RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

The diverse pharmacology and medicinal chemistry of Phosphoramidates – a review

Fabrício M. Oliveira,[a] Luiz C. A. Barbosa[ab*] and Fyaz M. D. Ismail[c]

The phosphoramidates consist of compounds which possess at least one amino group bound directly to the phosphorus atom and are, therefore, phosphoramide acid derivatives. The inherent chemical properties of the element phosphorus include polarizability, low to medium electronegativity and derivatives exhibit low coordination numbers thereby allowing synthesis of a diverse range of compounds. In line with their physicochemical properties, phosphorus compounds have widespread industrial applications and also demonstrate a diverse range of biological activities. In last two decades, notably, phosphoramidates have been evaluated for both their antitumor and antiviral efficacy. This brief review describes the most promising examples of this class which possesses antiviral, antitumor, antibacterial, antimalarial and anti-protozoal activity, as well as urease, acetyl and butyrylcholinesterase enzyme inhibitor activity.

Introduction

The element phosphorus was discovered in 1669 by Henning Brandt and since then, significant discoveries have been made regarding its biological function.[1]

The advent of suitable synthetic methodology coupled with advances in analytical science has accelerated research into both isolating and designing both organic and organometallic compounds with evermore diverse applications. Structural incorporation of a phosphorus atom within biomolecules essential for life, notably DNA and RNA, are especially abundant in cells. This element plays a pivotal role within ATP, which is used by most living organisms for storing and translocating energy (Fig. 1). Phosphorus compounds also serve as cofactors of multiple enzyme systems whereas others act as essential buffering agents within the cellular mileau.[1,2]

The inherent properties of the element phosphorus include: a) polarizability, b) low to medium electronegativity and c) low coordination number, thereby, allowing the synthesis of a diverse range of compounds. Biological and medicinal activities of organophosphorus compounds are modulated, in part, by the nature of the element bound to phosphorus and variations in its own oxidation state. In the last few decades, the phosphoramidates in particular have attracted the attention of many investigators. This class is characterized by having at least one $-NR^1R^2$ group (where R^1 and R^2 = alkyl, aryl, heteroaryl) bound to phosphorus, itself derived from phosphoramidic acid (Fig. 2).[2]

Amongst the large array of activities and properties reported for phosphoramidates, the following are particularly noteworthy: biological activities and potential agricultural applications,[3-5] flame retardant properties,[6,7] their use as antirust additives within lubricating oils[8] and, notably, pharmaceuticals used for treating and controlling various diseases.[1,9-31] Considering the increasing interest in this class of compound, especially within the pharmaceutical arena, this review discusses the most significant examples of phosphoramidates imbued with antiviral, antitumor, antibacterial, anti-malarial, anti-protozoal activities, as well as examples of selected compounds that act as inhibitors of urease, butyryl and acetyl cholinesterase. Hopefully, this review will encourage further attention upon this class of compounds, especially that involving the discovery of new drugs for the treatment of various diseases.
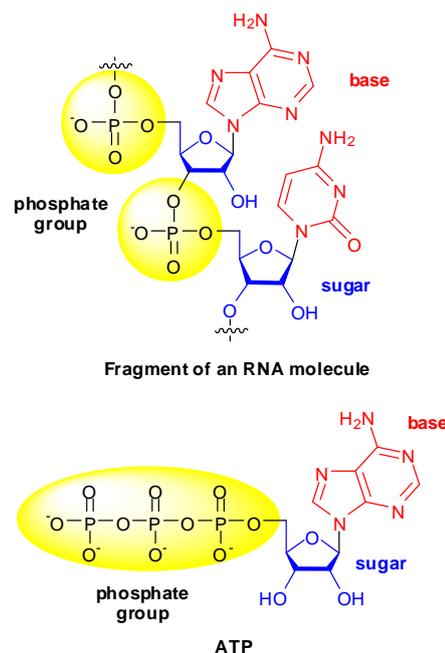
Fig. 1 Fragment of an RNA molecule and the structural formula of adenosine triphosphate (ATP).

[a] Departament of Chemistry, Universidade Federal de Viçosa, Viçosa, MG, 36570-000, Brazil.
[b] Departament of Chemistry, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos 6627, Pampulha, Belo Horizonte, MG, 31270-901, Brazil. Email address: lcab@ufmg.br; phone number +55 3138993068; fax +55 3138993065; www.luizclaudiobarbosa.com.br
[c] Medicinal Chemistry Research Group, Institute for Health Research, School of Pharmacy & Biomolecular Sciences, Byrom Street, Liverpool John Moores University, Liverpool, Merseyside, L3 3AF, UK.
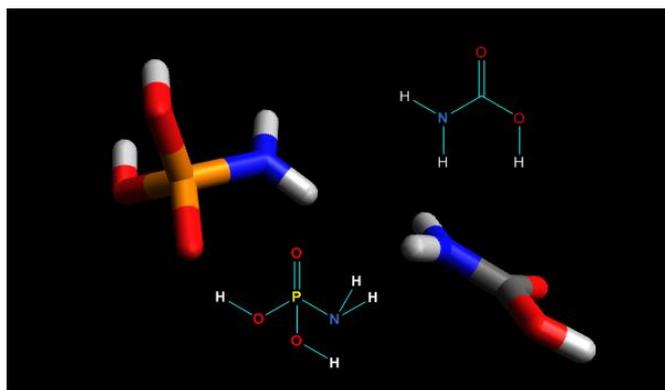
Fig. 2 a) Tetrahedral structure of phosphoramidic acid; b) planar structure of carbamic acid.

The use of vaccines during the last decades has assisted humankind in the fight against diseases caused by viruses such as rubella, measles, mumps and polio. However, for some viral pathogens such as the *Human papilloma virus* (HPV) and human herpes virus (HHV), amongst others, currently, there is neither any small molecule (denoted here as drug) nor vaccine effective for managing such infections. For other diseases, such as herpes simplex virus (HSV) and *Varicella-zoster virus* (VZV), although some effective drugs are available, in many cases off target-side effects in some patients can pose problems, and in such cases, it is imperative to discover new, more efficacious drugs.[28,32] For instance, two- drugs Telaprevir and Boceprevir were approved in 2011 for treating hepatitis C virus infection (HCV) in genotype 1 patients. However, these drugs do not exert effect on genotypes 2-6.[28] In these cases, the treatment also requires regular injections of pegalated α-interferon (PEG-IFN)[‡] with daily oral administration of ribavirin (a nucleoside analogue which possesses useful and broad antiviral activity). However, daily administration places additional burden upon the patient arising from management of uncontrolled side effects (Fig. 3). Amongst the large array of possible scaffolds for antiviral drugs development, the phosphoramidates promise considerable potential for development into putative drugs.[28]

[‡] Interferons (IFNs) are proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites or tumor cells. They facilitate communication between cells to trigger the protective defences of the immune system allowing eradicate of either pathogens or tumors. The pegylated form of interferon-alpha, with greater stability and in vivo activity, has substantially improved sustained virological response rates when compared with unmodified interferon-alpha.
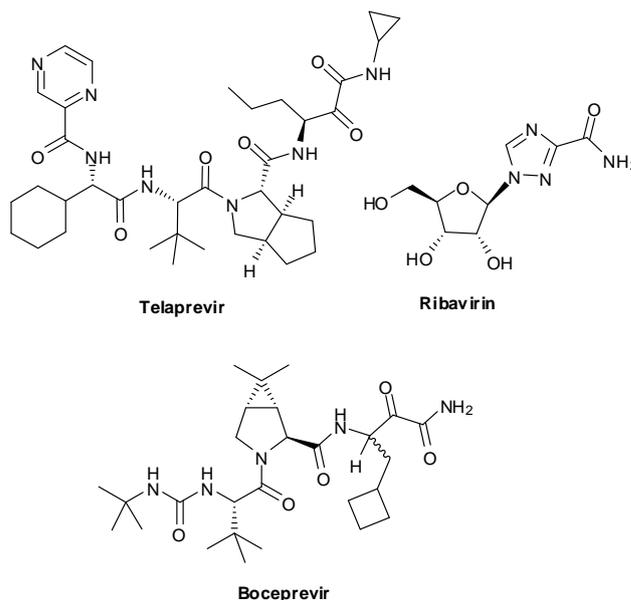
Fig. 3 Structure of Telaprevir, Boceprevir and Ribavirin, drugs approved for treating HCV infection in genotype 1 patients.

Nucleoside analogs are considered an important class of inhibitors of the viral polymerase of several therapeutic targets especially HCMV, HSV, HIV and HBV.[33] Recently some investigators have evaluated phosphoramidate groups covalently linked to nucleosides, a strategy known as 'Phosphoramidate protide'.[12-14,16,19,20,24,26,31,34-38] This approach was introduced by McGuigan *et al.* (1992) as a means of improving the therapeutic potential of a prototype drug.[23] The introduction of this group favors the phosphorylation process *in vivo*, by converting the nucleosides into their active forms as triphosphates derivatives, commonly known as nucleotides. These phosphorylated analogues have significantly advanced both the chemotherapy of neoplasms and antiviral treatments.[23,39] McGuigan *et al.*[19] have synthesized several phosphorylated nucleoside analogs that proved to be efficient phosphate releasing agents. The phosphoramidate derivatives of 4'-azidocytidine (**R1479**) were effective in inhibiting hepatitis C virus (HCV). From such compounds, pro-nucleoside derivatives were evolved that were up to 3.4 times more potent (**1**; EC_{50} = 0.38 μM) than the standard compounds (**R1479**; EC_{50} = 1.28 μM) (Fig. 4). Sofosbuvir (Fig. 4) is a drug for the treatment of HCV approved by FDA in December of 2013. This compound suppresses various HCV genotypes in patients exposed to the drug for up to 12 weeks without causing any serious side effects. Clinical trials revealed a cure rate of 90% when sofosbuvir (Fig. 4) is combined with peginterferon and ribavirin. Notably, with conventional treatment that utilizes only peginterferon and ribavirin, the cure rate is less than 50%.[40]
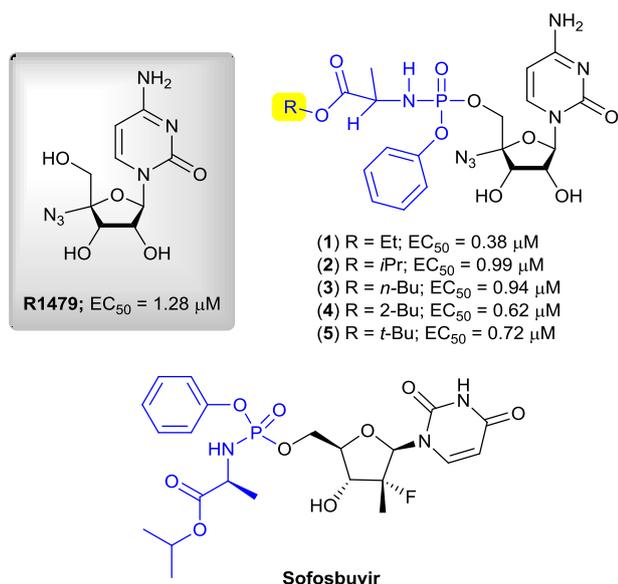
Fig. 4 Structural formulas of compounds with anti-HCV activity.

(**1**) R = Et; EC$_{50}$ = 0.38 µM
(**2**) R = iPr; EC$_{50}$ = 0.99 µM
(**3**) R = n-Bu; EC$_{50}$ = 0.94 µM
(**4**) R = 2-Bu; EC$_{50}$ = 0.62 µM
(**5**) R = t-Bu; EC$_{50}$ = 0.72 µM

R1479; EC$_{50}$ = 1.28 µM

Sofosbuvir

	EC$_{50}$ (µM)		
	HSV-1	HSV-2	HSV-1 TK⁻
(**6**) Aryl = p-F-Ph; AA = L-Ala; R^2 = Bn	0.9	0.5	1.5
(**7**) Aryl = Naph; AA = DMG; R^2 = Bn	1.4	1.6	3.2
(**8**) Aryl = Ph; AA = L-Leu; R^2 = Bn	0.8	0.7	1.4
(**9**) Aryl = Ph; AA = L-Ile; R^2 = Bn	1.1	1.1	1.4

	EC$_{50}$ (µM)		
	HIV-1 CEM	HIV-2 CEM	HIV-1 MT-4
(**10**) Aryl = Naph; AA = L-Ala; R^2 = Bn	15	8.9	0.8
(**11**) Aryl = Naph; AA = L-Ala; R^2 = n-Pr	6.6	24	10
(**12**) Aryl = Naph; AA = L-Ala; R^2 = Me	10	13	4.7
(**13**) Aryl = Naph; AA = L-Ala; R^2 = Et	12	42	1.7
(**14**) Aryl = Naph; AA = L-Ala; R^2 = i-Pr	6.2	12	5.4
(**15**) Aryl = Naph; AA = L-Ala; R^2 = Me	17	26	15
(**16**) Aryl = Naph; AA = L-Ala; R^2 = Bn	16	11	5.7

Fig. 5 Structure of Acyclovir® and synthetic pro-nucleosides actives against HSV-1 TK- and HIV viruses (Derudas et al. 2009).[34]

Acyclovir® (also commercially available as Zovirax®) and a series of derivatives were synthesized by Derudas et al.[34] Acyclovir (ACV), a synthetic purine nucleoside analogue, possesses inhibitory activity *in vitro* and *in vivo* against human herpes viruses including *Herpes simplex virus* (HSV) types 1 and 2, *Varicella-zoster virus* (VZV), *Epstein-Barr virus* (EBV) and *Cytomegalovirus* (CMV).[41] ACV is considered inactive against virus HSV thymidine kinase deficient (HSV-1 TK⁻) (EC$_{50}$ = 50 µM), whereas in the case of nucleoside, pro-activity was increased 35 fold (EC$_{50}$ = 1.4 µM) (Fig. 5). As for activities against HSV-1 and HSV-2, the synthetic compounds proved slightly less active than the standard product ACV employed as a control. Against HSV-1, ACV revealed an EC$_{50}$ = 0.4 µM, whilst for compounds **6-9** the activities were as follows: compound **6**, EC$_{50}$ = 0.9 µM; **7**, 1.4 µM; **8**, 0.8 µM; **9**, 1.1 µM. For HSV-2, ACV had an EC$_{50}$ = 0.2 µM and for the synthetic phosphorous compounds the EC$_{50}$ values were: compound **6**, 0.5 µM; **7**, 1.6 µM; **8**, 0.7 µM; **9**, 1.1 µM. In the same study, the investigators found that pro-nucleoside derivatives **10-16**, incorporating the amino acid alanine, showed high activity (EC$_{50}$ values lay between 0.8 and 42 µM) on three cultures of HIV (HIV-1 CEM, HIV-2 CEM and MT-4) (Fig. 5), whilst ACV proved relatively inactive (EC$_{50}$ > 250 for all cultures). The pro-nucleosides **6-9** were also evaluated against HIV cultures, but failed to demonstrate any useful activity against these infections.[34]

Phosphoramidate 2'-C-methyl guanosine monophosphate Pro-drugs **18a-i**, were active against HCV (Fig. 6). Notably, the addition of a phosphorous group to nucleosides (**17a-i**) enhanced biological activity over 100 fold. Amongst the compounds synthesized, the insertion of the amino acid alanine produced the most favorable modification, with EC$_{50}$ lying within the nanomolar range (Fig. 6).[20] The phosphoramidate derivatives (**20-30**) of 4'-azidouridine (**AZU**, **19**, Fig. 7), synthesized by Perrone et al.,[26] were up to 450 times more active against HCV when compared with their corresponding, non-phosphorylated, precursors. Data are presented as EC$_{50}$ values (representing the concentration of compounds reducing HCV replication by 50%) and CC$_{50}$ values (representing the concentration of compounds reducing cell viability by 50%) as determined using the WST assay. All phosphoramidates tested (**20-30**) were non-toxic towards HCV in the replication assay (CC$_{50}$ > 100 µM). In this series, the most active compound was **20** (EC$_{50}$ = 0.22 µM), with the phosphorus atom in either the *R* or *S* configuration. In general, it was observed that compounds bearing naphthyl group (**20-22**) were more active when compared with their corresponding analogues (**23-30**) bearing a phenyl group (Fig. 7).

REVIEW

(17a-i) → **(18a-i)**
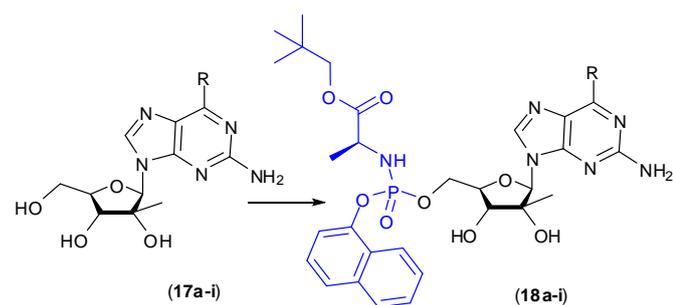
		EC$_{50}$ (µM)	
	R	Series **17**	Series **18**
a	Cl	8.5	0.012
b	OMe	4.6	0.010
c	OEt	8.8	0.08
d	NHMe	13	0.035
e	SMe	11	0.037
f	O(CH$_2$)$_3$OMe	12	0.043
g	NHCH$_2$Bn	24	0.270
h	NHBn	30	0.79
i	OCH(CH$_3$)$_2$	9.3	0.023

Fig. 6 HCV Replicon activity of 6-substituted 2′-C-methylguanosine nucleosides (**17a-i**) and ProTides (**18a-i**).
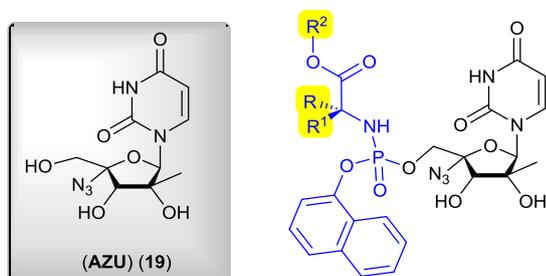
AA = *L*-Ala; ester = Bn

(**19**) 4′azidouridine; EC$_{50}$ > 100 µM
(**20**) Phosphorus configuration: *S/R*; EC$_{50}$ = 0.22 µM
(**21**) Phosphorus configuration: *R*; EC$_{50}$ = 0.39 µM
(**22**) Phosphorus configuration: *S*; EC$_{50}$ = 0.43 µM
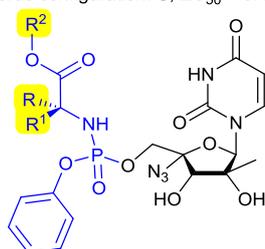
(**23**) AA = *L*-Ala; ester = Et; EC$_{50}$ = 1.3 µM
(**24**) AA = *L*-Ala; ester = Bu; EC$_{50}$ = 1.2 µM
(**25**) AA = *L*-Ala; ester = 2-Bu; EC$_{50}$ = 0.63 µM
(**26**) AA = *L*-Ala; ester = *i*-Pr; EC$_{50}$ = 1.77 µM
(**27**) AA = *L*-Ala; ester = Bn; EC$_{50}$ = 0.61 µM
(**28**) AA = Phe; ester = Et; EC$_{50}$ = 1.37 µM
(**29**) AA = Gly; ester = Bn; EC$_{50}$ = 1.6 µM
(**30**) AA = *D*-Ala; ester = Bn; EC$_{50}$ = 1.2 µM

Fig.7 HCV Replicon activity of 4′-azidouridine nucleosides derivatives (**20-30**).

In an attempt to increase the concentration of the bioactive ribavirin monophosphate in the cells, some phosphorylated derivatives of ribavirin (Fig. 8) were prepared.[12] Subsequent

pharmacological evaluation revealed that compound (**31**) was active against *Vesicular stomatitis virus* (EC$_{50}$ = 20 µM), *Vaccinia virus* (EC$_{50}$ = 100 µM) and Influenza B (EC$_{50}$ = 14 µM); compound (**32**) was active against *Punta Toro virus* (EC$_{50}$ = 100 µM) and (**33**) showed a better effect against *Vesicular stomatitis virus* (EC$_{50}$ = 20 µM), *Vaccinia virus* (EC$_{50}$ = 20 µM) and *Punta Toro virus* (EC$_{50}$ = 58 µM). These values are comparable to the activity of ribavirin (*Vesicular stomatitis virus*, EC$_{50}$ = 22 µM; *Vaccinia virus*, EC$_{50}$ = 22 µM; *Influenza B*, EC$_{50}$ = 9 µM; *Punta Toro virus*, EC$_{50}$ = 183 µM), clearly indicating that phosphorylation, in this case, did not improve the desired bioactivity. Studies using cell lysate coupled with molecular modeling investigations revealed a weak activation of ProTide in comparison with the free monophosphate, which may provide one explanation for the observed biological activity.

Stavudine (d4T), an analogue of thymidine, is the fourth antiretroviral drug to achieve world-wide commercialization.[41] In 2011, Román *et al*. synthesized several diasteroisomericaly pure derivatives of d4T phosphoramidates (**34-37**). Antiviral activities against HIV-1 and HIV-2 were evaluated and the corresponding EC$_{50}$ against type HIV-1 (IIIB) are shown in Fig. 9. Since the phosphorous center is chiral, all compounds can exist in the S_P and R_P forms. All isomers were examined and, in general, compounds with S_P stereochemistry proved superior to the corresponding R_P analogues, compound **36** being a notable exception.[35]

(**31**) R = CH$_3$
(**32**) R = CH$_2$Ph
(**33**) R = H

Fig. 8 Phosphorilated derivatives of ribavirine.

(S_P)-(**34**) Ar = *p*-Cl-Ph; R = CH$_3$; EC$_{50}$ = 0.021 µM
(R_P)-(**34**) Ar = *p*-Cl-Ph; R = CH$_3$; EC$_{50}$ = 0.13 µM
(S_P)-(**35**) Ar = *p*-Cl-Ph; R = Bn; EC$_{50}$ = 0.042 µM
(R_P)-(**35**) Ar = *p*-Cl-Ph; R = Bn; EC$_{50}$ = 0.14 µM
(S_P)-(**36**) Ar = *p*-Br-Ph; R = CH$_3$; EC$_{50}$ = 0.45 µM
(R_P)-(**36**) Ar = *p*-Br-Ph; R = CH$_3$; EC$_{50}$ = 0.19 µM
(S_P)-(**37**) Ar = *p*-Br-Ph; R = Bn; EC$_{50}$ = 0.029 µM
(R_P)-(**37**) Ar = *p*-Br-Ph; R = Bn; EC$_{50}$ = 0.11 µM

Fig. 9 Structural formulas of diasteroisomericaly pure derivatives of d4T phosphoramidates actives HIV-1 (IIIB)

Some reports have demonstrated that phosphoramidate derivatives of dioxolane sugar nucleosides (DOA) possessing pyrimidines improved the antiviral potency *in vitro*.[42,43] The phosphoramidates **38** and **39**, synthesized by Bondada *et al*.[44] had evaluated their activities against HIV-1 and HBV. The compound **38** displayed up to a 1070-fold greater potency versus HIV-1 compared to their corresponding parent nucleoside (**40**), and, notably, were up to 12-fold more potent versus HBV (Fig. 10). The improved and significant dual antiviral activity of these novel phosphoramidate nucleosides was partially explained by the increased intracellular formation of the adenosine dioxolane triphosphate. Cytotoxicity was absent for compound **38** when tested at concentrations of 100 μM in human PBM cells, Vero cells (kidney epithelial cells from the African green monkey) and CEM cells (a human-T-cell-derived cell line), whereas compound **39** showed a value of $CC_{50} = 65$ μM for Huh7 cells (Human Hepatoma Cell).

Recently, Cho *et al*.[9] have formulated a structure-activity relationship (SAR) of C-nucleosides containing the 2'-C-Me as putative NS5B inhibitors. In search of compounds with a better selectivity compared to their precursors, structural changes were introduced within both the heteroaromatic portion base and sugar moiety, coupled with a monophosphate prodrug approach. Judicious modification of pharmacokinetic parameters led to the discovery of phosphoramidate **GS-6620** (Fig. 11), the first C-nucleoside clinical candidate. During phase I clinical trials **GS-6620** showed potent anti-HCV activity in HCV-infected patients, but with an unexpectedly high variability in PK/PD investigations.[10]
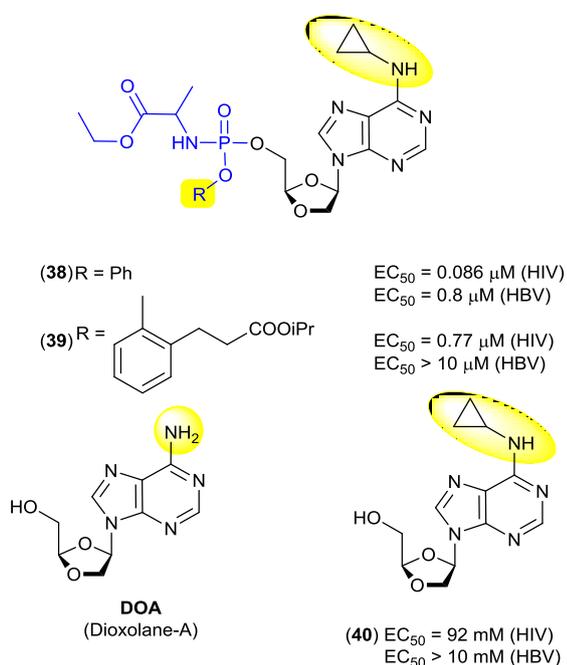
(**GS-6620**)
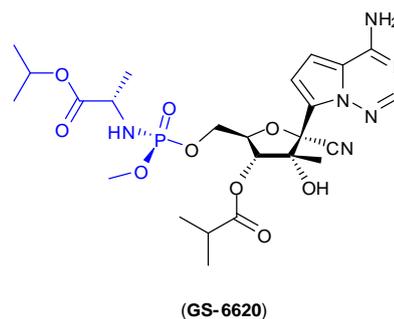
Fig. 11 Phosphoramidate prodrug possessing HCV replicon activity.

In a study aimed at improving efficacy against HCV, several phosphoramidate derivatives 2'-methycytidine were prepared (Fig. 12). Although Valopicitabine 1 (**NM283**) proved highly efficacious, phase II clinical trials revealed some undesirable side effects. In the case of derivative **41**, a thirty fold increase in activity was observed in the replication test, whilst compound **42** proved to be only ten times more active. However, measurements of triphosphate levels in mouse liver after oral administration, showed low levels of nucleoside triphosphate (NTP) for both compounds. In contrast, compound **41**, after six hours of subcutaneous administration,[13] exhibited high levels of NTP.

The anti-influenza activity of 6-modified 2'-fluoro-2'-phosphoramidate deoxyriboside ProTides was evaluated *in vitro* (Fig. 13). Compounds **43-46** showed EC_{99} value ranging from 12 μM to 15 μM. Xenobiotic metabolism experiments with carboxypeptidase Y and whole-cell lysates demonstrated that the 2'-fluoro-2'-deoxyguanosine ProTides are readily cleaved by cellular enzymes to release the 6-modified 2'-fluoro-2'-deoxyguanosine 5'-monophosphate. Further conversion into 2'-fluoro-2'-deoxyguanosine 5'-triphosphate, the active metabolite that inhibits the viral polymerase then occurs. Antiviral results were supported by drug metabolism studies and calculations suggest that the clogP value (in the range of 2–3) may be favourable. These compounds proved inactive against HSV-1, HSV-2 TK-deficient HSV-1, as well as *Vaccinia* and *Vesicular stomatitis viruses*.[25]

(**38**) R = Ph

$EC_{50} = 0.086$ μM (HIV)
$EC_{50} = 0.8$ μM (HBV)

(**39**) R = [structure] COOiPr

$EC_{50} = 0.77$ μM (HIV)
$EC_{50} > 10$ μM (HBV)

DOA
(Dioxolane-A)

(**40**) $EC_{50} = 92$ mM (HIV)
$EC_{50} > 10$ mM (HBV)

Fig. 10 Adenosine dioxolane nucleoside phosphoramidates as antiviral agents for inhibiting Human Immunodeficiency and Hepatitis B Viruses
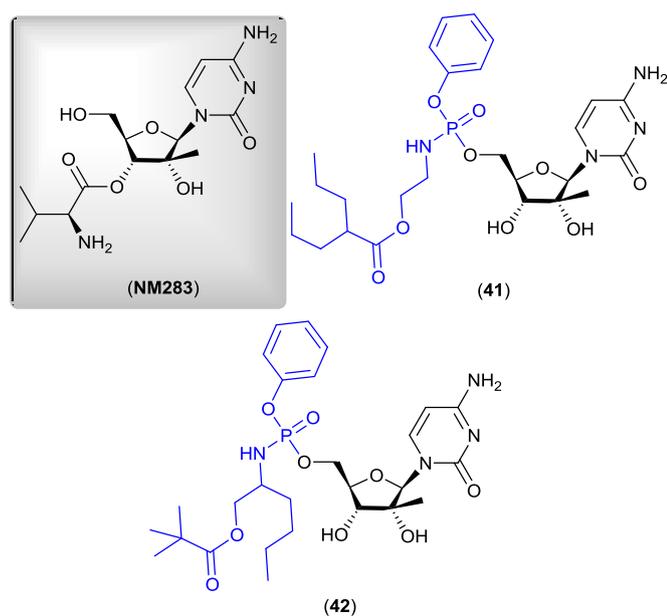
Fig. 12 Structure of **NM283** and 5'-Phosphoramidate prodrugs of 2'-C-methylcytidine inhibitors of NS5B.

(43) R = OCH$_3$, X = Me; EC$_{99}$ = 15 μM
(44) R = OCH$_2$CH$_3$, X = Me; EC$_{99}$ = 14 μM
(45) R = OCH$_2$CH$_3$, X = Et; EC$_{99}$ = 12 μM
(46) R = Cl, X = Bn; EC$_{99}$ = 12 μM
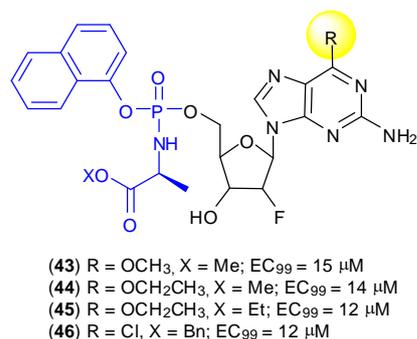
Fig 13. Phosphoramidate ProTides with anti-influenza virus activity.

The synthesis of numerous phosphoramidates with antiviral activities has been described in the literature. Recently, Sofia *et al.*[28] published a review on inhibitors of hepatitis C virus NS5B RNA-dependent RNA-polymerase highlighting in particular the phosphoramidates. Several other research groups are also engaged in the search for promising bioactive phosphorylated molecules but have only achieved sporadic success.[13,14,16,25,29,31,36-38,43,45-47]

Antitumoral Activity

Cancer is a disease characterized by abnormal cell division and migration, accounting for approximately 13% of all human deaths worldwide. In 2008, 12.4 million cases and 7.6 million deaths have been reported. The forecast is that by 2030 there will be 27 million cancer patients.[48,49] Consequently, there is an urgent need to develop new, cost effective and safe drugs (chemotherapy agents) which may be used in combination with surgery and radiation therapy to reduce overall mortality.

Notably, "Phosphoramidate protide" has also been used as a strategy for the development of compounds possessing antitumor activity. This strategy combines, generally, the advantage of high water solubility in many cases, with low toxicity (to normal cells) of such compounds.[50] For instance, brivudine (**BVdU**) (Fig. 14), a potent inhibitor of *Herpes simplex virus* type 1 (HSV-1) and *Varicella-zoster virus* (VZV), has served as a model for the development of newer, active, prototypes drugs.[51,52] The incorporation of a phosphoramidate group within this scaffold led to the discovery of Thymectacin (**NB1011**, **47a**) by the company New Biotics. This compound selectively acts on tumor cells that express high levels of thymidylate synthase.[53,54] Subsequently, the replacement of the methyl by a benzyl group within Thymectacin (**47a**) increased the potency against colon cancer HT115 by 175 fold (**47b**, Fig. 14).[24] Cogiatu *et al.*[11] have synthesized a number BVdU phosphoramidate derivatives (**48-54**) (Fig. 14) possessing high cytotoxic activity against the cell lines MDAMB231 (breast cancer) and PC-3 (prostate cancer) cell lines. Compounds **48-50** and **53**, tested as diastereisomer mixtures, were the most active on MDAMB231 cells, with EC$_{50}$ ranging from 0.32 μM to 2.7 μM whilst compounds **49-54** showed EC$_{50}$ values in the range of 1.4-6.9 μM upon PC-3. These compounds were more active than the standard control NB1011 (EC$_{50}$ = 79 and 155 μM on MDAMB231 and PC-3, respectively). For compound **53**, the mixture was separated by reversed phase chromatography and each diastereoisomer was tested separately against the MDAMB231 (breast cancer) cell line. The rapidly eluting diastereoisomer (*R* stereochemistry on P center) was less active than the mixture with an EC$_{50}$ = 7.4 μM, whilst the slow eluting diastereoisomer (*S* stereochemistry on the P center) was 10 times more potent than the mixture, with an EC$_{50}$ = 0.5 μM highlighting the importance of investigating pharmacology of individual stereoisomers.

Some fluorinated pyrimidine nucleosides[55] have been used as chemotherapeutic agents since 1957 in the treatment of ovarian, breast and gastrointestinal tumors.[56,57] The fluorinated pyrimidine 5-fluorouracil-2'-deoxynucleoside (**FUDR**) is active against liver metastases, and is beneficially metabolized within the host.[58] In recent studies, McGuigan *et al.*[22] reported the synthesis of **39** FUDR phosphate pro-drugs derivatives (Fig. 15) and measured their relative cytotoxic activity within tumor cell lines L1210/0, L1210/TK-, Cem/0, Cem/TK-, HeLa, HeLa/TK-. The effect on L1210/0 infected with *Mycoplasma hyorhinis* (L1210.Hyor) was also reported. In general, the compounds showed ability to release the nucleoside monophosphate within intact cells whilst maintaining activity in tumor cell cultures infected with mycoplasma. The ProTide's stability in both acidic and neutral pH, as well as host plasma, is of great importance, especially considering that some compounds have proven cytotoxicity within the namomolar range.
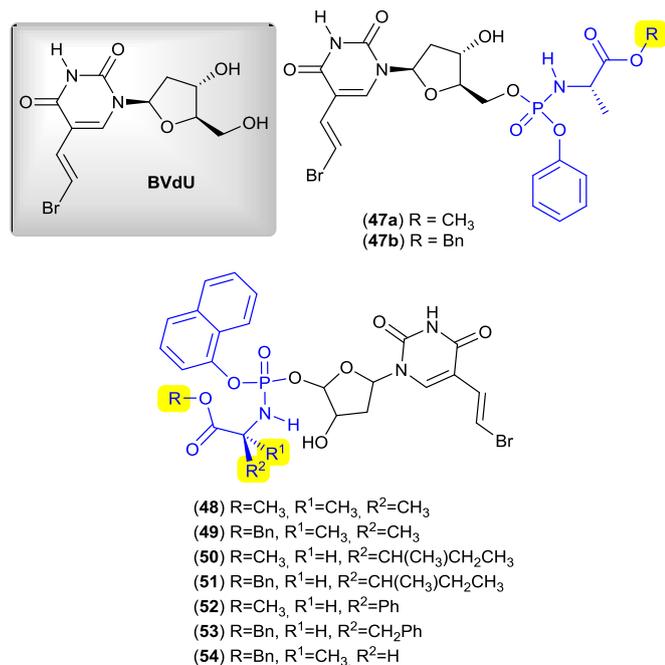
Fig. 14 Structure of Brivudine (BVdU) and various pro-nucleoside derivatives.

(47a) R = CH$_3$
(47b) R = Bn

(48) R=CH$_3$, R^1=CH$_3$, R^2=CH$_3$
(49) R=Bn, R^1=CH$_3$, R^2=CH$_3$
(50) R=CH$_3$, R^1=H, R^2=CH(CH$_3$)CH$_2$CH$_3$
(51) R=Bn, R^1=H, R^2=CH(CH$_3$)CH$_2$CH$_3$
(52) R=CH$_3$, R^1=H, R^2=Ph
(53) R=Bn, R^1=H, R^2=CH$_2$Ph
(54) R=Bn, R^1=CH$_3$, R^2=H

R^1, R^2 = amino acid side chain
R^3 = Alkyl, cycloalkyl, aryl
Ar = Ph or 1-Naph
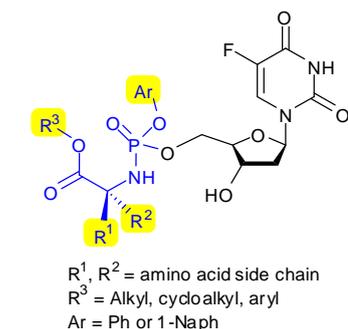
Fig. 15 General structure of FUDR Prodrugs described of McGuigan et al.[22]

(55) R = CH$_2$CH$_3$
(56) R = -CH$_2$CCH

Fig. 16 3´-azido-2´,3´-dideoxy-5-fluorouridine phosphoramidates with anticancer activity.

In 2013, Lewandowska et al.[59] reported the synthesis of 4-chlorophenyl N-alkyl phosphoramidate diesters of 3´-azido-2´,3´-dideoxy-5-fluorouridine (**AddFU**) (Fig. 16) and evaluation of their cytotoxic activity in three human cancer cell lines: cervical (HeLa), oral (KB) and breast (MCF-7). The most active compound for all cell lines was compound **56**, which displayed IC$_{50}$ = 1.64 μM, IC$_{50}$ = 1.64 μM and IC$_{50}$ = 1.88 μM for HeLa, KB and MCF-7 cells, respectively. This compound was more potent than the parent **AddFU** (IC$_{50}$ = 11.06 μM, IC$_{50}$ = 8.11 μM and IC$_{50}$ = 10.32 μM, respectively). Phosphoramidate **56** also exhibited relatively high activity for these cell lines, displaying IC$_{50}$ = 4.21 μM for HeLa, IC$_{50}$ = 3.81 μM for KB and IC$_{50}$ = 5.41 μM for MCF-7. The authors suggested that the mechanism of inhibition of these compounds differs from that described by McGuigan et al.,[22] when devoid of the ester motif, which initiates the hydrolysis process to release the nucleoside analogue 5´-monophosphate.

Other notable examples of compounds demonstrating useful antineoplastic activities include triethylenethiophosphoramides and cyclophosphamides (Fig. 17). For instance, Thio-TEPA®, which has been previously used against a variety of neoplasms, demonstrated consistent efficacy against breast and ovarian adenocarcinomas as well as superficial papillary bladder carcinoma. The pharmacological effect is considered similar to mechlorethamine hydrochloride (**57**), the first systemic chemotherapeutic agent to be approved in the United States for the treatment of Hodgkin's disease, lymphosarcoma, chronic myelocytic or lymphocytic leukemia, polycythemia vera, bronchogenic carcinoma and mycosis fungoides (MF), the most common type of cutaneous T-cell lymphoma (CTCL).[1,60] The cyclophosphamide (2H-1,3,2-oxazaphosphorin-2-amine, N,N-bis(2-chloroethyl)tetrahydro-2-oxide is the active principle of Endoxan®. This genotoxic nitrogen mustard is used in the treatment of many cancers and is also endowed with marked immunosuppressant properties.[1,61] In an attempt to alleviate carcinogenic potential and find more selective agents, it has served as a template for the synthesis of **TH-302** (Fig. 17). This nitro-1H-imidazole has proven effective in the control of pancreatic tumor cells MIA PaCa-2, whilst also surviving microsomal metabolism. In addition, when combined with Gemcitabine®, it has proven particularly effective when tested in mice. Such attractive preclinical properties of **TH-302** have prompted Phase 1 clinical trials for the treatment of solid tumors.[62] The presence of the nitro group may engender activity in hypoxic conditions found in these neoplasms. Another series of compounds derived from cyclophosphamide, namely trofosfamide (**58**), mafosfamide (**59**), and ifosfamide (**60**) (Fig. 17) represent some of the most widely used anticancer chemotherapeutic agents.[1]

Wang et al.[30] prepared phosphoramidate derivatives of haematoporphine dihydrochloride (**HPD**), a drug with anti-tumor properties marketed under the name Apiabasilon®. For instance, compound (**61**), shows selectivity against the liver tumor cancer cell line (BEL-7402), possessing activity in the micromolar range in vitro (IC$_{50}$ = 8.41 μM; Fig. 18).
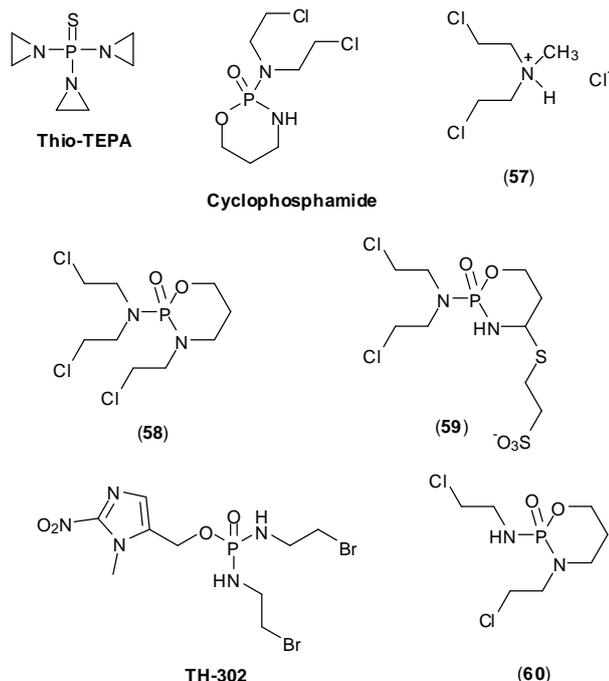
Fig. 17 Chemical structures of some highly effective anticancer agents.
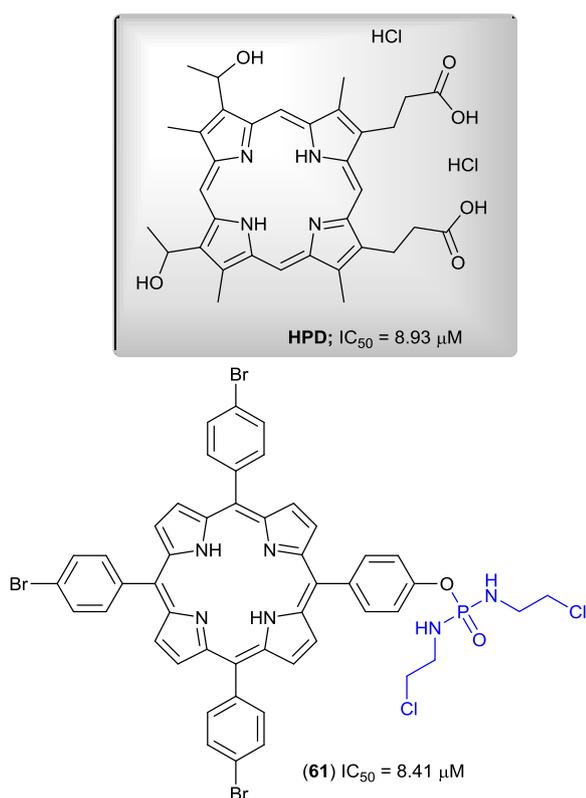
Fig. 18 Structure of the momomeric photosensitiser found within the mixture denoted HpD64 and its posporamide analogue of chlorin (**61**). The IC$_{50}$ reported are against the liver tumor cell line BEL-7402

Some highly potent anticancer heterocyclic phosphoramidates were synthesized by Kiran *et al.*[15] Tests *in vitro*, involving three human tumor cell lines NCI-H460 (large

cell lung), MCF-7 (breast adenocarcinoma) and SF-268 (central nervous system glioblastoma), cyclic compounds like **62** (IC$_{50}$ = 2.8-4.5 μM) showed greater activity when compared to acyclic products such as **63** (IC$_{50}$ = 71.3-90.4 μM) (Fig. 19). The investigators did not offer an explanation as to why such cyclic compounds were highly active, and considering the limited number of analogues prepared, further investigations, especially those involving substitutions on the aromatic rings may produce compounds with superior activity.
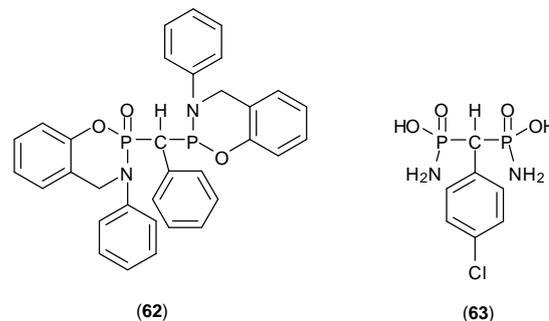
Fig. 19 Anticancer phosphoramidates reported by Kiran *et al.* (2008).

Ghosh *et al.*[64] reported that 7-benzylguanosine monophosphate (GMP-7BN) (**64**) was a potent antagonist of eukaryotic initiation factor 4E (eIF4E) (K$_d$ = 0.8 μM). However, this compound possesses a negative charge at physiological pH preventing penetration across the cell membrane.[50] Consequently, phosphoramidate derivatives of **64** were prepared and the pro-drug **65** was also active against eIF4E (Fig. 20). Furthermore, it was found that the zwitterion **65** was very efficient in crossing the membrane of MDA-231 breast cancer and lung cancer (H460, H383 and H2009) cell lines. Hydrolysis of the indolyl side chain once inside the cells provided the active anion form **64** which was then entrapped within the cells.[65]
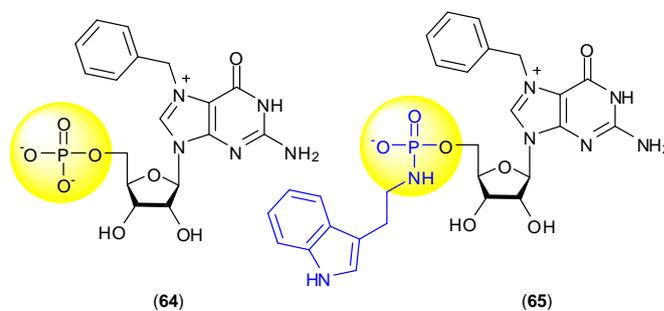
Fig. 20 Structures of 7Bn-GMP and 7Bn-GMP pro-drug.

Antibacterial activity

The incidence of clinically multi-resistant antibiotics bacterial infections caused by bacteria has led to an unacceptable increase in mortality rates. An absence of effective treatments makes it vitally important that both health professionals and the public are sufficiently informed to take

necessary precautions so as to maximize the effectiveness and lifetime of currently available antibiotics. Meanwhile, both the pharmaceutical sector and academic investigators continue to search for both novel targets and mechanisms of drug action rational drug design thereby facilitating development of new and effective antibacterial agents.[66-68] In this context, amongst the several classes of compounds with putative antibacterial activity, phosphoramidates possess considerable potential for further development.

In 2011, Gholivand and Dorosti[69] reported the synthesis of two new *N*-phosphinyl ureas (Fig. 21) and evaluated their antibacterial activities against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* using the disc diffusion method and also obtained the minimum inhibitory concentration (MIC) for each compound. Compound **66** exhibited high activity against all of the microorganisms under test (MIC = 1.17-18.8 µg.cm^{-3} comparable with MIC of Genatamycin used as a positive control) whilst compound **67** was virtually inactive (MIC > 385 µg.cm^{-3}).
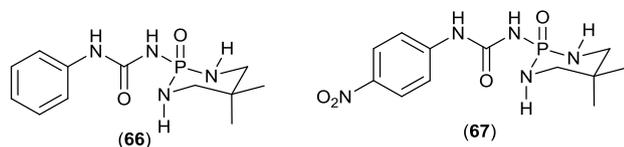
Fig. 21 Two novel *N*-phosphinyl ureas with antibacterial activity.

Prasad *et al.*[27] have synthesized a series of heterocyclic phosphoramidates (**68-76**), which showed moderate activity against two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) and four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Klebsiella pneumoniae*). Their antibacterial activity was evaluated at concentrations of 20 µg/ml and 40 µg/ml with Ciprofloxacin® a commercial quinolone antibiotic, as a comparator (Fig. 22). Compounds **71-74** inhibited bacterial growth at a concentration of 20 µg/mL for both Gram-positive and Gram-negative bacteria. Compounds **73** and **74** exhibited the highest activity. Other diphenylphosphoramidates (**77-78**), have been prepared by Madhala and coworkers.[70] When tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, they were shown to be twice as effective as Cyprofloxacin® (Fig. 22).

Antiurease activity

Urease is a nickel-dependent enzyme present in various organisms, such as plants, fungi and bacteria, especially *Proteus mirabilis*, *Klebsiella aerogenes* and *Helicobacter pylori* and, consequently, is of considerable biological significance considering its ubiquity in organisms causing diseases of the gastrointestinal and urinary tracts. By neutralizing gastric acid through ammonia production from urea hydrolysis, *H. Pylori*, can maintain 50% to 60% of the metabolic activity at pH = 2.5[71-74] and is considered the leading cause of stomach ulcers. It has been estimated that around half

of the world population is infected with this microorganism, and in some developing countries this can affect up to 85% of the adult population.[72] During the search for novel enzyme inhibitors of urease phosphoramidates showed excellent activity. Amongst putative urease inhibitors, *N*-(but-1-yl)thiophosphoric triamide (**NBPT**, Fig. 23) excels in inhibiting the enzyme.[71,74] NBPT is a pro-drug since it has to be oxidized to unmask the active form: *N*-(but-1-yl)phosphoric triamide (**NBPTO**, Fig. 23).[71] In an *in silico* investigation of the active site of urease from *H. pylori*, a molecular modelling study of phenylphosphorodiamidate (**PPD**, Fig. 23) demonstrated that this compound interacts with His221 through a hydrogen bond involving its oxygen atoms. A hydrophobic interaction between the benzene group with residues of Ala168, Ala169 and Ala365[75] is also involved. A deeper understanding of such non-covalent interactions may well lead to the design of superior inhibitors.
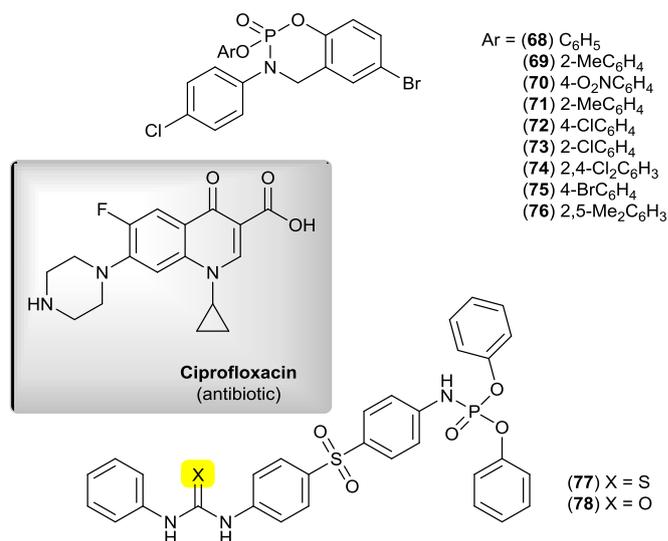
Fig. 22 Structural formulas of Cyprofloxacin® and some synthetic phosphoramidates with antibacterial activity.
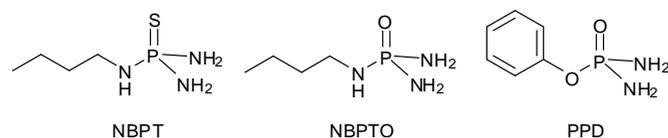
Fig. 23 Chemical structures of phosphoramidates efficient as urease inhibitors.

During 2008, Dominguez *et al.*[76] described the synthesis of various phosphoramidates all highly active in the nanomolar range against *Jack bean* urease *in vitro* (Fig. 24). The reference compounds NBPT and PPD used, showed IC$_{50}$ equal to 100 and 3 nM, respectively. Although no clear structure-activity relationship was found, this series of compounds represents a useful template for further investigation as these are currently the most potent urease inhibitors reported so far.
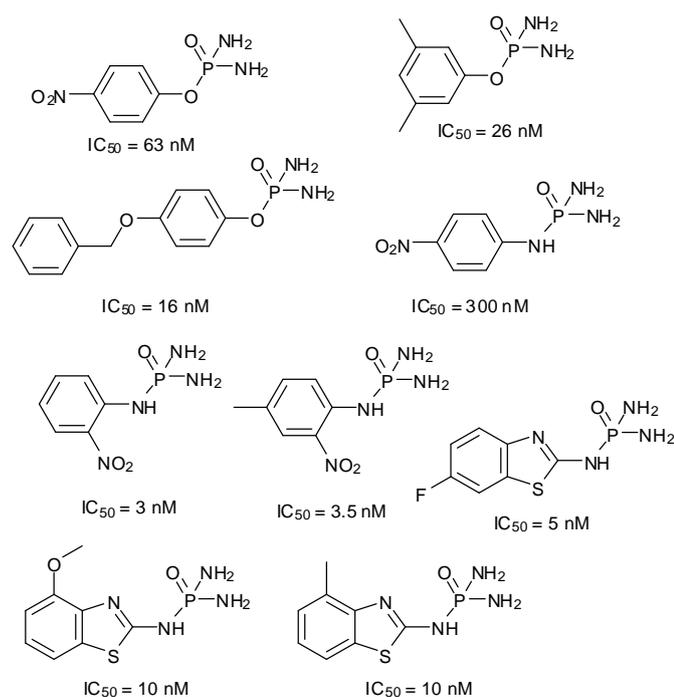
Fig. 24 Aromatic phosphoramidates with high urease inhibitory activity.77.

Miscellaneous

As this survey has shown, the phosphoramidates encompass a diverse range of biological activities. These have, therefore, led several investigators to explore the potential application of this class of compounds against tropical diseases especially malaria and leishmaniasis. Recent investigations include synthesis of nitrobenzyl phosphoramide mustards (compounds **79-90**, Fig. 25) that proved active against *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*. These protozoan parasites cause major health problems in many countries of the developing world spread across three continents.[77-79] The highest biological activity was associated with compounds **84** and **85**, which showed IC$_{50}$ values in the namomolar range against *T. brucei*.[80] Compound **86** revealed selectivity towards *T. brucei*[81] and served as a prototype for the development of novel phosphonates with EC$_{50}$ lying between 1.7-4.2 µM (structures not shown).[82] Disappointingly, these compounds showed low bioavailability due to their inability to cross cell membranes, therefore, only reaching the site of drug action in sub-therapeutic concentrations *in vivo*. Thus, Ruda *et al.*[83] prepared a series of phosphoramidate derivatives **86** with better pharmacokinetic properties. Among these compounds **87-90** showed EC$_{50}$ values equal to 90, 40, 50 and 8 nM, respectively, against *T. brucei*.

Malaria is a parasitic disease caused by the protozoan of the *Plasmodium genus*, with the most virulent species been *Plasmodium falciparum*. This disease is responsible for about

800,000 deaths per year, many of them including children and pregnant women, and most of them in sub Saharan Africa.[84] Amongst a number of compounds described by Mara *et al.*,[18] **91-94** showed good efficacy against *P. falciparum* (IC$_{50}$ = 39-50 µM) (Fig. 26). The bio-isosteric replacement of isopropylamino group by other alkylamino substituents resulted in new more pharmacologically potent phosphoramidates **95-104** (Fig. 26). Amongst such compounds, **95**, **97**, **99** and **104** (IC$_{50}$ = 17-32 µM) proved most active.[18] Other examples of phosphoramidates (**106-109**) with antimalarial activity were published recently which employed diphosphonic acid **105** as the lead structure.[85] Compound **108** (IC$_{50}$ = 3.4 µM) was more potent than **106** (IC$_{50}$ = 6.6 µM), **107** (IC$_{50}$ = 9.7 µM) and **109** (IC$_{50}$ = 42 µM) cells on D6 (wild-type *P. falciparum* strain proved most sensitive to these drugs). In cell lines W2 (chloroquine-and pyrimethamine-resistant *P. falciparum* strain), compound **108** showed IC$_{50}$ value of 4.0 µM as **106**, **107** and **109** had values equal to 7.7, 7.1 and 48 µM, respectively. Compound **109** has little or no effect since it was cytostatic (CC$_{50}$ > 300 µM) on the cell lines A549 (human lung carcinoma cells), C32 (human melonoma cells) and C32TG (and thioguanine-resistant mutant of HGPRT-deficient cell line C32). The pro-drug **107** shows low levels of cytotoxicity against C32 line cell (CC$_{50}$ = 130 µM) and C32TG (CC$_{50}$ = 109 µM). According to the authors, these values are not considered cytotoxic, since these pro-drugs fail to enter mammalian cells. Compound **105** (Fig. 26) represents a promising prototype for the development of new phosphoramidates as pro-drugs which may well possess useful antimalarial activity.[85]

(**79**) R = H; IC$_{50}$ = 3.4 µM
(**80**) R = 2-OCH$_3$; IC$_{50}$ = 1.2 µM
(**81**) R = 2-CF$_3$; IC$_{50}$ = 0.15 µM
(**82**) R = 2-F; IC$_{50}$ = 0.27 µM
(**83**) R = 3-F; IC$_{50}$ = 3.2 µM
(**84**) R = 2,6-diF; IC$_{50}$ = 0.007 µM
(**85**) R = 2-Cl; IC$_{50}$ = 0.008 µM

(**87**) R^1 = H, R^2 = *i*-Pr, R^3 = *t*-Bu; EC$_{50}$ = 90 nM
(**88**) R^1 = 4-CH$_3$, R^2 = *i*-Pr, R^3 = CH$_2$CH$_3$; EC$_{50}$ = 40 nM
(**89**) R^1 = 2,3-CH$_3$, R^2 = *i*-Pr, R^3 = CH$_2$CH$_3$; EC$_{50}$ = 50 nM
(**90**) R^1 = 2,3-CH$_3$, R^2 = *i*-Pr, R^3 = *t*-Bu; EC$_{50}$ = 8 nM
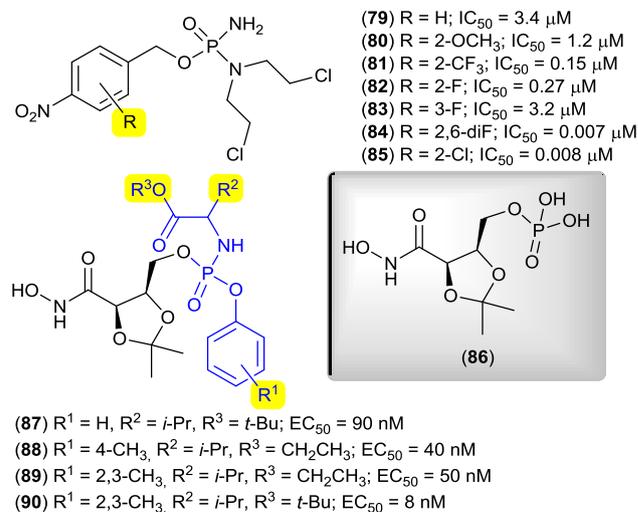
Fig. 25 Compounds with activity against *T. brucei*.
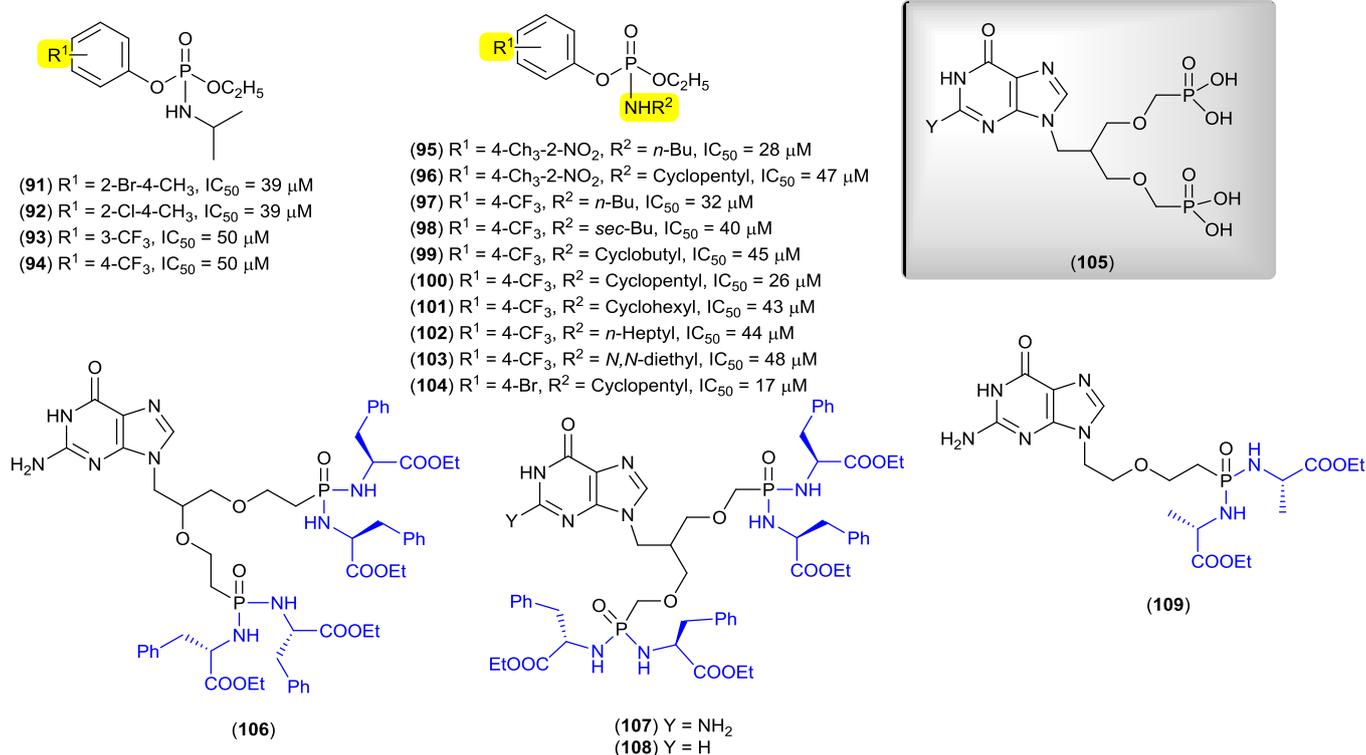
Fig. 26 Chemical structure of compounds with activity against *P. falciparum*.

(91) R^1 = 2-Br-4-CH$_3$, IC$_{50}$ = 39 μM
(92) R^1 = 2-Cl-4-CH$_3$, IC$_{50}$ = 39 μM
(93) R^1 = 3-CF$_3$, IC$_{50}$ = 50 μM
(94) R^1 = 4-CF$_3$, IC$_{50}$ = 50 μM

(95) R^1 = 4-Ch$_3$-2-NO$_2$, R^2 = *n*-Bu, IC$_{50}$ = 28 μM
(96) R^1 = 4-Ch$_3$-2-NO$_2$, R^2 = Cyclopentyl, IC$_{50}$ = 47 μM
(97) R^1 = 4-CF$_3$, R^2 = *n*-Bu, IC$_{50}$ = 32 μM
(98) R^1 = 4-CF$_3$, R^2 = *sec*-Bu, IC$_{50}$ = 40 μM
(99) R^1 = 4-CF$_3$, R^2 = Cyclobutyl, IC$_{50}$ = 45 μM
(100) R^1 = 4-CF$_3$, R^2 = Cyclopentyl, IC$_{50}$ = 26 μM
(101) R^1 = 4-CF$_3$, R^2 = Cyclohexyl, IC$_{50}$ = 43 μM
(102) R^1 = 4-CF$_3$, R^2 = *n*-Heptyl, IC$_{50}$ = 44 μM
(103) R^1 = 4-CF$_3$, R^2 = *N,N*-diethyl, IC$_{50}$ = 48 μM
(104) R^1 = 4-Br, R^2 = Cyclopentyl, IC$_{50}$ = 17 μM

(105)

(106)

(107) Y = NH$_2$
(108) Y = H

(109)

The major health problems caused by parasites are currently confined to the developing world but climate change may well shift these zones of infection to the more developed areas. Sadly this area of world health has attracted less attention from the pharma sector than desired. In contrast, within several regions of the world, Alzheimer's disease has attracted considerable attention from both pharmaceuticals companies and academic institutions since increasing longevity has led to the emergence of such conditions. Currently, cholinesterase inhibitors (ChEI) are the main drugs commercially available for the treatment of Alzheimer's disease, which is a leading cause of dementia worldwide, with approximately 24 million cases reported annually.[86,87] The rationale behind the use of these inhibitors is based on the assumption that a cholinergic deficit is associated with the disease, and aims at increasing the availability of synaptic acetylcholine by inhibiting its main catalytic enzymes, acetyl (AChE) and butyrylcholinesterase (BChE).[86,88] In 2010, Gholivand *et al*.[89] synthesized and evaluated the effect of compounds **110-114** (Fig. 27) on the aforementioned enzymes. As a result, these compounds revealed a mixed type mechanism of action with moderate activity against BChE (IC$_{50}$ ranging from 0.43 to 2.45 mM) and AChE (IC$_{50}$ ranging from 1.95 to 18.62 mM).

(110) R = C$_6$H$_5$-; X=O
(111) R = C$_6$H$_5$O-; X=O
(112) R = C$_6$H$_5$C(O)NH-; X=O
(113) R = C$_6$H$_5$C(O)NH-; X=CH$_2$

(114) R = C$_6$H$_5$O-; X=CH$_2$
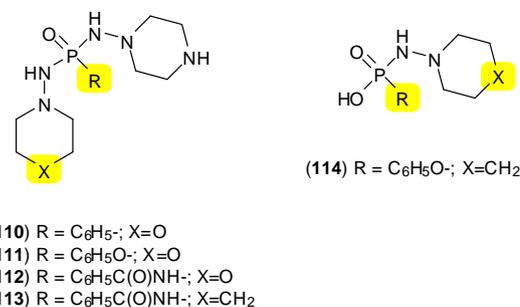
Fig. 27 Chemical structure of compounds with activity against AChE and BChE.

Rational drug design using Phosporamidates

The previous sections demonstrate that the incorporation of a phosphoramidate at a critical point within the agonist or antagonist is essential for success. During a drug design campaign, bioisosteric substitution should be carried out at a known xenobiotic transformation point inferred from *studies in vitro* using the relevant receptor, usually, but not exclusively an enzyme, which can now be achieved using recombinant methods. For instance, since folate hydrolase I and glutamate carboxypeptidase II, PSMA possess proteolytic activities toward γ-glutamyl folic acid derivatives and the neuropeptide N-acetylaspartylglutamate.[90] Studies involving PSMA substrate specificity revealed that acidic residues at the P1 and P19 positions are suitable targets for modification, and several

folate-like and *N*-acetylaspartylglutamate-like dipeptides with modest hydrolytic efficiency were reported.[91] Later, by adapting the dipeptide motif for PSMA targeting, Lapi *et al.* developed a library of tetrahedral phosphoramidates for PSMA inhibition (Fig. 28).[92]
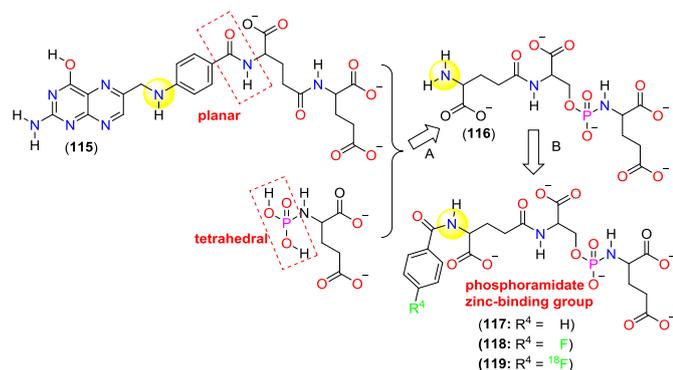
Fig. 28 Rational approaches towards the design of inhibitors active *in vivo*.

Through adopting a *seco*-analogue approach (molecular pruning), Wu *et al.* systematically identified several potent inhibitors[93] (Fig. 29 & 30) and proposed a pharmacophore model involving the zinc binding site which pseudo-irreversibly bind to PSMA.[94]
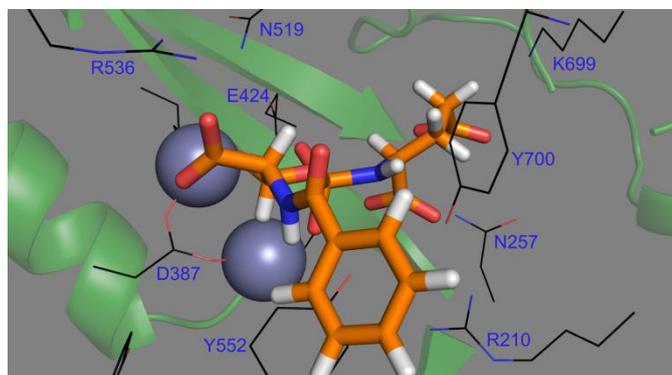
Fig. 29 Computational docking of phosphoramidate inhibitors into the active site of PSMA binding with Zinc represented by two spheres: (See panel a, inhibitor 1a: (((S)-2-benzamido-2-carboxylatoethoxy)oxidophosphoryl)-L-glutamatej; yellow; re-rendered using PyMol from Ref 93; see original paper for details of interactions with relevant amino acids).

Fig. 30 Computational docking of phosphoramidate inhibitors into the active site of PSMA binding with Zinc represented by two spheres:: (See panel c, inhibitor 1j, phosphonato-D-glutamate, yellow; re-rendered using PyMol from Ref 93; see original paper for details of interactions with relevant amino acids).

An ^{18}F labelled analogue of phosphoramidate (**117**, R^4 = H) showed their suitability in PSMA-targeted delivery for prostate cancer using positron emission tomography[95] allowing them to track its biodistribution data in murine xenografts *in vivo*[9]. The uptake of ^{18}F-fluorobenzamido-phosphoramidate *in vivo* **119** in both PSMA+ and PSMA− tumor models by PET and *ex vivo* biodistribution study revealed that phosphoramidate probe **119** rapidly localized at the LNCaP PSMA+ tumor, whereas there was significantly less activity within the PC-3 PSMA− tumor model (Fig 31).
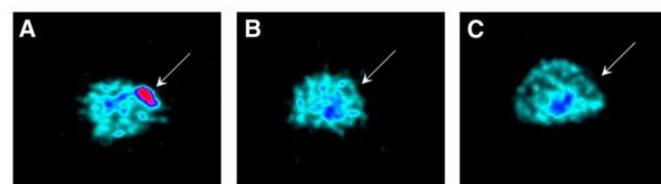
Fig. 31 PET transaxial images of male nude mice bearing subcutaneous LNCaP (A and C) and PC-3 (B) tumor xenografts at 2 h after injection of ^{18}F-fluorobenzamido-phosphoramidate **(3)**. Arrows indicate tumor placement. Blocked LNCaP is shown in C. Reproduced with permission from Ref 96).

As an imaging agent, the ^{18}F-labeled phosphoramidate, had high uptake in the PSMA-expressing tumor and low background and some of the accumulation seen in the kidney may be specific to the mouse. Such findings clearly demonstrate that the leading phosphoramidate is a PSMA-specific targeting ligand *in vivo* and uptake was specific to PSMA. Consequently, these findings will allow further development of this class of compounds and also provide a sensitive and specific imaging agent for prostate cancer.

Knowledge of drug action through crystallography is the ideal guide for rational synthesis. For instance, the mode of binding to thermolysin of the unsubstituted phosphoramidate inhibitor *N*-phosphoryl-L-leucinamide was studied by single crystal x-ray diffraction methods (Fig. 32) and these were considered transition state analogues.[97]
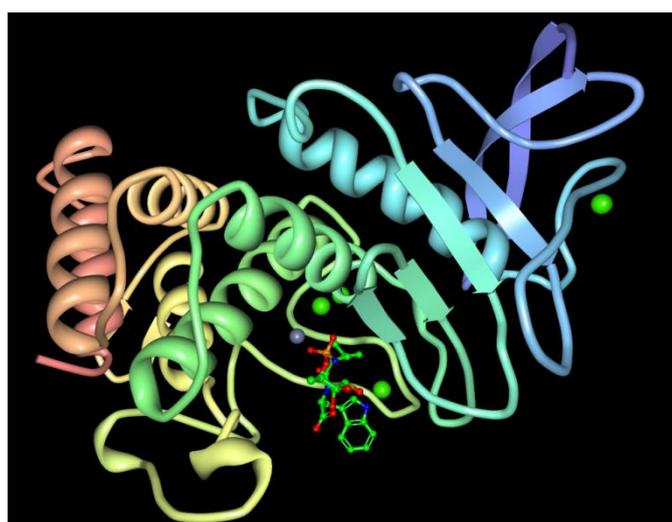
Fig. 32 binding to thermolysin of the unsubstituted phosphoramidate inhibitor *N*-phosphoryl-L-leucinamide.

In general, the bond that is known to be cleaved is usually modified and, in our experience, often tends to be the weakest bond possessing dimensions that are normally longer than those averages culled from crystal structure databases [98] (F. M. D. Ismail & M. G. B. Drew Unpublished study).

Conclusion

In this review, we briefly reported promising pharmacological activities displayed by the phosphoramidate class of compounds. Although various applications have been discussed in the literature, the biological activity of this class deserves further investigation since they reveal considerable potential for improving drug action especially with respect to antitumor and antiviral activities. Rational drug design will be improved as the structure of the target receptors and underlying biochemistry and genetics becomes available. By a careful consideration of ADMET approaches, more robust series of compounds can be developed especially using 3D-QSARs.

Acknowledgements

Abbreviations Used

7Bn-GMP, 7-benzyl guanosine monophosphate; A549 cell, human lung carcinoma cells; AChE, Acetylcholinesterase; ACV, Acyclovir; AddFU, 3´-azido-2´,3´-dideoxy-5-fluorouridine; ADMET, absorption, distribution, metabolism, and excretion - toxicity in pharmacokinetics; Ala, alanine; APM, amiprophos methyl; ATP, adenosine triphosphate; AZU, 4'-azidouridine; BChE, Butyrylcholinesterase; BEL-7402 cell, liver tumor cancer cell line; BVdU, brivudine; C32 cell, human melonoma cells; C32TG, thioguanine-resistant mutant of HGPRT-deficient cell line C32; CEM cell, Human T cell lymphoblast-like cell line; ChEI, cholinesterase inhibitors; d4T, stavudine; DNA, deoxyribonucleic acid; DOA, dioxolane sugar nucleosides; EBV, *Epstein-Barr virus*; eIF4E, eukaryotic initiation factor 4E; MIC, minimum inhibitory concentration; FUDR, 5-fluorouracil 2´-deoxynucleoside; Gly, glycine; H460, H383 and H2009 cells, human lung cancer cell lines; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HCV, hepatitis C virus; HeLA cell, human cervical cancer cell lines; HHV, human herpes virus; HIV, human immunodeficiency virus; HPD, haematoporphine dihydrochloride; HPV, *Human papilloma virus*; HSV, herpes simplex virus; HSV-1 TK-, HSV-deficient thymidine kinase; KB cell, human oral cancer cell lines; MCF-7 cell, human breast cancer cell lines; MDA-231 cell, human membrane of breast cancer cell lines; MDAMB231 cell, human breast cancer cell lines; MIC, minimum inhibitory concentration; MT-4 cell, human T cell line MT4; NBPT, *N*-(n-butyl) thiophosphoric triamide; NBPTO, *N*-(n-butyl)phosphoric triamide; NCI-H460 cell, human large cell lung cancer cell lines; NS5B, non-structural protein 5B; NTP, nucleoside triphosphate; PC-3 cell, human prostate cancer cell lines; PEG-IFN, pegalated α-interferon; Phe, phenylalanine; PK/PD, pharmacokinetic/ pharmacodynamics; PSMA, prostate-specific membrane antigen, PPD, phenylphosphorodiamide; RNA, ribonucleic acid; SF-268 cell, human central nervous system glioblastoma cell lines; VZV, *Varicella-zoster virus*.

References

1   V. M. R. d. Santos, C. L. Donnici, J. B. N. DaCosta and J. M. R. Caixeiro, *Quim. Nova*, 2007, **30**, 159-170.

2   F. Westheimer, Science, 1987, **235**, 1173-1178.

3   T. Katagi, *J. Agric. Food. Chem*., 1993, **41**, 496-501.

4   F. M. Oliveira, L. C. A. Barbosa, R. R. Teixeira, A. J. Demuner, C. R. A. Maltha, M. C. Picanço, G. A. Silva and V. F. Paula, *J. Pestic. Sci.*, 2012, **37**, 85-88.

5   V. F. Paula, L. C. A. Barbosa, R. R. Teixeira, M. C. Picanco and G. A. Silva, *Pest Manag. Sci*., 2008, **64**, 863-872.

6   Y. B. Kiran, C. D. Reddy, D. Gunasekar, C. N. Raju, L. C. A. Barbosa, D. C. O. Marney and L. J. Russel, *J. Fire Sci*., 2007, **25**, 193-215.

7   T.-M. Nguyen, S. Chang, B. Condon, R. Slopek, E. Graves and M. Yoshioka-Tarver, *Ind. Eng. Chem. Res*., 2013, **52**, 4715-4724.

8   J. Yan, J. Bu, X. Bai, J. Li, T. Ren and Y. Zhao, *J. Eng. Tribol*., 2012, **226**, 377–388.

9   A. Cho, L. Zhang, J. Xu, D. Babusis, T. Butler, R. Lee, O. L. Saunders, T. Wang, J. Parrish, J. Perry, J. Y. Feng, A. S. Ray and C. U. Kim, *Bioorg. Med. Chem. Lett*., 2012, **22**, 4127-4132.

10 A. Cho, L. Zhang, J. Xu, R. Lee, T. Butler, S. Metobo, V. Aktoudianakis, W. Lew, H. Ye, M. Clarke, E. Doerffler, D. Byun, T. Wang, D. Babusis, A. C. Carey, P. German, D. Sauer, W. Zhong, S. Rossi, M. Fenaux, J. G. McHutchison, J. Perry, J. Feng, A. S. Ray and C. U. Kim, *J. Med. Chem*., 2013, In press.

11 C. Congiatu, C. McGuigan, W. G. Jiang, G. Davies and M. D. Mason, *Nucleos. Nucleot. Nucl*., 2006, **24**, 485-489.

12 M. Derudas, A. Brancale, L. Naesens, J. Neyts, J. Balzarini and C. McGuigan, *Bioorg*. *Med*. *Chem*., 2010, **18**, 2748-2755.

13 M. Donghi, B. Attenni, C. Gardelli, A. D. Marco, F. Fiore, C. Giuliano, R. Laufer, J. F. Leone, V. Pucci, M. Rowley and F. Narjes, *Bioorg. Med. Chem. Lett*., 2009, **19**, 1392-1395.

14 C. Gardelli, B. Attenni, M. Donghi, M. Meppen, B. Pacini, S. Harper, A. Di Marco, F. Fiore, C. Giuliano, V. Pucci, R. Laufer, N. Gennari, I. Marcucci, J. F. Leone, D. B. Olsen, M. MacCoss, M. Rowley and F. Narjes, *J. Med. Chem*., 2009, **52**, 5394-5407.

15 Y. B. Kiran, C. Devendranath Reddy, D. Gunasekar, C. Suresh Reddy, A. Leon and L. C. A. Barbosa, *Eur. J. Med. Chem*., 2008, **43**, 885-892.

16 J. Liu, J. Du, P. Wang, D. Nagarathnam, C. L. Espiritu, H. Bao, E. Murakami, P. A. Furman and M. J. Sofia, *Nucleos. Nucleot. Nucl*., 2012, **31**, 277-285.

17 W. Liu, L. Zhang, H. Zhou, C. Yang, Z. Miao and Y. Zhao, *Nucleos. Nucleot. Nucl.*, 2013, **32**, 161-173.

18 C. Mara, E. Dempsey, A. Bell and J. W. Barlow, *Bioorg. Med. Chem. Lett*., 2011, **21**, 6180-6183.

19 C. McGuigan, M. R. Kelleher, P. Perrone, S. Mulready, G. Luoni, F. Daverio, S. Rajyaguru, S. L. Pogam, I. Najera, J. A. Martin, K. Klumpp and D. B. Smith, *Bioorg. Med. Chem. Lett*., 2009, **19**, 4250-4254.

20 C. McGuigan, K. Madela, M. Aljarah, A. Gilles, S. K. Battina, C. V. Ramamurty, C. Srinivas Rao, J. Vernachio, J. Hutchins, A. Hall, A. Kolykhalov, G. Henson and S. Chamberlain, *Bioorg. Med. Chem. Lett*., 2011, **21**, 6007-6012.

21 C. McGuigan, K. Madela, M. Aljarah, A. Gilles, A. Brancale, N. Zonta, S. Chamberlain, J. Vernachio, J. Hutchins, A. Hall, B. Ames, E. Gorovits, B. Ganguly, A. Kolykhalov, J. Wang, J. Muhammad, J. M. Patti and G. Henson, *Bioorg. Med. Chem. Lett*., 2010, **20**, 4850-4854.

22 C. McGuigan, P. Murziani, M. Slusarczyk, B. Gonczy, J. Vande Voorde, S. Liekens and J. Balzarini, *J. Med. Chem*., 2011, **54**, 7247-7258.

23 C. McGuigan, R. N. Pathirana, N. Mahmood and A. J. Hay, *Bioorg. Med. Chem. Lett.*, 1992, **2**, 701-704.

24 C. McGuigan, J. C. Thiery, F. Daverio, W. G. Jiang, G. Davies and M. Mason, *Bioorg. Med. Chem.*, 2005, **13**, 3219-3227.

25 S. Meneghesso, E. Vanderlinden, A. Brancale, J. Balzarini, L. Naesens and C. McGuigan, *ChemMedChem*, 2013, **8**, 415-425.

26 P. Perrone, G. M. Luoni, M. R. Kelleher, F. Daverio, A. Angell, S. Mulready, C. Congiatu, S. Rajyaguru, J. A. Martin, V. Leveque, S. Le Pogam, I. Najera, K. Klumpp, D. B. Smith and C. McGuigan, *J. Med. Chem*., 2007, **50**, 1840-1849.

27 G. S. Prasad, B. H. Babu, K. R. K. K. Reddy, P. R. Haranath and C. S. Reddy, *Arkivoc*., 2006, **2006**, 165-170.

28 M. J. Sofia, W. Chang, P. A. Furman, R. T. Mosley and B. S. Ross, *J. Med. Chem.*, 2012, **55**, 2481-2531.

29 F. Valiyev, F. Y. Tsai, A. A. Saboury, H. J. Liu, A. A. Moosavi-Movahedi and G. H. Hakimelahi, *J. Iran. Chem. Soc.*, 2008, **5**, 228-237.

30 Z. W. Wang, C. C. Guo, W. Z. Xie, C. Z. Liu, C. G. Xiao and Z. Tan, *Eur. J. Med. Chem*., 2010, **45**, 890-895.

31 N. F. Zakirova, A. V. Shipitsyn, M. V. Jasko, M. M. Prokofjeva, V. L. Andronova, G. A. Galegov, V. S. Prassolov and S. N. Kochetkov, *Bioorg. Med. Chem*., 2012, **20**, 5802-5809.

32 E. De Clercq, *Nat. Rev. Drug Discov*., 2002, **1**, 13-25.

33 E. Ichikawa and K. Kato, *Curr. Med. Chem*., 2001, **8**, 385-423.

34 M. Derudas, D. Carta, A. Brancale, C. Vanpouille, A. Lisco, L. Margolis, J. Balzarini and C. McGuigan, *J. Med. Chem*., 2009, **52**, 5520-5530.

35 C. Arbelo Román, P. Wasserthal, J. Balzarini and C. Meier, *Eur. J. Org. Chem*., 2011, **2011**, 4899-4909.

36 Q. B. Cui, G. X. Zhang, P. Yu and Y. Q. Wang, *Yao Xue Xue Bao*, 2011, **46**, 1015-1018.

37 L. Borrello, U. Chiacchio, A. Corsaro, V. Pistarà and D. Iannazzo, *Arkivoc*., 2009, **2009**, 112-124.

38 S. A. Harris, C. McGuigan, G. Andrei, R. Snoeck, E. De Clercq and J. Balzarini, *Antivir. Chem. Chemother*., 2001, 12, 293-300.

39 J. Hilfinger, *Mol. Pharm*., 2013, **10**, 429-429.

40 D. A. Herbst, Jr. and K. R. Reddy, *Expert Opin. Investig. Drug*., 2013, **22**, 527-536.

41 E. De Clercq, *Int. J. Antimicrob*. *Agents*, 2009, **33**, 307-320.

42 P. Wang, S. Rachakonda, V. Zennou, M. Keilman, C. Niu, D. Bao, B. S. Ross, P. A. Furman, M. J. Otto and M. J. Sofia, *Antivir. Chem. Chemother*., 2012, **22**, 217-238.

43 Y. Liang, J. Narayanasamy, R. F. Schinazi and C. K. Chu, *Bioorg. Med. Chem.*, 2006, **14**, 2178-2189.

44 L. Bondada, M. Detorio, L. Bassit, S. Tao, C. M. Montero, T. M. Singletary, H. Zhang, L. Zhou, J.-H. Cho, S. J. Coats and R. F. Schinazi, *ACS Med. Chem. Lett.*, 2013, **4**, 747-751.

45 Y. Mehellou, J. Balzarini and C. McGuigan, *Org. Biomol. Chem*., 2009, **7**, 2548-2553.

46 I. Zlatev, A. Giraut, F. Morvan, P. Herdewijn and J.-J. Vasseur, *Biorg. Med. Chem*., 2009, **17**, 7008-7014.

47 F. M. Uckun, T. K. Venkatachalam, D. Erbeck, C. L. Chen, A. S. Petkevich and A. Vassilev, *Bioorg. Med. Chem*., 2005, **13**, 3279-3288.

48 J. Li, F. Chen, M. M. Cona, Y. Feng, U. Himmelreich, R. Oyen, A. Verbruggen and Y. Ni, *Target. Oncol*., 2012, **7**, 69-85.

49 D. S. Shewach and R. D. Kuchta, *Chem. Rev.*, 2009, **109**, 2859-2861.

50 C. R. Wagner, V. V. Iyer and E. J. McIntee, *Med. Res. Rev*., 2000, **20**, 417-451.

51 S. J. Keam, T. M. Chapman and D. P. Figgitt, *Drugs*, 2004, **64**, 2091-2097; discussion 2098-2099.

52 X. Rabasseda, *Drugs Today*, 2003, **39**, 359-371.

53 S. T. Neuteboom, P. L. Karjian, C. R. Boyer, M. Beryt, M. Pegram, G. M. Wahl and H. M. Shepard, *Mol. Cancer Ther*., 2002, **1**, 377-384.

54 R. H. Wilson, *Oncologist*, 2006, **11**, 1018-1024.

55 F. M. D. Ismail, *J. Fluorine Chem*., 2002, **118**, 27-33.

56 J. L. Grem, *Invest. New Drugs*, 2000, **18**, 299-313.

57 C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven and J. Scheiner, *Nature*, 1957, **179**, 663-666.

58 J. Homsi and C. R. Garrett, *Cancer Control*, 2006, **13**, 42-47.

59 M. Lewandowska, P. Ruszkowski, D. Baraniak, A. Czarnecka, N. Kleczewska and L. Celewicz, *Eur. J. Med. Chem*., 2013, **67**, 188-195.

60 S. R. Lessin, M. Duvic, J. Guitart and et al., *JAMA Dermatol.*, 2013, **149**, 25-32.

61 Q. Sun, R.-T. Li, W. Guo, J.-R. Cui, T.-M. Cheng and Z.-M. Ge, *Bioorg. Med. Chem. Lett*., 2006, **16**, 3727-3730.

62 J. X. Duan, H. Jiao, J. Kaizerman, T. Stanton, J. W. Evans, L. Lan, G. Lorente, M. Banica, D. Jung, J. Wang, H. Ma, X. Li, Z. Yang, R. M. Hoffman, W. S. Ammons, C. P. Hart and M. Matteucci, *J. Med. Chem.*, 2008, **51**, 2412-2420.

63 R. L. Lipson, E. J. Baldes and A. M. Olsen, *Dis. Chest*, 1964, **46**, 676-679.

64 B. Ghosh, A. O. Benyumov, P. Ghosh, Y. Jia, S. Avdulov, P. S. Dahlberg, M. Peterson, K. Smith, V. A. Polunovsky, P. B. Bitterman and C. R. Wagner, *ACS Chem. Biol*., 2009, **4**, 367-377.

65 S. Li, Y. Jia, B. Jacobson, J. McCauley, R. Kratzke, P. B. Bitterman and C. R. Wagner, *Mol. Pharm*., 2013, **10**, 523-531.

66 K. Bush, P. Courvalin, G. Dantas, J. Davies, B. Eisenstein, P. Huovinen, G. A. Jacoby, R. Kishony, B. N. Kreiswirth, E. Kutter, S. A. Lerner, S. Levy, K. Lewis, O. Lomovskaya, J. H. Miller, S. Mobashery, L. J. V. Piddock, S. Projan, C. M. Thomas, A. Tomasz, P. M. Tulkens, T. R. Walsh, J. D. Watson, J. Witkowski, W. Witte, G. Wright, P. Yeh and H. I. Zgurskaya, *Nat. Rev. Microbiol*., 2011, **9**, 894-896.

67 O. Cars, A. Hedin and A. Heddini, *Drug Resist. Update*., 2011, **14**, 68-69.

68 L. J. V. Piddock, *Lancet Infect. Dis*., 2012, **12**, 249-253.

69 K. Gholivand and N. Dorosti, *Monatsh. Chem*., 2011, **142**, 183-192.

70 G. Madhavaa, K. V. Subbaiahb, R. Sreenivasulu and C. N. Rajua, *Der Pharmacia Lettre*, 2012, **4**, 1194-1201.

71 Z. Amtul, A. U. Rahman, R. A. Siddiqui and M. I. Choudhary, *Curr. Med. Chem*., 2002, **9**, 1323-1348.

72 M. J. Duckworth, A. S. Okoli and G. L. Mendz, *Expert Rev. Anti Infect. Ther*., 2009, **7**, 835-867.

73 C. Follmer, *J. Clin. Pathol*., 2010, **63**, 424-430.

74 B. Krajewska, *J. Mol. Catal. B: Enzym*., 2009, 59, 9-21.

75 W.-J. Mao, P.-C. Lv, L. Shi, H.-Q. Li and H.-L. Zhu, *Biorg. Med. Chem.*, 2009, **17**, 7531-7536.

76 M. J. Domínguez, C. Sanmartín, M. Font, J. A. Palop, S. San Francisco, O. Urrutia, F. Houdusse and J. M. García-Mina, *J. Agric. Food. Chem*., 2008, **56**, 3721-3731.

77 D. Bahia, L. M. Oliveira, F. M. Lima, P. Oliveira, J. F. Silveira, R. A. Mortara and J. C. Ruiz, *Biochem. Biophys. Res. Commun*., 2009, **390**, 963-970.

78 D. C. Turnock and M. A. J. Ferguson, *Eukaryot Cell*, 2007, **6**, 1450-1463.

79 M. Parsons, E. A. Worthey, P. N. Ward and J. C. Mottram, *BMC Genomics*, 2005, **6**, 127.

80 L. Hu, X. Wu, J. Han, L. Chen, S. O. Vass, P. Browne, B. S. Hall, C. Bot, V. Gobalakrishnapillai, P. F. Searle, R. J. Knox and S. R. Wilkinson, *Bioorg. Med. Chem. Lett*., 2011, **21**, 3986-3991.

81 C. Dardonville, E. Rinaldi, M. P. Barrett, R. Brun, I. H. Gilbert and S. Hanau, *J. Med. Chem*., 2004, **47**, 3427-3437.

82 S. J. Hecker and M. D. Erion, *J. Med. Chem*., 2008, **51**, 2328-2345.

83 G. F. Ruda, P. E. Wong, V. P. Alibu, S. Norval, K. D. Read, M. P. Barrett and I. H. Gilbert, *J. Med. Chem*., 2010, **53**, 6071-6078.

84 WHO, 2010.

85 D. T. Keough, P. Spacek, D. Hockova, T. Tichy, S. Vrbkova, L. Slavetinska, Z. Janeba, L. Naesens, M. D. Edstein, M. Chavchich, T. H. Wang, J. de Jersey and L. W. Guddat, *J. Med. Chem*., 2013, **56**, 2513-2526.

86 C. Ballard, S. Gauthier, A. Corbett, C. Brayne, D. Aarsland and E. Jones, *Lancet*, 2011, **377**, 1019-1031.

87 C. Reitz, C. Brayne and R. Mayeux, *Nat. Rev. Neurol*., 2011, **7**, 137-152.

88 R. Brady and J. Weinman, *Dement. Geriatr. Cogn. Disord*., 2013, **35**, 351-363.

89 K. Gholivand, Z. Hosseini, S. Farshadian and H. Naderi-Manesh, *Eur. J. Med. Chem*., 2010, **45**, 5130-5139.

90 J. T. Pinto, B. P. Suffoletto, T. M. Berzin, C. H. Qiao, S. Lin, W. P. Tong, F. May, B. Mukherjee, W. D. Heston, *Clin Cancer Res.* 1996; **2**, 1445–1451.

91 C. Barinka, M. Rinnova, P. Sacha, C. Rojas, P. Majer, B. S. Slusher, J. Konvalinka, *J. Neurochem.*, 2002, **80**, 477-487.

92 J. P. Mallari, C. J. Choy, Y. Hu, A. R. Martinez, M. Hosaka, Y. Toriyabe, J. Maung, J. E. Blecha, S. F. Pavkovic, C. E. Berkman, *Bioorg. Med.Chem.,* 2004, **12**, 6011–6020.

93 L. Y. Wu, M. O. Anderson, Y. Toriyabe, J. Maung, T. Y. Campbell, C. Tajon, M. Kazak, J. Moser, C. E. Berkman, *Bioorg. Med .Chem*. 2007, **15**, 7434-7443.

94 T. Liu, Y. Toriyabe, M. Kazak, C. E. Berkman, *Biochemistry*, 2008, **47**, 12658–12660.

95 F.M.D. Ismail, M.G.B. Drew, M.J. Dascombe. *Chimica Oggi: Chemistry Today*, 2009, **27**, 18-22..

96 S. E. Lapi, H. Wahnishe, D. Pham, L. Y. Wu, J. R. Nedrow-Byers, T. Liu, K. Vejdani, H. F. VanBrocklin, C. E. Berkman, E. F. Jones, *J. Nucl. Med.*, 2009, **50**, 2042-2048.

97 D. E. Tronrud, A. R. F. Monzingo, B. W. Matthews. *Eur. J. Biochem*. 1986, **157**, 261-268.