formerly Hoechst AG)) were originally developed as blockers of cation-Cl⁻ cotransport carriers [32] and can be separated into two groups. The furosemide-like molecules (**1**), which are synthesised by nucleophilic substitution of a leaving group X (e.g. -F or -Cl) in position 2 [33]:



and, the most effective derivatives (with some IC_{50} values < 100 nM), the piretanide-like molecules (**2**), which are prepared by a selective borane-reduction of the carbonyl compound and subsequent saponification of the ester [34]:



For a rational approach to developing combinatorial libraries of putative NPP inhibitors based on similar compounds to those described above, two factors can be taken into account from the data published by Kirk and Horner [30]. They reported that *R*-enantiomers of NPPB derivatives are more effective at inhibiting NPP activity than *S*-enantiomers and that the length of the uncharged groups added to the anionic "head group" alter potency (the longer the uncharged groups, the greater the potency of the inhibitor).

If starting from scratch, a simple starting scaffold can be determined by screening small anionic aromatic compounds. This is illustrated in (Table 2). At a concentration of 1 mM, 3-nitrobenzoic acid (the anionic "head group" of NPPB) has

its own intrinsic ability to block NPP activity (inhibiting by over 50%). Interestingly, 3-nitrophenol inhibits approximately 30% of NPP activity at 1 mM and, if this group is used to replace 3-nitrobenzoic acid in NPPB, the inhibitory potency of the resulting compound is reduced by more than 50-fold (unpublished observation from this laboratory). With this in mind, the results in (Table 2) suggest that while a single carboxylate group is important for inhibitor potency, twin carboxylate groups on the "head group" offer no benefit. Furthermore, salicylic acid would be an excellent starting point.

Table 2. The effect of Small Aromatic Compounds on NPP Activity in *P. falciparum*-Infected Human RBCs

Compound ^a	Structure	NPP activity (% of control) ^b
3-Nitrobenzoic acid		48 ± 1
Salicylic acid		33 ± 1
3-Nitrophenol		70 ± 2
Catechol		84 ± 2
Isophthalic acid		95 ± 3
Phenol		94 ± 3
Benzoic acid		80 ± 3
Phthalic acid		98 ± 1
Benzene		98 ± 1

^aCompounds were all present at a concentration of 1 mM.

^bThe influx of [¹⁴C]choline was used as a marker for NPP activity. NPP activity is expressed as a percentage of that measured in the absence of additional compounds. The data are averaged from three experiments, each on RBCs from a different donor, and are shown as the mean ± S.E.M.

4. PROBLEMS TO OVERCOME

Although, there are now excellent inhibitors of the NPP, there are several problems that, if addressed, would aid greatly rational approaches to designing new agents based upon these blockers and their development as antimalarials.

4.1. The Molecular Identities of the NPP

At present, the number, derivation and molecular structure(s) of the pathway(s) that form the NPP are unknown and this makes screening for inhibitors of the NPP very complex. If the number of types of pathways that form the NPP is one (as has been suggested by several groups [16, 35, 36]), then there is less of a problem. However, several reports have suggested that more than one type of pathway forms the NPP [37-40]. If so, it will not only be important to determine how critical each pathway is for parasite survival but it will also affect the choice of the test substrate used for profiling the effect of putative NPP inhibitors (i.e. specific test substrates would be required to profile each type of pathway present).

In the absence of molecular structures for the NPP, the range of molecular tools, which have now been developed specifically for *P. falciparum* [41], cannot be used for the characterisation of the NPP. In terms of screening and inhibitor development, molecular identities would ultimately enable stable transfection of the NPP into easy-to-handle expression systems. This would allow the movement away from expensive and potentially dangerous parasite cultures and, if more than one type of pathway does form the NPP, isolation of each. In addition, more complex binding-site interaction studies would be possible (combined with site-directed point mutations).

There is also the interesting question of whether the NPP are inactive, native RBC pathways induced by the parasite or parasite-derived. If the former is true, these pathways may be present in RBC progenitor cells (e.g. the erythroleukemic

cell line, K562), which could be used directly both for easier screening assays and for genetic manipulations. In addition, if the NPP are native to the RBC, it may also be harder to achieve selective inhibition compared with targeting a parasite-encoded protein but, if achieved, it may also be harder for the parasite to develop resistance.

4.2. Serum Binding and the Required Level of NPP Activity for Parasite Growth

The disappointing feature of the NPP inhibitors reported to date is that, while they are highly effective at inhibiting the NPP, they fail to inhibit parasite development in growth assays unless much higher concentrations are used [30, 31]. This point is illustrated in (Fig. 1). The figure shows dose-response curves for the effect of the furosemide-based analogue H161 on NPP activity (using choline as the test substrate) and parasite growth (the growth assay curve is to the far right of the transport inhibition curves).

One reason for this discrepancy is due to the presence of human serum (8.5% v/v) in the growth assays, which is not normally present in transport assays. The centre curve on (Fig. 1) shows the effect of having 8.5% v/v human serum present in the transport assay. This shifts the inhibition curve to the right towards the growth curve. The furosemide- and NPPB-based inhibitors are all lipophilic anions, which will bind to the proteins contained in serum (predominantly albumin) and effectively reduce their free concentration. Further evidence for this effect can be seen in (Table 3), which shows the effect of displacement agents (drugs that occupy large numbers of albumin binding sites) on the activity of the NPP in the presence of human serum and in the presence of human serum and NPPB. In the presence of 8.5% serum, the displacement agents inhibit, at most, 25% of NPP activity. However, if 10 μ M NPPB is also present, which alone inhibits less than 10% of NPP activity in the presence of 8.5% v/v human serum, NPP activity is further

Table 3. The Effect of Displacement Agents on the Ability of NPPB to Inhibit NPP Activity in the Presence of 8.5% v/v Human Serum

Displacement agent ^a	NPP activity (% of control) ^b	NPP activity in the presence of 10 μ M NPPB (% of control)	% difference
Control	100	91 \pm 1	9
Aspirin	87 \pm 2	75 \pm 2	12
Phenylbutazone	74 \pm 1	37 \pm 1	37
Tolbutamide	75 \pm 1	48 \pm 1	27
Ibuprofen	77 \pm 3	35 \pm 1	42
Valproic acid	94 \pm 1	64 \pm 1	30
Sulphisoxazole	83 \pm 1	65 \pm 1	18
Trichloroacetic acid	92 \pm 1	68 \pm 1	24
Warfarin	78 \pm 1	46 \pm 2	32

^aDisplacement agents were all present at a concentration of 1 mM.

^bThe influx of [¹⁴C]choline was used as a marker for NPP activity. NPP activity is expressed as a percentage of that measured in the absence of additional compounds. The data are averaged from three experiments, each on RBCs from a different donor, and are shown as the mean \pm S.E.M.

reduced by more than 10% (in each case) and suggests NPPB is binding to the same sites. This would need to be addressed when producing new inhibitor libraries.

Serum binding alone cannot explain why higher inhibitor concentrations are required to block parasite growth than block NPP activity (if it did, the presence of serum would shift the transport inhibition curve directly on to the growth inhibition curve in (Fig. 1)). This leaves the possibility that the NPP have a built-in overcapacity and do not need to operate at 100% to allow normal parasite growth (work from within this laboratory suggests that NPP can be reduced by 40% before parasite survival is affected in culture conditions [31]). If this is the case, it may be more appropriate to determine IC₉₀ values rather than IC₅₀ values for putative inhibitors.

4.3. Inhibitor Specificity

A general problem with inhibitors that target anion transport pathways is that they tend to lack specificity [42, 43] and this is also the case for the majority of NPP inhibitors. For example, the most potent inhibitor of the NPP reported to date is H156, with an IC₅₀ value of 41 nM [31], but it is nearly as effective at inhibiting the RBC anion exchanger, Band 3, with an IC₅₀ value of 50 nM [43]. However, with just a library of 16 NPPB analogues, Kirk and Horner [30] identified 5-nitro-2-(3, 3-diphenylpropylamino) benzoic acid as selective for the NPP when compared with three other anion transport pathways. This suggests that this problem is primarily due to the lack of research on anion transport inhibitors (compared with, for example, cation channel inhibitors) and that larger libraries will almost certainly produce specific inhibitors.

5. THE NPP AS DRUG DELIVERY ROUTES

While inhibition (and possibly increased activation) of the NPP remains an important goal for the development of new antimalarials, it is worth noting that the NPP have also been touted as selective routes for targeting malariacidal agents at the internal parasite [2, 44-46]. It is therefore worth reviewing the science behind this possibility as it may affect future antimalarial design.

Saliba and Kirk [46] reported that Pepstatin A (a protease inhibitor that reduces parasite growth) gains entry to the infected cell via the NPP and added further support for this conclusion by reporting that partial inhibition of the NPP also reduces the antimalarial effect of Pepstatin A. Several more compounds that inhibit parasite growth have also been shown to gain access to the parasite via the NPP. These include pentamidine [47], bisquaternary ammonium compounds [48] and toxic L-nucleosides [49]. The latter report highlights the selective role of the NPP as the L-nucleosides are extremely toxic to the parasite but are unable to enter normal mammalian cells.

As the number of parasite targets increase (aided by the recent publication of the *P. falciparum* genome [50]), the use of the NPP as a delivery route may become increasingly important. Two points should be noted when designing compounds that selectively use the NPP. The first is the use of monovalent cationic moieties because monovalent cations

permeate via the NPP, are generally far less permeable to biological membranes than anions and electroneutral compounds and can accumulate within parasites (due to the negative membrane potential of the parasite plasma membrane [51]). The second is the use of non-physiological stereo-isomers (e.g. the toxic L-nucleosides) because the NPP are not stereo-selective (while most human carrier transport pathways are).

6. CONCLUSIONS

The evidence to date suggests that the NPP are highly promising chemotherapeutic targets and/or selective routes for targeting the internal parasite. However, the NPP have yet to be exploited in the fight against malaria. There are now a number of highly potent inhibitors of the NPP, which could be used as potential lead compounds for the development of new inhibitor libraries. It is also worth noting that while this review has concentrated on inhibitors of the NPP, compounds that increase activity of the NPP may also have antimalarial capabilities (discussed by Kirk [13]). New libraries will allow exploration of more detailed structure-activity relationship studies (aided by the development of new HTS protocols), which will not only enhance our knowledge of malaria but might eventually aid the treatment of this devastating disease.

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ABBREVIATIONS

- NPP = New permeability pathways
 RBC = Red blood cell
 PVM = Parasitophorous vacuole membrane
 NPPB = 5-nitro-2-(3-phenylpropylamino) benzoic acid

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