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Research paper

Design, synthesis and biological evaluation of 2-substituted-6-[(4-substituted-1-piperidyl)methyl]-1H-benzimidazoles as inhibitors of ebola virus infection

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ABSTRACT

Novel 2-substituted-6-[(4-substituted-1-piperidyl)methyl]-1H-benzimidazoles were designed and synthesized as Ebola virus inhibitors. The proposed structures of the new prepared benzimidazole-piperidine hybrids were confirmed based on their spectral data and CHN analyses. The target compounds were screened *in vitro* for their anti-Ebola activity. Among tested molecules, compounds **26a** ($EC_{50=}$0.93 μM, SI = 10) and **25a** ($EC_{50=}$0.64 μM, SI = 20) were as potent as and more selective than Toremifene reference drug (EC_{50} = 0.38 μM, SI = 7) against cell line. Data suggests that the mechanism by which **25a** and **26a** block EBOV infection is through the inhibition of viral entry at the level of NPC1. Furthermore, a docking study revealed that several of the NPC1 amino acids that participate in binding to GP are involved in the binding of the most active compounds **25a** and **26a**. Finally, *in silico* ADME prediction indicates that **26a** is an idealy drug-like candidate. Our results could enable the development of small molecule drug capable of inhibiting Ebola virus, especially at the viral entry step.

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1. Introduction

The family Filoviridae includes five genera: *Cuevavirus*, *Marburgvirus*, *Ebolavirus*, *Striavirus* and *Thamnovirus* [1,2]. Since 1967, when Marburg virus was first isolated, numerous filovirus hemorrhagic fever (HF) outbreaks have occurred, often with high fatality rates. The most virulent Ebolavirus species is Zaire ebolavirus (EBOV) with mortality rate of up to 90%, [3]. Filoviruses cause HF, associated with immune suppression and a systemic inflammatory response in human and non-human primates. Filoviruses are enveloped, non-segmented, negative-sense, single-stranded RNA viruses [4]. To begin the infection cycle, the virus must transport its genetic information through the membrane of a target cell. The envelope glycoprotein (GP) of filovirus mediates binding to cellular receptors and the subsequent fusion of the virus envelope with the host cell membrane [5–8]. Study of the host factors that promote or restrict Ebola replication is of great interest as it may thus lead to the development of novel therapeutics. It has been reported that filovirus GP interacts with multiple molecules for entry into host cells [4]; the entry mechanism is complex, involving not only cell-surface molecules but also intracellular proteins. Several cell-surface molecules are thought to participate in viral entry [4,6,9,10] including the cellular lectins DC-SIGN, DC-SIGNR, LSECtin, ASPGR-1 and hMGL [11–13]. The T-cell immunoglobulin and mucin domain-containing protein 1 (TIM-1) was also shown to be a factor with a role in virus entry [14]. Another known host cell factor is the Tyro3/Axl/Mer (TAM) family of tyrosine kinase receptors [15] . Other molecules involved in filovirus entry are α3 and β1 integrins [16] as well as cathepsins (L and B) [17]. If promising results with antibody therapies have been reported, and very recently one has been approved for the treatment of EBOV infection [18] one important application for an anti-EBOV small molecule could be the

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treatment of persistently-infected patients, as antibodies may be unable to access the immunologically-privileged sites that harbor the virus. Thus, some representative antifilovirus small molecules, belonging to several chemical classes [19,20], are shown in Fig. 1; they target virus genome replication such as nucleosides BCX4430 and GS-5734 (remdesivir) (two non-obligate RNA chain terminator), or virus entry such as CID23631927 (a cathepsin L inhibitor), the benzimidazole FGI-103 (mechanism to be established), toremifene and terconazole (two Niemann-Pick C1-dependent inhibitors). In 2011, Côté et al. identified the benzylpiperazine adamantane diamide-derived (3.47) as inhibitor of Ebola virus entry [21]. Compound 3.47, alongside a genetic screening study, was used to identify Niemann-Pick C1 protein (NPC1) as an essential receptor required for EBOV entry [21,22]. Compound 3.47 is reported to blocked binding of EBOV-GP to NPC1; however, this inhibitor has unfavourable properties for *in vivo* application. In 2013, Shoemaker et al. demonstrated the inhibition of EBOV entry by multiple cationic amphiphiles [23]. The mechanism of this inhibition was NPC1-dependent, but different from that described for the compound 3.47. More recently, Rong et al. reported some 4-(aminomethyl)benzamides (CBS1118) as potent entry inhibitors of Ebola virus [24]. Toremifene, and other selective estrogen receptor modulators, were first identified as inhibitors of EBOV infection in screens of approved drugs, with toremifene conferring a statistically significant survival benefit in the mouse model of infection [25–27]. Crystallographic and biochemical studies have subsequently shown that toremifene's mechanism of action is through binding in a large cavity in the EBOV glycoprotein and destabilizing its structure [28–30]. Thus, there are multiple steps and targets within the EBOV entry mechanism that might be subject to therapeutic intervention.

Several of the virus entry inhibitors are sharing common moieties, such as benzimidazole, piperazine and piperidine. Based on this finding, we report herein the synthesis of *hitherto* unknown 2-substituted-6-[(4-substituted-1-piperidyl)methyl]-1H-benzimidazoles (Fig. 1); those compounds are targeting NPC1 involved in Ebola virus entry. The main scaffold is formed by a benzimidazole moiety, which has been an important pharmacophore and privileged structure in medicinal chemistry [31], linked to a piperidine ether and its isostere 4-methylpiperidine. Modifications are done at C2 position on the benzymidazole moiety with substituted phenyl group having (or not) a trifluoromethyl- and trifluoromethyl ether due to its peculiar properties (steric and electronic effects and enhanced lipophilicity) [32] (blue dashed rectangle). We investigate also the influence of [(phenoxy-methyl)phenyl group bearing a enzymatic stable phosphonate diester (green dashed rectangle) bounded at C4 of the piperidine moiety by either X = O (4-aryloxy-, serial A) or X = CH_2 (4-benzyl-, serial B). All compounds were evaluated for their ability to inhibit EBOV entry.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of 2-substitued-6-[(4-aryloxy-1-piperidyl)methyl]-1H-benzimidazoles (***serial A***)

The chosen strategy to the desired 2-substituted-6-[(4-substituted-1-piperidyl)methyl]-1H-benzimidazoles was to follow

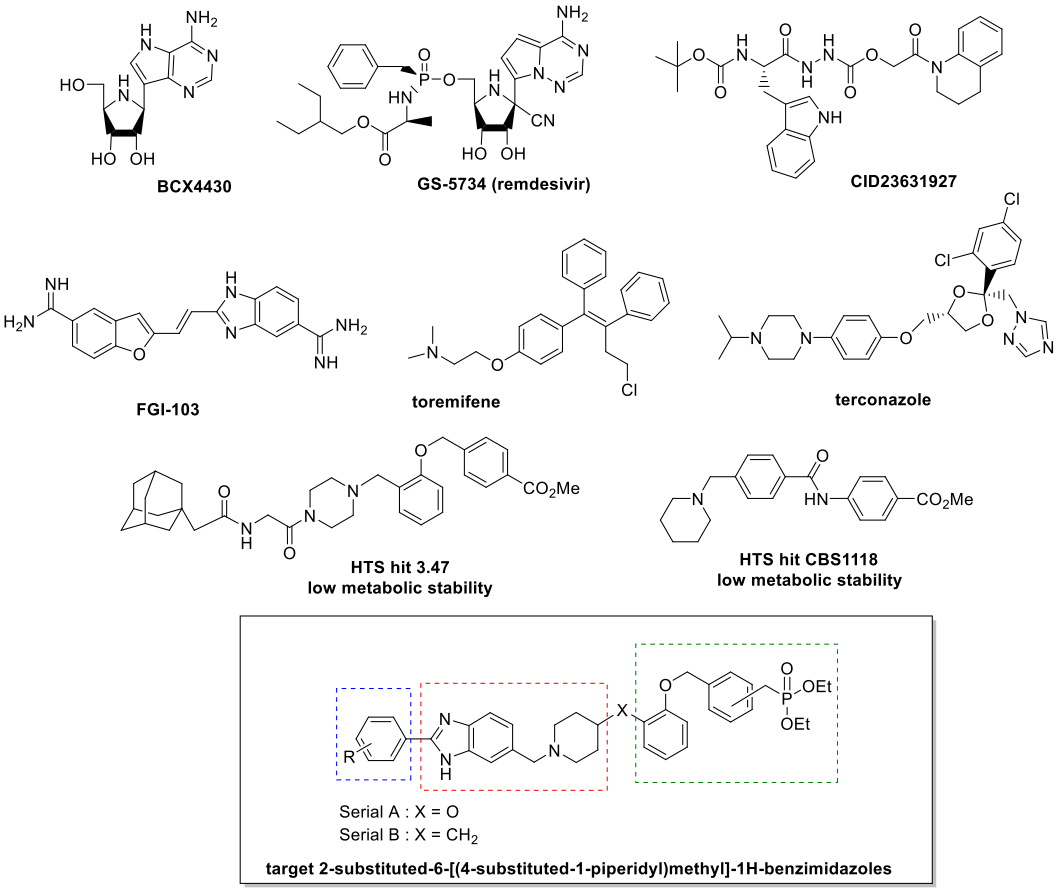


Fig. 1. Some anti-ebola molecules and general structure of our new inhibitors.

a convergent synthesis ending with the coupling of a (4-substituted)-1-piperidine fragment at C6 position of a 6-(chloromethyl)-1H-benzimidazole fragment, under SN conditions. Thus, the synthesis of the (4-substituted)-1-piperidine fragment started with either the α,α-dibromo-m-xylene (**1a**) or the α,α-dibromo-p-xylene (**1b**), which were converted to their phosphonate analogs **2a** (*para* position) and **2b** (*meta* position), respectively, respectively, by SN of bromine with diethylphosphite. Beside, catechol (**3**) was monoprotected to **4**. Then, the nucleophilic substitution with **2a** and **2b** in the presence of a **4** afforded the compounds **5a** and **5b**, respectively, after 20 min at 80 °C under microwave irradiation. Removal of the pyrane protecting group by a treatment with pyridinium *p*-toluenesulfonate (PPTS) during 3 h in ethanol afforded **6a** and **6b**, respectively, in excellent yields (Scheme 1).

The protected piperidine-4-one (**7**) was reduced to **8** and activated as tosyl leaving group analog **9**. After a brief optimization of the solvent, the activation and the substrate, it appears that the nucleophilic substitution of tosylpiperidine **9** with **6a** and **6b**, respectively, runs in optimal conditions with dimethylacetamide (DMA) under microwave irradiation (Scheme 2) and gave the protected piperidines **10a** and **10b**, respectively, in good yields. After deprotection of the carbamate group with trifluoroacetic acid, the (4-aryloxy)-1-piperidines **11a** *(para*-position*)* and **11b** *(meta*-position*)* were isolated, respectively.

The 6-(chloromethyl)-1H-benzimidazole fragment was synthesized from methyl 3,4-diaminobenzoate and various benzaldehydes **12a-e** via one-step process using $NaHSO_3$ 40% as oxidant, (Scheme 3). The desired compounds **13a-e** were isolated in a range of 76–97% yields. After reduction of **13a-e**, the (2-phenyl-3H-benzimidazol-5-yl)methanol derivatives **14a-e** were isolated in quantitative yields. The final coupling was done by (1) *in situ* chlorination of the benzylic alcohol of **14a-e**, in the presence of thionyl chloride and subsequent substitution with piperidine fragment **11a,b** under basic conditions. The final 2-substitued-6-[(4-aryloxy-1-piperidyl)methyl]-1H-benzimidazoles **15a-e** and **16a-e** were isolated in moderate yields (over two-steps).

2.1.2. Synthesis of 2-substituted-6-[(4-benzyl-1-piperidyl)methyl]-1H-benzimidazoles (***serial B***)

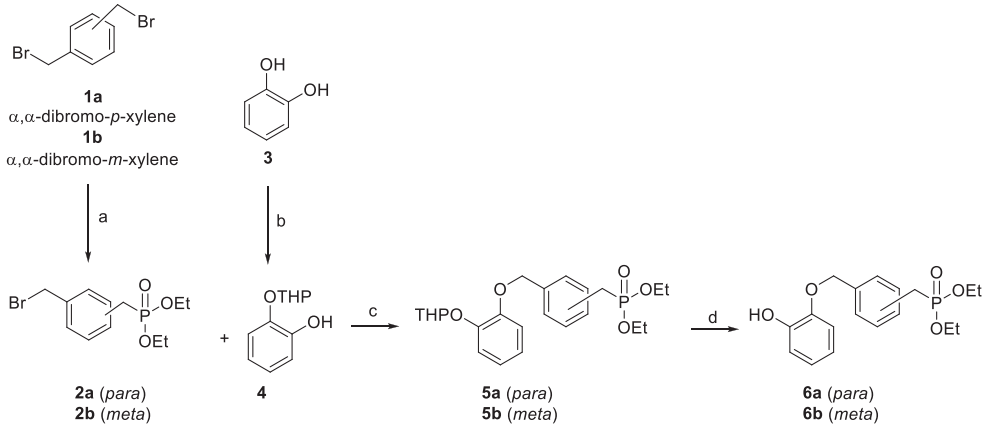
Facing some difficulties to build these 2-substituted-6-[(4-benzyl-1-piperidyl)methyl]-1H-benzimidazoles through the similar above described approach, we considered an alternative approach based first on the synthesis of the 2-(4-piperidylmethyl) phenol (**22**), followed then on its selective functionalization of either the piperidine moiety or the phenol moiety, (Scheme 4). Thus, the 2-bromophenol **17** was protected to its tetrahydropyranyl ether **18**. The transmetallation of **18** with *n*-BuLi followed by the nucleophilic addition on *N*-Boc-piperidine-4-carboxaldehyde afforded **19**. Unfortunately, the dehydroxylation/phenol deprotection of **19** with TFA/Et_3SiH gave the desired **22** in only 21% poor yield, probably due to some removal of BOC under acidic traces. In order to circumvent this problem, **17** was protected as benzyl ether **20**, which, following a similar pathway, afforded **21** in 65% yield. Final dehydroxylation/phenol deprotection of **21** by hydrogenation under pressure (10 bars) during 24 h gave **22** in quantitative yields.

22 was then reacted with 1-(bromomethyl)-4-(diethoxyphosphorylmethyl)benzene (**2a**) or its meta analog **2b** in DMA at 140 °C under microwave irradiation to afford **23a** and **23b**, respectively, (Scheme 5). Subsequent deprotection of the carbamate protecting group was realized in acidic conditions and led **24a** and **24b**, respectively. Finally, 2-substituted-6-[(4-benzyl-1-piperidyl)methyl]-1H-benzimidazoles **25a** and **26a-e** were obtained, respectively, as above described by (1) *in situ* chlorination of the benzylic alcohol of **14a-e**, in the presence of thionyl chloride and subsequent substitution with piperidine fragment **24a,b** under basic conditions.

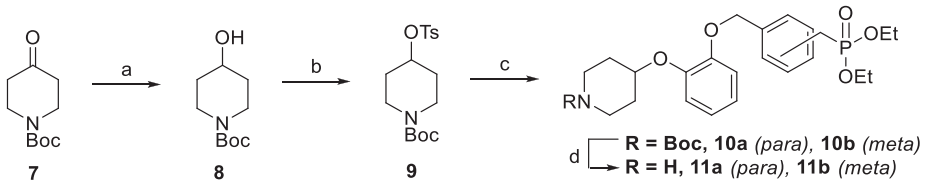
2.2. Antiviral evaluation

The anti-EBOV activity and cytotoxicity of our molecules were assessed using an infectious recombinant reporter EBOV and by cell viability assay, respectively (Table 1), toremifene being used as positive control [25].

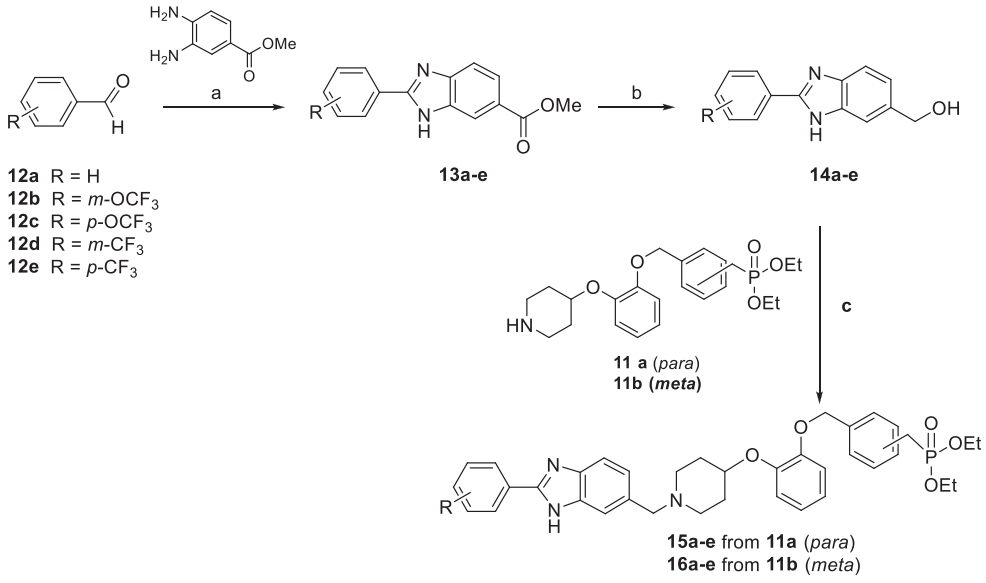
As a result, all compounds having our benzimidazole-piperidine scaffold were found to possess a good potency (EC_{50} μM range). The introduction of CF_3- and CF_3O- groups (for increased lopophilicity) didn't improve the SI. Analogs bearing a piperidine-ether moiety (*e.g.* 2-substitued-6-[(4-aryloxy-1-piperidyl)methyl]-1H-benzimidazoles - serial A) appear to be less active than the 2-substituted-6-[(4-benzyl-1-piperidyl)methyl]-1H-benzimidazoles (Serial B). Compounds **16a** ($EC_{50=}$1.88 μM, SI = 7) and **26b** ($EC_{50=}$0.87 μM, SI = 5) displayed good inhibitory activity, and a SI comparable or nearly equipotent to toremifene (EC_{50} = 0.38 μM; CC_{50} = 2.50 μM and SI = 7). Among those molecules, compound **26a** ($EC_{50=}$0.93 μM, SI = 10) and **25a** ($EC_{50=}$0.64 μM, SI = 20) are of great interest, meanwhile compound **25a** stood out as the most potent against EBOV. **25a** and **26a** are isomers of position and they both belong to serial B. To test if **25a** and **26a** inhibited EBOV entry, we used HIV pseudotype particles bearing the EBOV GP. Both **25a** and **26a** inhibited the EBOV GP-mediated entry of these particles into



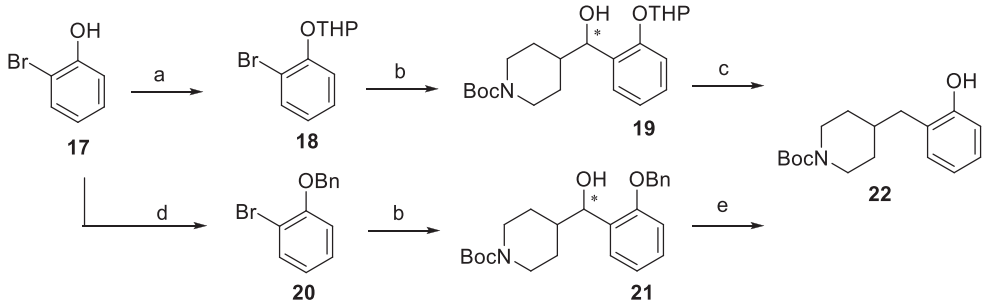
Scheme 1. Synthesis of compounds 6a and 6b. Reagents and conditions: (a) diethylphosphite, DMF, 150 °C, MW, 2 min, 90% (b) DHP, PPTS, DCM, r.t., 3 h, 88–94% (c) NaI, K_2CO_3, DMF, MW, 80 °C, 20 min, 72% (d) PPTS, EtOH, 55 °C, 3 h, 99%.



Scheme 2. Synthesis of (4-aryloxy)-1-piperidines **11a** and **11b**. Reagents and conditions: (a) NaH, MeOH, rt, 20 h, 95% (b) TsCl, Et_3N, DCM, r.t., 20 h, 98% (c) **6a** and **6b**, K_2CO_3, DMA, MW, 140 °C, 30 min, 72% (d) TFA in DCM 1:2, r.t., 1 h.



Scheme 3. Synthesis of 2-substitued-6-[(4-aryloxy-1-piperidyl)methyl]-1H-benzimidazoles **15a-e** and **16a-e**. Reagents and conditions: (a) $NaHSO_3$ 40%, EtOH, 100 °C, 1–4 h, 76–97% b) $LiAlH_4$, THF, r.t., 2–4 h, 94–99% (c) 1. $SOCl_2$, reflux, 2.5 h, 2.**11a** or **11b**, DIPEA, CH_3CN, r.t., 19 h, 21–40% (over two-steps).

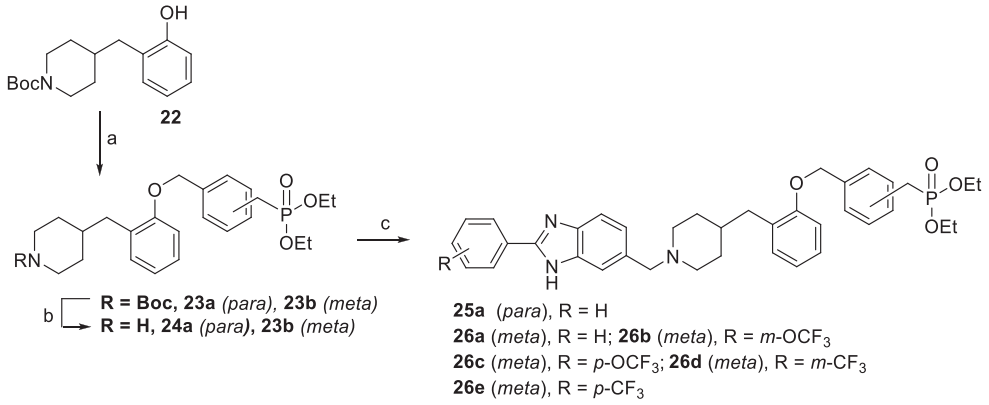


Scheme 4. Synthesis of compound **22**. Reagents and conditions: (a) PPTS, DHP, DCM, r.t., 12 h, quant. (b) 1. *n*-BuLi, THF, −78 °C, 30 min, 2.1-Boc-piperidine-4-carboxaldehyde, THF, r.t., 3 h, 60–65%. c) TFA, Et_3SiH, DCM, rt, 1 h, (21% from **19**) and (65% from **21**) (d) BnCl, K_2CO_3, DMF, 70 °C, 12 h, quant. (e) H_2, Pd/C, EtOAc, rt, 24 h, 10 bars, quant.

Huh7 cells, with EC_{50} and CC_{50} values similar to those seen with infectious EBOV (Fig. 2A). The EBOV entry receptor is NPC1, which functions in the cellular trafficking of cholesterol. We looked to see the effect of **25a** and **26a** on the subcellular localization of cholesterol in HeLa cells. Both of these compounds induced a dramatic accumulation of cholesterol to intracellular vacuoles, similar to that seen with U18666A, a known inhibitor of EBOV entry that acts through NPC1 (Fig. 2B). This effect was associated with minimal cytotoxicity in HeLa cells (Fig. 2C). Together, this data suggests that the mechanism by which **25a** and **26a** block EBOV infection is through the inhibition of viral entry at the level of NPC1.

2.3. Chemoinformatics

In order to explain these data, **25a** and **26a** were docked in the active site of induced fit optimized NPC1 crystallized structure (Fig. 3). The recently crystallized structure of NPC1 (PDB id: 5U73) [33] was used for the initial docking model generation in Schrödinger Suite (Protein Preparation Wizard Tool [34] and Glide [35] at default settings, except the size of the grid box set as 30x30 × 30 Å). Because the model showed highly unsatisfactory efficiency in retrospective virtual screening experiments, cluster-specific models were next obtained in an induced-fit docking approach. All 1637 compounds active against NPC1 fetched from the ChEMBL



Scheme 5. Synthesis of 2-substituted-6-[(4-benzyl-1-piperidyl)methyl]-1H-benzimidazoles **25a** and **26a-e**. Reagents and conditions: (a) **2a** or **2b**, K_2CO_3, DMA, MW, 140 °C, 30 min, (b) TFA, r.t., 1 h, 88–94% (c) 1.**14a-e**, $SOCl_2$, reflux, 2.5 h, 2. DIPEA, ACN, r.t., 19 h, 18–24%.

[36] and the PDSP databases [37] (Ki or equivalent less than 1000 nM for ChEMBL ligands or marked as an active for PDSP compounds) were clustered with the Hierarchical Clustering tool in Canvas [38]. After manual refinements, compounds were split into 20 distinct chemical classes. Centroids from each cluster were used for the induced-fit docking procedure [39] which generated one model per cluster. Screening efficiency (tested as previously, with the application of all actives and 88k decoys generated with the application of DUD-E formalism [40]) was significantly improved and the best performing model (AUC increased from 0.501 for the crystal structure to 0.722 for the best-optimized model) was used for the docking studies of compounds **25a** and **26a**.

Analysis of the docking modes showed that several of the NPC1 amino acids that participate in binding to GP [41–43] are involved in the binding of compounds **25a** and **26a**. The benzimidazole moiety of both compounds formed a hydrogen bond with Q421 and pi-pi stacking interaction with Y423. Both compounds created additional pi-pi stacking interaction: compound **25a** with F504 and compound **26a** with F503. Moreover, compound **26a** was hydrogen-bonded with Y423.

The #stars parameter, of the QikProp ADME prediction program, can be used to help determine the drug-likeness of a particular compound. The #stars values of approved drugs are generally found in the acceptable range of 0–5. The most favorable value #star = 0 was found for compound **26a**. This value indicated **26a** is an ideally drug-like molecule (Table 2). Investigated compounds showed few violations of Lipinski's rule of five (RuleOfFive) and Jorgensen's rule of three (RuleOfThree). However, these guidelines for defining drug-like properties and oral availability are not always entirely predictive for an optimal lead molecule. All compounds showed good predicted human oral absorption. Their low aqueous solubility (ClQPlogS) and high - predicted octanol/water partition coefficient (QPlogPo/w) indicated the hydrophobic properties. The distribution of the compounds was simulated by calculation of binding affinities to human serum albumin (QPlogKhsa). The estimated values have been found in the acceptable range for almost all our compounds. To predict the drug cytotoxicity, the estimation of IC_{50} values for blockage of HERG K+ channel was used, and every compound was found to be in the acceptable range. All molecules showed excellent predicted Caco-2 cell (model for the gut-blood barier) permeability and great predicted MDCK cell (good mimic for the blood-brain barrier) permeability. Thus, these compounds are predicted to cross the blood/brain barrier (QPlogBB) and may potentially exhibit some activity in the central nervous system.

3. Conclusions

We have developed an efficient synthesis for the generation of functionalized 2-substituted-6-[(4-substituted-1-piperidyl) methyl]-1H-benzimidazoles as entry inhibitors of Ebola virus. The exploration of our scaffolds was focused on the piperidine moiety as center core, and we also investigated the influence of various substitution at the benzimidazole moiety. Our compounds also exhibited a potent anti-EBOV activity, with four compounds with a submicromolar activity, and two of them **25a** and **26a** with an excellent selectivity index of, respectively, 10 and 20 (2–3 times more selective than the FDA-approved anti-cancer drug Toremifene). Data suggests that the mechanism by which **25a** and **26a** block EBOV infection is through the inhibition of viral entry at the level of NPC1. Finally, the ADME-Tox profile of our compounds showed an excellent *in silico* profile for almost all synthesized compounds, with good calculated permeability and solubility, low HERG toxicity, high permeability and good protein plasma interaction. Altogether, these compounds deserve to be further optimized and developed as potential antifiloviral drugs.

4. Experimental

4.1. Chemistry

Commercially available chemicals were of reagent grade and used as received. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60F254, E. Merck). Column chromatography was performed on Silica Gel 60 M (0.040e0.063 mm, E. Merck). The ^1H and ^{13}C NMR spectra were recorded on a Varian InovaUnity 400 spectrometer (400 MHz) in (d4) methanol, $CDCl_3$, shift values in parts per million relative to $SiMe_4$ as internal reference. High Resolution Mass spectra were performed on a Bruker maxis mass spectrometer by the "Fédération de Recherche".

4.1.1. General procedure 1 (for the synthesis of 2a and 2b)

The microwave vial was charged with α,α-dibromo-m/*p*-xylene (0.40 g, 1.5 mmol, 2 equiv), phosphite derivative (0.75 mmol, 1 equiv) and DMF (0.8 mL). The reaction mixture was heated at 150 °C for 2 min through microwave activation. Then, mixture was poured into water and product was extracted with EtOAc. Combined organic phases were washed with water and brine, then dried over $MgSO_4$ and concentrated under vacuum. Purification by column chromatography on silica gel (PE/EtOAc or DCM/MeOH) gave the

Table 1
Anti-EboV activity of benzimidazole analogs.

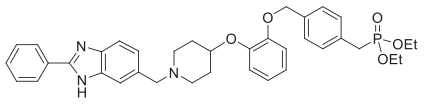
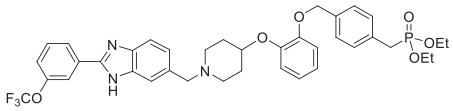
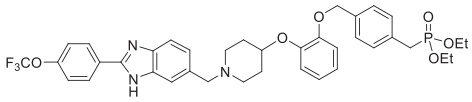
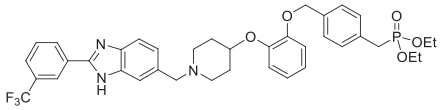
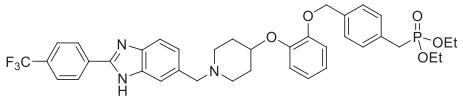
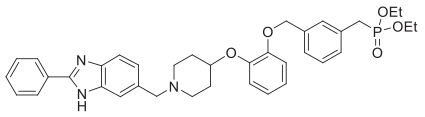
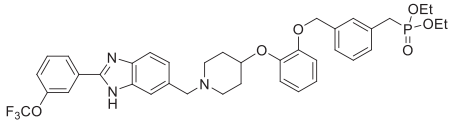
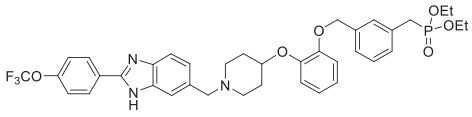
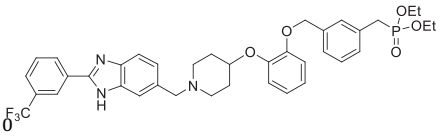
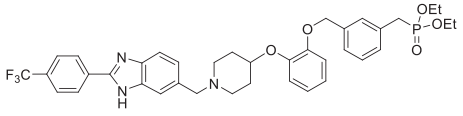
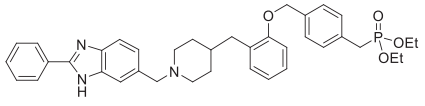
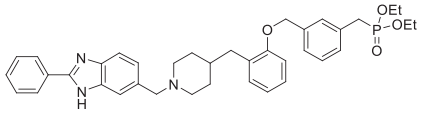
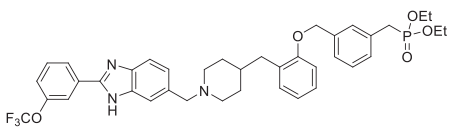
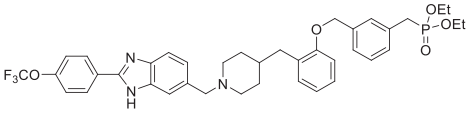
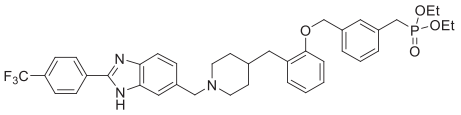
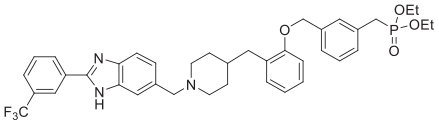
Number	Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
15a		2.95	14.72	5
15b		2.95	14.72	5
15c		1.19	4.20	4
15d		29.67	100	3
15e		1.73	3.82	2
16a		1.88	13.14	7
16b		2.08	9.56	5
16c		1.97	4.23	2
16d		2.49	4.59	2
16e		8.73	15.50	2
25a		**0.64**	**13.21**	**20**
26a		**0.93**	**9.17**	**10**
26b		0.87	4.45	5

Table 1 (*continued*)

Number	Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
26c		1.11	4.20	4
26d		1.40	4.22	3
26e		1.57	4.56	3
27	Toremifene	0.38	2.50	7

[a] Effective concentration required to inhibit virus-expressed reporter fluorescence by 50%.
[b] Cytotoxic concentration required to cause a loss of cell viability by 50%.
[c] Selectivity Index defined as CC_{50}/EC_{50}.

desired product.

4.1.1.1. Diethyl 4-(bromomethyl)benzylphosphonate (2a). The title compound was prepared from α,α-dibromo-*p*-xylene (**1a**) (0.40 g, 1.5 mmol, 2 equiv) and triethyl phosphite (0.13 mL, 0.75 mmol, 1 equiv) following the general procedure 1. Compound **2a** (0.21 g, 88%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (d, J = 8.1 Hz, 2H), 7.32–7.23 (m, 2H), 4.49 (s, 2H, Br–CH_2), 4.09–3.89 (m, 4H, O–CH_2), 3.16 (d, J = 21.8 Hz, 2H, CH_2–P), 1.26 (t, J = 7.1 Hz, 6H, CH_3).

4.1.1.2. Diethyl 3-(bromomethyl)benzylphosphonate (2b). The title compound was prepared from α,α-dibromo-*m*-xylene (**1b**) (0.40 g, 1.5 mmol, 2 equiv) and triethyl phosphite (0.13 mL, 0.75 mmol, 1 equiv) following the general procedure 1. Compound **2b** (0.23 g, 94%) was obtained as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.21 (m, 4H), 4.49 (s, 2H, Br–CH_2), 4.12–3.94 (m, 4H, O–CH_2), 3.16 (d, J = 21.7 Hz, 2H, CH_2–P), 1.26 (t, J = 7.1 Hz, 6H, CH_3).

4.1.2. 2-(Oxan-2-yloxy)phenol (4)

Catechol **3** (2 g, 18.2 mmol, 1 equiv) and dihydropyran (1.66 mL, 18.2 mmol, 1 equiv) were added to a solution of pyridinium p-toluenesulfonate (46 mg, 0.182 mmol, 1 mmol%) in DCM (35 mL). The solution was then stirred at room temperature for 3 h, and the solvent were removed under reduced pressure. The resulted mixture was then dissolved in EtOAc, washed twice with NaHCO3, once with brine, dried over MgSO4 and concentrated to afford pure compound **4** (3.30 g, 94%) as a yellowish oil, [44]. CAS: 21645-25-0.

4.1.3. Diethyl ({4-[2-(oxan-2-yloxy)phenoxymethyl]phenyl}methyl) phosphonate (5a)

In a 10–20 mL microwave vial, compound **4** (1.12 g, 1 eq., 5.74 mmol) was dissolved in DMF (18 mL). To this solution, potassium carbonate (1.58 g, 2 eq., 11.48 mmol), compound **2a** (2.03 g, 1.1 eq., 6.32 mmol) and few crystals of sodium iodide (catalytic amount) were added. The mixture was then stirred 20 min at 80 °C under microwave irradiation. After evaporation of all volatiles, the residue was purified by silica gel chromatography to afford desired product **5a** as a colorless oil. (1.42 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 7.8 Hz, 2H, H^{Ar}), 7.30 (dd, J = 8.2, 2.5 Hz, 2H, H^{Ar}), 7.15 (m, 1H, H^{Ar}), 6.93 (m, 3H, H^{Ar}), 5.43 (s, 1H, $H^{anomeric}$), 5.09 (t, J = 2.1 Hz, 2H, CH_2–O), 4.01 (m, 4H, CH_2–O-Phosph), 3.60 (m, 1H, H^5), 3.15 (d, J = 21.6 Hz, 2H, CH_2–P), 2.16–1.79 (m, 3H, $H^{2/3/4/5}$), 1.74–1.56 (m, 4H, $H^{2/3/4/5}$), 1.23 (t, J = 7.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.45 (C^{quat}), 147.16 (C^{quat}), 136.13 (d, J = 3.6 Hz, C^{quat}), 131.11 (d, J = 9.3 Hz, C^{quat}), 129.83 (d, J = 6.8 Hz, C^{Ar}), 127.42 (d, J = 3.0 Hz, C^{Ar}), 122.58 (C^{Ar}), 121.84 (C^{Ar}), 118.56 (C^{Ar}), 115.58 (C^{Ar}), 97.61 ($C^{Anomeric}$), 71.06 (CH_2–O), 62.12 (d, J = 6.8 Hz, CH_2–O–P), 61.99 (C^5), 33.55 (d, J = 138.1 Hz, CH_2–P), 30.39 ($C^{2/3/4}$), 25.30 ($C^{2/3/4}$), 18.72 ($C^{2/3/4}$), 16.36 (d, J = 5.9 Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.27. HRMS-ESI (*m/z*) $[M+Na]^+$calcd for $C_{23}H_{31}NaO_6P$ 457.1756, found 457.1760.

4.1.4. Diethyl ({3-[2-(oxan-2-yloxy)phenoxymethyl]phenyl}methyl) phosphonate (5b)

Compound **4** (665 mg, 1.1 eq., 3.43 mmol) was dissolved in DMF (10 mL) in a 10–20 mL microwave vial. To this solution were added potassium carbonate (868 mg, 2 eq., 6.28 mmol), compound **2b** (1 g, 1eq., 3.14 mmol) and few crystals of sodium iodide (catalytic amount). The solution was then stirred 20 min at 80 °C under microwave irradiation. After evaporation of all volatiles, the residue was purified by silica gel chromatography to afford desired product **5b** as a colorless oil. (974 mg, 72%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.30 (m, 4H, H^{Ar}), 7.07 (s, 1H, H^{Ar}) 6.88 (m, 3H, H^{Ar}), 5.08 (s, 2H, CH_2–O-Ar), 3.97 (m, 5H, CH_2–O–P, O–CH–O), 3.57 (m, 1H, CH_2–O THP) 3.12 (d, J = 21.7 Hz, 2H, CH_2–P), 2.11–1.44 (m, 7H, CH_2 THP), 1.20 (t, J = 7.1 Hz, 6H, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.21. HRMS-ESI (*m/z*) $[M+Na]^+$ calcd for $C_{23}H_{31}NaO_6P$ 457.1756, found 457.1760.

4.1.5. Diethyl {[4-(2-hydroxyphenoxymethyl)phenyl]methyl} phosphonate (6a)

To a solution of phosphonate **5a** (1.16 g, 1 eq., 2.67 mmol) in ethanol (27 mL) pyridium p-toluenesulfonate (34 mg, 5 mol%, 0.13 mmol) was added. The mixture was then stirred at 55 °C for 3 h, followed by the evaporation of all volatiles. The residue was then purified by flash chromatography, eluting Petroleum Ether/EtOAc 65:35 to 1:1, to give unprotected compound **6a** as a white solid. (936 mg, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 4H, H^{Ar}), 6.89 (m, 4H, H^{Ar}), 5.68 (s, 1H, OH), 5.08 (s, 2H, CH_2–O), 4.02 (ddq, J = 10.2, 7.0, 3.0 Hz, 4H, CH_2–O–P), 3.17 (d, J = 21.7 Hz, 2H, CH_2–P), 1.25 (t, J = 7.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.96 (C^{quat}), 145.74 (C^{quat}), 135.02 (d, J = 3.8 Hz, C^{quat}), 131.95 (d, J = 9.3 Hz, C^{quat}), 130.10 (d, J = 6.5 Hz, C^{Ar}), 128.03 (d, J = 3.2 Hz, C^{Ar}), 121.89 (C^{Ar}), 120.08 (C^{Ar}), 114.79 (C^{Ar}), 112.33 (C^{Ar}), 70.83 (CH_2–O),

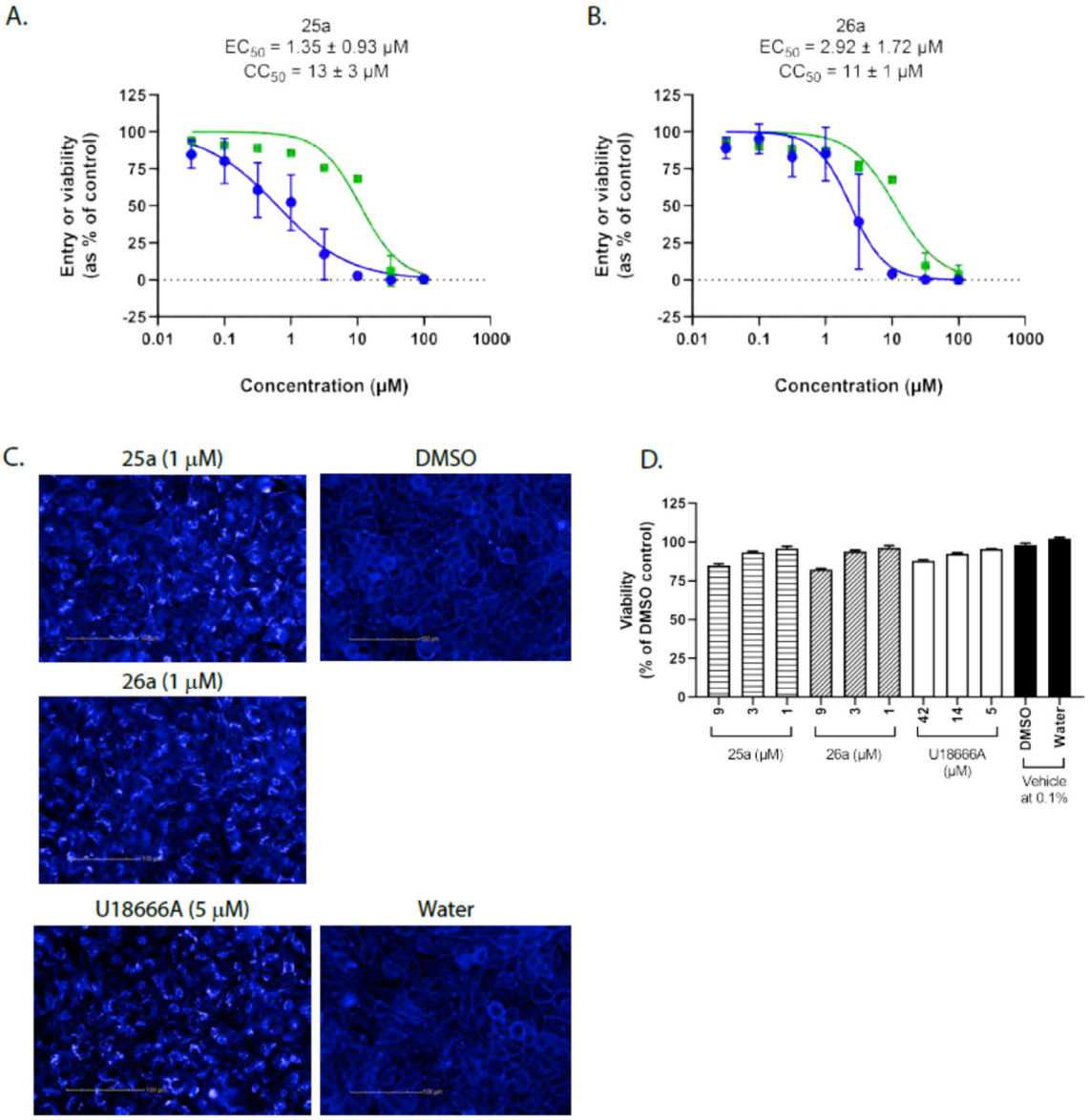


Fig. 2. Compounds block EBOV GP-mediated entry and alter the subcellular distribution of intracellular cholesterol, consistent with inhibition of NPC1 activity. (A & B) Compounds block the entry of HIV pseudotype particles bearing the EBOV GP. Data represent the mean ± standard deviation from 3 biological repeats. Blue circles, entry; green, viability for (A) compound **25a** and (B) compound **26a**. (C) Compounds alter the distribution of intracellular cholesterol, consistent with an inhibition of NPC1 activity. Immunofluorescence micrographs of HeLa cells treated with compounds **25a**, **26a** or their DMSO-vehicle, or U18666A or its water-vehicle, then stained with filipin to visualize intracellular cholesterol. Bars are equivalent to 100 μm. (D) Minimal cytotoxicity in HeLa cells treated with these compounds. Cellular viability, relative to the DMSO vehicle-control, as determined by CellTiter-Glo. Data represent the mean ± standard deviation from 3 biological repeats.

62.17 (d, $J = 6.9$ Hz, CH_2—O—P), 33.54 (d, $J = 138.0$ Hz, CH_2—P), 16.38 (d, $J = 6.0$ Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.08. HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{18}H_{24}O_5P$351.1362, found 351.1353.

4.1.6. Diethyl {[3-(2-hydroxyphenoxymethyl)phenyl]methyl} phosphonate (6b)

To a solution of phosphonate **5b** (1.60 g, 1 eq., 3.6 mmol) in ethanol (37 mL) pyridium p-toluenesulfonate (45 mg, 5 mol%, 0.18 mmol) was added. The mixture was then stirred at 55 °C for 3 h, followed by the evaporation of all volatiles. The residue was then purified by flash chromatography, eluting Petroleum Ether/EtOAc 65:35 to 1:1, to give unprotected compound **6b** as a light brown oil. (1.28 g, quant.) ^1H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 4H, H^{Ar}), 6.89 (m, 4H, H^{Ar}), 5.75 (s, 1H, OH), 5.10 (s, 2H, CH_2—O), 4.01 (ddq, $J = 8.2$, 7.1, 3.2 Hz, 4H, CH_2—O—P), 3.17 (d, $J = 21.7$ Hz, 2H, CH_2—P), 1.23 (t, $J = 7.1$ Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 145.99 (C^{quat}), 145.76 (C^{quat}), 136.77 (d, $J = 3.2$ Hz, C^{quat}), 132.28 (d, $J = 9.0$ Hz, C^{quat}), 129.79 (d, $J = 6.7$ Hz, C^{Ar}), 129.11 (d, $J = 6.6$ Hz, C^{Ar}), 128.91 (d, $J = 3.0$ Hz, C^{Ar}), 126.26 (d, $J = 3.5$ Hz, C^{Ar}), 121.88 (C^{Ar}), 120.08 (C^{Ar}), 114.86 (C^{Ar}), 112.37 (C^{Ar}), 70.91 (CH_2—O), 62.17 (d, $J = 6.7$ Hz, CH_2—O—P), 33.77 (d, $J = 138.2$ Hz, CH_2—P), 16.37 (d, $J = 5.9$ Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.05. HRMS-ESI (*m/z*): $[M+H]^+$calcd for $C_{18}H_{24}O_5P$ 351.1362, found 351.1360.

4.1.7. tert-Butyl 4-hydroxypiperidine-1-carboxylate (8)

tert-Butyl 4-oxopiperidine-1-carboxylate **7** (5 g, 1 eq., 25.1 mmol) was dissolved in MeOH (50 mL) under inert atmosphere. This solution was cooled at 0 °C and sodium borohydride was subsequently added portionwise (950 mg, 1 eq., 25.1 mmol). The mixture was allowed to reach room temperature and stirred for

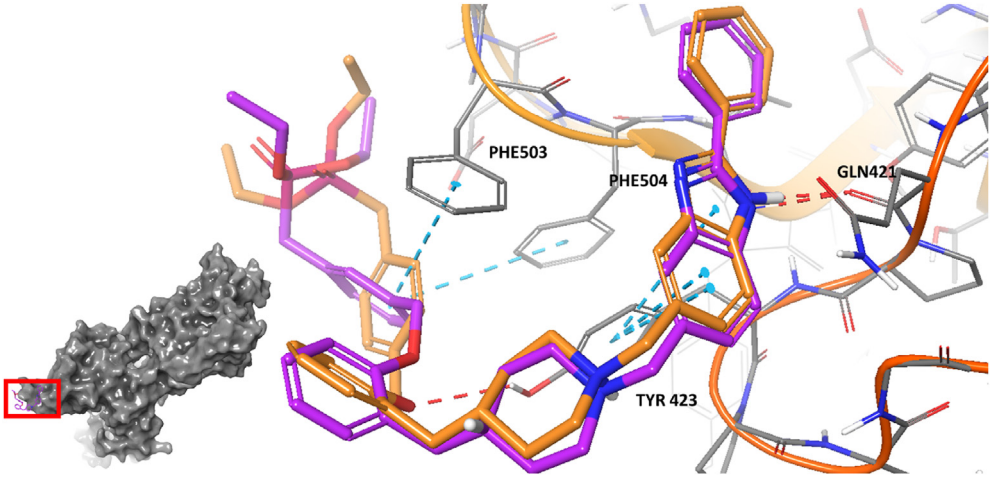


Fig. 3. The docking binding poses of compound **25a** (orange) **26a** (purple) and in the active site of NPC1 (PDB ID: 5U73). Residues within a distance of 4 Å from compounds have been shown. Hydrogen bond and pi-pi stacking interactions between NPC1 and docked compounds are in red and blue, respectively. In the left bottom corner, whole NPC1 protein is shown, with the binding site highlighted in the red box.

Table 2
ADME profile of synthesized compounds.

	#stars	QPlogPo/w	QPlogKhsa	QPlogHERG	QPPCaco	QPPMDCK	Percent Human Oral Absorption	Rule of Five	Rule of Three
Range or recommended values	0–5	−2.0–6.5	−1.5–1.5	Below −5	<25 poor, >500 great	<25 poor, >500 great	<25 poor, >80% high	Max 4	Max 3
3.47 [22]	3	4.54	0.54	−5.21	60.06	71.04	72.39	1	1
15a	2	6.31	1.04	−8.16	608.80	321.19	87.82	2	0
15b	4	7.61	1.40	−7.90	736.35	1285.39	96.89	2	1
15c	7	7.83	1.44	−8.33	752.54	1879.53	100	2	1
15d	8	7.93	1.55	−8.33	748.73	1490.82	100	2	1
15e	3	7.67	1.37	−7.71	1397.40	3433.29	100	2	1
16a	4	6.90	1.18	−8.72	1113.77	625.09	95.94	2	1
16b	7	8.07	1.49	−8.34	1033.88	2646.59	100	2	1
16c	4	7.64	1.29	−7.66	1455.18	3833.36	100	2	1
16d	7	7.88	1.44	−8.64	1113.92	2744.82	100	2	1
16e	6	7.86	1.44	−8.58	1111.66	2721.34	100	2	1
25a	2	6.81	1.27	−6.72	1063.19	584.78	95.09	2	0
26a	0	6.32	1.02	−6.05	1741.80	997.06	96.08	2	0
26b	1	7.08	1.19	−5.86	1676.22	4173.72	100	2	0
26c	1	7.53	1.36	−6.66	1728.33	3415.32	100	2	1
26d	5	8.07	1.51	−7.51	1423.42	3672.55	100	2	2
26e	1	7.36	1.26	−6.27	1731.97	3660.05	100	2	1

20h. The solution was carefully quenched at 0 °C with 2 N sodium hydroxide (20 mL), followed by evaporation of all volatiles. The mixture was then dissolved in EtOAc (40 mL) and water (20 mL). The aqueous phase was extracted with EtOAc (3 x 40 mL), the organic phases were washed with H_2O (20 mL), brine (20 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Purification by silica gel column chromatography (Petroleum Ether/EtOAc 8:2) afforded desired product **8** as a white solid. (4.8 g, 95%), [45]. CAS: 109384-19-2.

4.1.8. tert-Butyl 4-[(4-methylbenzenesulfonyl)oxy]piperidine-1-carboxylate (9)

To a solution of compound **8** (5 g, 1 eq., 24.8 mmol) in DCM (50 mL), triethylamine (13.9 mL, 4 eq., 99.4 mmol) and p-toluenesulfonyl chloride (9.47 g, 2 eq., 49.7 mmol) were added. The mixture was stirred 20 h at room temperature followed by the concentration of this solution under reduced pressure. The residue was then dissolved in DCM (20 mL) and water (20 mL), extracted with DCM (20 mL), washed with 1 N HCl (40 mL), extracted twice with DCM (2 x 40 mL), dried over $MgSO_4$ and evaporated. The crude was then purified by silica gel column chromatography (Petroleum ether/EtOAc 8:2) to afford desired product **9** as a white crystalline solid. (8.7 g, 99%), [46]. CAS: 118811-07-7.

4.1.9. tert-Butyl-4-{[2-({4-[(diethoxyphosphoryl)methyl]phenyl} methoxy)phenyl]-methyl }piperidine-1-carboxylate (10a)

In a 10–20 mL microwave vial, phosphonate compound **6a** (512 mg, 1 eq., 1.46 mmol) was dissolved in dimethylacetamide (13 mL). To this solution, potassium carbonate (404 mg, 2 eq., 2.92 mmol) and compound **9** (778 mg, 1.5 eq., 2.19 mmol) were then added and this mixture was stirred at 140 °C during 30 min under microwave irradiation. The resulting residue was dissolved in EtOAc (20 mL) and water (25 mL), and the aqueous phase was extracted thrice with EtOAc (3 x 20 mL). The organic phases were then washed 5 times with water (5 x 25 mL), once with brine (25 mL) and dried over magnesium sulfate. The residue was concentrated under reduced pressure and purified by silica gel column chromatography, eluting DCM/MeOH 99:1, to afford the

intermediate **10a** as colorless oil which was directly engaged in the next step.

4.1.10. tert-Butyl-4-{[2-({3-[(diethoxyphosphoryl)methyl]phenyl} methoxy) phenyl]-methyl}piperidine-1-carboxylate (10b)

In a 2–5 mL microwave vial, phosphonate compound **6b** (100 mg, 1 eq., 0.29 mmol) was dissolved in dimethylacetamide (DMA, 2.5 mL). To this solution potassium carbonate (81 mg, 2 eq., 0.57 mmol) and compound **9** (152 mg, 1.5 eq., 0.43 mmol) were afterwards added. This reaction mixture was stirred at 140 °C for 30 min under microwave irradiation. The resulting residue was dissolved in EtOAc (10 mL) and water (5 mL), and the aqueous phase was extracted thrice with EtOAc (3 x 10 mL). The organic phases were then washed 5 times with water (5 x 5 mL), once with brine (5 mL) and dried over magnesium sulfate. The crude was concentrated under reduced pressure and purified by silica gel column chromatography, eluting DCM/MeOH 99:1, to afford desired product **10b** as colorless oil. (100 mg, 71%). ^{1}H NMR (400 MHz, DMSO) δ 7.29 (m, 3H, H^{Ar}), 7.19 (s, 1H, H^{Ar}), 7.01 (m, 2H, H^{Ar}), 6.85 (m, 2H, H^{Ar}), 5.03 (s, 2H, CH_2–O), 4.41 (m, 1H, H^4 pip), 3.87 (ddq, J = 6.9, 6.1, 0.4 Hz, 4H, CH_2–O–P), 3.56 (m, 2H, H^2, H^6 pip), 3.17 (d, J = 21.5 Hz, 2H, CH_2–P),3.15 (m, 2HH^2, H^6 pip),1.79 (s, 2H, H^3, H^5 pip), 1.51 (s, 2H, H^3, H^5 pip), 1.35 (s, 9H, CH_3 Boc), 1.07 (dt, J = 6.9, 0.4 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 154.36 (C=O), 150.05 (C^{quat}), 147.18 (C^{quat}), 137.83 (d, J = 3.0 Hz, C^{quat}), 132.88 (d, J = 8.7 Hz, C^{quat}), 129.61 (d, J = 6.8 Hz, C^{Ar}), 129.07 (d, J = 6.8 Hz, C^{Ar}), 128.74 (d, J = 2.5 Hz, C^{Ar}), 126.00 (d, J = 3.7 Hz, C^{Ar}), 122.57 (C^{Ar}), 121.79 (C^{Ar}), 118.59 (C^{Ar}), 115.52 (C^{Ar}), 79.11 (C^{quat} Boc), 74.19 (C^4 pip), 70.43(CH_2–O), 61.83 (d, J = 6.5 Hz, CH_2–O–P), 39.74 (C^2, C^6 pip, under DMSO peak),32.71 (d, J = 134.0 Hz, CH_2–P), 31.00 (C^3, C^5 pip), 28.53 (CH_3 Boc), 16.64 (d, J = 5.9 Hz, CH_2–O–P). ^{31}P NMR (162 MHz, DMSO) δ 26.34. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{28}H_{41}NO_7P$ 534.2621, found 534.2615.

4.1.11. General procedure 2 (for BOC removal)

Trifluoroacetic acid (100 eq.) was added dropwise to a mixture of Boc-compound (1 eq.) in DCM (2:1 DCM/TFA v/v). The reaction was stirred at room temperature for 2h and then volatiles were removed under reduced pressure. The crude product was extracted with EtOAc, washed with $NaHCO_3$ until pH 7, dried over $MgSO_4$, filtrated and concentrated under vacuum. Pure compounds were obtained after purification by flash column chromatography with DCM/MeOH (95:5) as eluent.

4.1.11.1. Diethyl ({4-[2-(piperidin-4-yloxy)phenoxymethyl]phenyl} methyl) phosphonate (11a). Following general procedure 2, trifluoroacetic acid (11.1 mL, 100 eq., 146 mmol) was added to a solution of intermediate **10a** in DCM (20 mL). Pure compound **11a** was obtained after flash column chromatography (DCM/MeOH 95:5), affording desired product as an orange solid. (588 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (d, J = 8.0 Hz, 2H, H^{Ar}), 7.29 (d, J = 8.2, 2.5 Hz, 2H, H^{Ar}), 7.09 (m, 2H, H^{Ar}), 7.01 (t, J = 7.3 Hz, 1H, H^{Ar}), 6.94 (m, 1H, H^{Ar}), 5.10 (s, 2H, CH_2–O), 4.57 (quint., J = 4.3 Hz, 1H, H^4 pip), 4.04 (2x ddq, J = 7.7, 6.7, 2.3 Hz, 4H, CH_2–O–P), 3.38 (dt, J = 13.0, 7.0 Hz, 2H, H^2, H^6 pip), 3.26 (d, J = 21.8 Hz, 2H, CH_2–P), 3.09 (m, 2H, H^2, H^6 pip), 3.13 (tdd, J = 12.8, 5.0 Hz, 2H, H^3, H^5 pip), 2.03 (dt, J = 7.1,5.3 Hz, 2H, H^3, H^5 pip), 1.26 (t, J = 7.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 146.55 (C^{quat}), 135.15 (d, J = 3.8 Hz, C^{quat}), 129.78 (d, J = 6.6 Hz, C^{Ar}), 127.65 (d, J = 3.3 Hz, C^{Ar}), 123.08 (C^{Ar}), 121.41 (C^{Ar}), 118.82 (C^{Ar}), 114.95 (C^{Ar}), 70.80 (C^4 pip), 70.52 (CH_2–O), 62.35 (d, J = 7.0 Hz, CH_2–O–P), 40.24(C^2, C^6 pip), 31.86(d, J = 138.0 Hz, CH_2–P), 27.06(C^3, C^5 pip), 15.24(d, J = 6.1 Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 27.50. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{23}H_{33}NO_5P$ 434.2097, found 434.2100.

4.1.11.2. Diethyl ({3-[2-(piperidin-4-ylmethyl)phenoxymethyl] phenyl}methyl) phosphonate (11b). Following general procedure 2, trifluoroacetic acid (1.5 mL, 100 eq., 19.7 mmol) was added to a solution of compound **10b** (105 mg, 1 eq., 0.197 mmol) in DCM (3 mL). Pure compound **11b** was obtained after flash column chromatography (DCM/MeOH 95:5), affording desired product as an orange solid. (58 mg, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 2.3 Hz, 1H, H^{Ar}), 7.29 (m, 1H, H^{Ar}), 7.15 (m, 2H, H^{Ar}), 6.96 (m, 4H, H^{Ar}), 4.98 (s, 2H, CH_2–O), 4.60 (s, 1H, H^4 pip), 3.95 (2x ddq, J = 7.6, 6.6, 0.5 Hz, 4H, CH_2–O–P), 3.32 (td, J = 12.9, 3.8 Hz, 2H, H^2, H^6 pip), 3.12 (d, J = 21.8 Hz, 2H, CH_2–P), 3.09 (m, 2H, H^2, H^6 pip), 2.25 (tdd, J = 12.8, 4.7, 2.2 Hz, 2H, H^3, H^5 pip), 1.96 (dd, J = 13.1,2.9 Hz, 2H, H^3, H^5 pip), 1.22 (td, J = 7.1, 0.5 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.77 (C^{quat}), 146.38 (C^{quat}), 137.04 (d, J = 3.4 Hz, C^{quat}), 131.41 (d, J = 9.5 Hz, C^{quat}), 129.87 (d, J = 6.7 Hz, C^{Ar}), 128.91 (d, J = 6.0 Hz, C^{Ar}), 126.78 (d, J = 3.2 Hz, C^{Ar}), 124.51 (d, J = 5.0 Hz, C^{Ar}), 123.47(C^{Ar}), 121.87(C^{Ar}), 119.29(C^{Ar}), 114.14(C^{Ar}), 71.32(C^4 pip), 70.80 (CH_2–O), 63.09 (d, J = 6.9 Hz, CH_2–O–P), 39.44(C^2, C^6 pip), 32.96 (d, J = 139.5 Hz, CH_2–P), 26.66(C^3, C^5 pip), 16.06 (d, J = 6.2 Hz, CH_3). ^{31}P NMR (162 MHz, $CDCl_3$) δ 26.57. HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{23}H_{33}NO_5P$ 434.2097, found 434.2094.

4.1.12. General procedure 3 (for benzimidazole ring construction)

Solution of an aldehyde (**12a-e**, respectively), (1 equiv) in 40% $NaHSO_3$ (4 mL) was stirred for 1h at room temperature, then solution of methyl 3,4-diaminobenzoate (0.65 g, 3.94 mmol, 1 equiv) in EtOH (2 mL) was added. Resulting mixture was stirred at 100 °C for 1–4h, then concentrated. Residue was dissolved in water and extracted with EtOAc. Combined organic phases were dried over $MgSO_4$ and concentrated. The crude product was purified by flash column chromatography (PE/EtOAc or DCM/MeOH) to afford desired product.

4.1.12.1. 5-Methyl carboxylate-2-phenyl-benzimidazole (13a). The title compound was prepared from benzaldehyde **12a** (0.4 mL, 3.94 mmol, 1 equiv) following the general procedure 3. Compound **13a** (0.97 g, 97%) was obtained as a white solid. ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.18–8.10 (m, 2H), 8.00 (dd, J = 8.5, 1.5 Hz, 1H), 7.66 (d, J = 8.2 Hz, 1H), 7.53–7.45 (m, 3H), 3.97 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.97, 130.99, 129.44, 129.38, 127.08, 125.02, 124.70, 52.42 (CH_3). HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{13}N_2O_2$ 253.0972 found 253.0974.

4.1.12.2. 5-Methyl carboxylate-2-[4-(trifluoromethyl)phenyl]-benzimidazole (13b). The title compound was prepared from 4-trifluoromethyl-benzaldehyde (0.41 mL, 3.00 mmol, 1 equiv) following the general procedure 3. Compound **13b** (0.90 g, 93%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 13.51 (s, 1H, NH), 8.39 (d, J = 8.3 Hz, 2H), 8.35–7.99 (m, 1H), 7.95 (d, J = 8.3 Hz, 2H), 7.92–7.82 (m, 1H), 7.81–7.59 (m, 1H), 3.88 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 166.63, 133.31, 130.33, 127.43, 126.05, 126.01, 125.40, 122.69, 111.82, 52.05 (CH_3). ^{19}F NMR (376 MHz, DMSO) δ −61.30. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{16}H_{12}F_3N_2O_2$ 321.0845 found 321.0848. IR (cm^{-1}) 3296, 1688, 1618, 1443, 1319, 1289, 1239, 1225, 1163, 1114, 1100, 1064, 1019, 984, 850, 765, 749, 696, 653. Mp 260 °C.

4.1.12.3. 5-Methyl carboxylate-2-[3-(trifluoromethyl)phenyl]-benzimidazole (13c). The title compound was prepared from 3-trifluoromethyl-benzaldehyde (0.24 mL, 1.8 mmol, 1 equiv) following the general procedure 3. Compound **13c** (0.44 g, 76%) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 13.45 (s, 1H, NH), 8.53 (s, 1H), 8.49 (d, J = 7.8 Hz, 1H), 8.22 (s, 1H), 7.92–7.80 (m, 3H), 7.75–7.68 (m, 1H), 3.88 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 166.64, 152.21, 130.60, 130.55, 130.36, 130.03, 129.71, 126.85,

126.82, 125.35, 123.84, 123.66, 123.14, 123.10, 122.64, 52.05 (CH_3). ^{19}F NMR (376 MHz, DMSO) δ −61.32. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{16}H_{12}F_3N_2O_2$ 321.0845 found 321.0848. IR (cm^{-1}) 3296, 1694, 1628, 1538, 1459, 1438, 1331, 1306, 1285, 1270, 1221, 1182, 1166, 1121, 1108, 1098, 1070, 991, 919, 803, 772, 751, 700, 688, 650, 612.Mp 190 °C.

4.1.12.4. 5-Methyl carboxylate-2-[4-(trifluoromethoxy)phenyl]-benzimidazole (13d). The title compound was prepared from 4-trifluoromethoxybenzaldehyde (0.75 mL, 5.3 mmol, 1 equiv) following the general procedure 3. Compound **13d** (1.38 g, 78%) was obtained as a light yellow solid. ^1H NMR (400 MHz, DMSO) δ 8.31 (d, $J = 8.7$ Hz, 2H, H^{Ar}), 8.21 (s, 1H, H^{Ar}), 7.87 (dt, $J = 8.4$ Hz, 1H, H^{Ar}), 7.70 (d, $J = 8.7$ Hz, 1H, H^{Ar}), 7.59 3.88 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 167.15(C═O), 152.98(C^{quat}), 150.12(C^{quat}), 129.31(C^{Ar}), 129.25(C^{Ar}), 126.73(C^{Ar}), 124.11(C^{Ar}), 123.96(C^{Ar}), 121.99(C^{Ar}), 121.77(C^{Ar}), 52.50 (CH_3). ^{19}F NMR (376 MHz, DMSO) δ −56.64. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{16}H_{12}F_3N_2O_2$ 337.0839 found 337.0842.

4.1.12.5. 5-Methyl carboxylate-2-[3-(trifluoromethoxy)phenyl]-benzimidazole (13e). The title compound was prepared from 3-trifluoromethoxybenzaldehyde (0.75 mL, 5.3 mmol, 1 equiv) following the general procedure 3. Compound **13e** (1.61 g, 91%) was obtained as a light yellow solid. ^1H NMR (400 MHz, DMSO) δ 8.24 (d, $J = 7.6$ Hz, 2H, H^{Ar}), 8.16 (s, 1H, H^{Ar}), 7.88 (d, $J = 8.4$ Hz, 1H, H^{Ar}), 7.75 (m, 2H, H^{Ar}), 7.55 (d, $J = 7.6$ Hz, 1H, H^{Ar}), 3.89 (s, 3H, CH_3). ^{13}C NMR (101 MHz, DMSO) δ 167.12 (C═O), 153.88 (C^{quat}), 152.66 (C^{quat}), 149.31 (C^{quat}), 132.26 (C^{Ar}), 131.81 (C^{quat}), 126.20 (C^{Ar}), 124.27 (C^{Ar}), 123.31 (C^{Ar}), 119.39 (C^{Ar}), 52.53 (CH_3) ^{19}F NMR (376 MHz, DMSO) δ −56.71. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{16}H_{12}F_3N_2O_2$ 337.0839 found 337.0841.

4.1.13. General procedure 4 (for reduction with $AlLiH_4$)

To the mixture of $LiAlH_4$ (0.03 g, 0.8 mmol, 2 equiv) in dry THF (1 mL), cooled at 0 °C, solution of methyl 2-substitued-benzimidazole-5 carboxylate (1 equiv) in dry THF (1 mL) was slowly added. The ice bath was removed, and the reaction mixture was stirred for 2−4h. After completion EtOAc and water were added. Then, aqueous phase was extracted with EtOAc (3x) and combined organic phases were dried over $MgSO_4$ and concentrated to give desired product.

4.1.13.1. 5-Hydroxymethyl-2-phenyl-benzimidazole (14a). The title compound was prepared from **13a** (0.10 g, 0.4 mmol, 1 equiv) following the general procedure 4. Compound **14a** (0.09 g, quantitative) was obtained as a white solid. ^1H NMR (250 MHz, DMSO) δ 12.86 (s, 1H, NH), 8.16 (dd, $J = 8.0$, 1.3 Hz, 2H), 7.76−7.36 (m, 5H), 7.16 (d, $J = 8.1$ Hz, 1H), 5.20 (t, $J = 5.4$ Hz, 1H, OH), 4.60 (d, $J = 5.0$ Hz, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 130.24, 129.70, 128.91, 126.31, 63.40 (CH_2). HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{14}H_{13}N_2O$ 225.1022 found 225.1024.

4.1.13.2. 5-Hydroxymethyl-2-[4-(trifluoromethyl)phenyl]-benzimidazole (14b). The title compound was prepared from **13b** (0.40 g, 1.25 mmol, 1 equiv) following the general procedure 4. Compound **14b** (0.35 g, 94%) was obtained as a white solid.^1H NMR (400 MHz, DMSO) δ 8.37 (d, $J = 8.1$ Hz, 2H), 7.93 (d, $J = 8.3$ Hz, 2H), 7.62−7.49 (m, 2H), 7.21 (d, $J = 8.8$ Hz, 1H), 5.21 (s, 1H, OH), 4.62 (s, 2H, CH_2)· ^{13}C NMR (101 MHz, DMSO) δ 149.58, 134.01, 129.68, 126.95, 125.96, 125.92, 125.50, 122.79, 63.32 (CH_2)· ^{19}F NMR (376 MHz, DMSO) δ −61.16.HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{12}F_3N_2O$ 293.0896 found 293.0900.IR (cm^{-1}) 3131, 2944, 2877, 1621, 1434, 1316, 1281, 1164, 1116, 1065, 1010, 961, 876, 849, 808, 786, 749, 693, 646, 634.Mp 228 °C.

4.1.13.3. 5-Hydroxymethyl-2-[3-(trifluoromethyl)phenyl]-benzimidazole (14c). The title compound was prepared from **13c** (0.31 g, 0.96 mmol, 1 equiv) following the general procedure 4. Compound **14c** (0.27 g, 95%) was obtained as a white solid. ^1H NMR (400 MHz, MeOD) δ 8.44 (s, 1H), 8.34 (d, $J = 7.7$ Hz, 1H), 7.85−7.71 (m, 2H), 7.69−7.55 (m, 2H), 7.31 (dd, $J = 8.3$, 0.9 Hz, 1H), 4.75 (s, 2H, CH_2). ^{13}C NMR (101 MHz, MeOD) δ 151.92, 132.76, 132.44, 132.10, 131.14, 127.66, 127.62, 126.77, 124.41, 124.37, 124.07, 65.55 (CH_2). ^{19}F NMR (376 MHz, MeOD) δ −64.33. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{12}F_3N_2O$ 293.0896 found 293.0901. IR (cm^{-1}) 3063, 2866, 2824, 1437, 1399, 1328, 1312, 1281, 1168, 1119, 1072, 1002, 975, 820, 798, 720, 699, 686, 657, 650.Mp 202 °C.

4.1.13.4. 5-Hydroxymethyl-2-[4-(trifluoromethoxy)phenyl]-benzimidazole (14d). The title compound was prepared from **13d** (1.38 g, 4.11 mmol, 1 equiv) following the general procedure 4. Compound **14d** (1.21 g, 95%) was obtained as a white solid. ^1H NMR (400 MHz, DMSO) δ 12.94 (s, 1H, NH), 8.29 (d, $J = 8.4$ Hz, 2H, H^{Ar}), 7.61 (s, 1H, H^{Ar}), 7.56 (d, $J = 8.4$ Hz, 2H, H^{Ar}), 7.51 (s, 1H, H^{Ar}), 7.18 (s, 1H, H^{Ar}), 5.22 (s, 1H, OH), 4.61 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 150.30, 149.62, 144.25, 143.21, 138.14, 136.94, 135.50, 129.98, 128.77 (C^{Ar}), 124.35, 122.68 (C^{quat}), 121.91 (C^{Ar}), 121.80 (C^{Ar}), 121.49 (C^{quat}), 119.25, 118.20 (d, $J = 157.8$ Hz, C^{Ar}), 116.69, 110.53 (d, $J = 174.2$ Hz, C^{Ar}), 63.85 (CH_2). ^{19}F NMR (376 MHz, DMSO) δ −56.68. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{12}F_3N_2O$ 309.0890 found 309.0893.

4.1.13.5. 5-Hydroxymethyl-2-[3-(trifluoromethoxy)phenyl]-benzimidazole (14e). The title compound was prepared from **13e** (1.61 g, 4.80 mmol, 1 equiv) following the general procedure 4. Compound **14e** (1.41 g, 95%) was obtained as a white solid. ^1H NMR (400 MHz, DMSO) δ 13.05 (bs, 1H, NH), 8.20 (d, $J = 8.0$ Hz, 1H, H^{Ar}), 8.13 (s, 1H, H^{Ar}), 7.70 (t, $J = 8.0$ Hz, 1H, H^{Ar}), 7.58 (d, $J = 8.2$ Hz, 1H, H^{Ar}), 7.56 (s, 1H, H^{Ar}), 7.49 (d, $J = 8.0$ Hz, 1H, H^{Ar}), 7.20 (d, $J = 8.2$ Hz, 1H, H^{Ar}), 5.21 (bs, 1H, OH), 4.62 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 150.02 (C^{quat}), 149.33 (C^{quat}), 132.94 (C^{Ar}), 131.64 (C^{quat}), 125.70 (C^{Ar}), 122.54 (C^{Ar}), 121.86 (C^{Ar}), 119.31 (C^{quat}), 118.93 (C^{Ar}), 63.80 (CH_2). ^{19}F NMR (376 MHz, DMSO) δ −56.69. HRMS (ESI) *m/z* $[M+H]^+$ calcd for $C_{15}H_{12}F_3N_2O$ 309.0890 found 309.0892.

4.1.14. General procedure 5 (for coupling piperidine fragment with benzimidazole fragment)

To a flask containing benzimidazole derivative (1 eq.), $SOCl_2$ (29 eq.) was added and the resulted mixture was stirred for 2h30 at 80 °C. After cooling to room temperature, $SOCl_2$ was evaporated and the obtained solid was diluted with ACN (4 mL) and cooled to 0 °C. Then, to this solution, piperidine derivative (1 eq.) and diisopropylethylamine (5.3 eq.) were added. The ice bath was removed and the reaction mixture was allowed to stir for 16−19 h at room temperature. After concentration, the crude product was purified by column chromatography (DCM/MeOH) to give the desired product.

4.1.14.1. Diethyl ({4-[2-({1-[(2-phenyl-1H-1,3-benzodiazol-6-yl) methyl]piperidin-4-yl}oxy)phenoxymethyl]-phenyl}methyl)phosphonate (15a). The title compound was obtained following the general procedure 5 from benzimidazole **14a** (31 mg, 1 eq., 0.138 mmol), $SOCl_2$ (293 μL, 29 eq., 4.01 mmol) and then compound **11a** (60 mg, 1 eq., 0.138 mmol), diisopropylethylamine (124 μL, 5.3 eq., 0.73 mmol) in ACN (2 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **15a** was obtained as an amorphous creamy solid. (29 mg, 40%) ^1H NMR (400 MHz, MeOD) δ 8.12 (d, $J = 7.0$ Hz, 2H, H^{Ar}), 7.63 (m, 5H, H^{Ar}), 7.37 (m, 5H, H^{Ar}), 7.04 (m, 2H, H^{Ar}), 6.91 (dquint, $J = 7.3$, 1.9 Hz,2H, H^{Ar}), 5.06 (s, 2H, CH_2−O), 4.48 (s, 1H, H^4 pip), 4.00 (ddq,

J = 8.8, 7.0, 1.8 Hz, 4H, CH_2—O—P), 3.93 (s, 2H, CH_2—N), 3.24 (d, J = 21.7 Hz, 2H, CH_2—P), 3.06 (bs, 2H, H^2, H^6 pip), 2.76 (bs, 2H, H^2, H^6 pip), 1.97 (m, 4H, H^3, H^5 pip), 1.23 (t, J = 7.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 150.15 (C^{quat}), 147.01 (C^{quat}), 136.24 (d, J = 3.9 Hz, C^{quat}), 131.11 (d, J = 9.2 Hz, C^{quat}), 130.19 (C^{Ar}), 129.71 (d, J = 6.6 Hz, C^{Ar}), 129.42 (C^{Ar}), 128.79 (C^{Ar}), 127.59 (d, J = 4.0 Hz, C^{Ar}), 126.48 (C^{Ar}), 122.46 (C^{Ar}), 121.46 (C^{Ar}), 118.52 (C^{Ar}), 115.23 (C^{Ar}), 70.49 (CH_2—O), 62.30 (d, J = 6.9 Hz, CH_2—O—P), 62.09 (CH_2—N), 48.99 (C^2, C^6 pip), 32.00 (d, J = 138.2 Hz, CH_2—P), 29.14 (C^3, C^5 pip), 15.23 (d, J = 6.0 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.43 HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{37}H_{43}N_3O_5P$ 640.2941, found 640.2935.

4.1.14.2. Diethyl {[4-(2-{[1-({2-[4-(trifluoromethyl)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]oxy}phenoxy-methyl)phenyl] methyl}phosphonate (15b). The title compound was obtained following the general procedure 5 from benzimidazole **14b** (0.16 mmol), $SOCl_2$ (340 μL, 29 eq., 4.68 mmol) and then compound **11a** (70 mg, 1 eq., 0.16 mmol), diisopropylethylamine (144 μL, 5.3 eq., 0.85 mmol) in ACN (4 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **15b** was obtained as an amorphous creamy solid. (28 mg, 32%) ^{1}H NMR (400 MHz, MeOD) δ 8.42 (bs, 1H, H^{Ar}), 8.33 (d, J = 8.0 Hz, 1H, H^{Ar}), 7.70 (m, 5H, H^{Ar}), 7.38 (d, J = 8.0 Hz, 2H, H^{Ar}), 7.28 (m, 3H, H^{Ar}), 6.98 (m, 2H, H^{Ar}), 6.88 (m, 2H, H^{Ar}), 5.01 (s, 2H, CH_2—O), 4.37 (d, J = 7.4 Hz, 1H, H^4 pip), 3.98 (m, 4H, CH_2—O—P), 3.75 (s, 2H, CH_2—N), 3.20 (d, J = 21.8 Hz, 2H, CH_2—P), 2.89 (bs, 2H, H^2, H^6 pip), 2.51 (bs, 2H, H^2, H^6 pip), 1.89 (m, 4H, H^3, H^5 pip), 1.20 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 150.89 (C^{quat}), 150.05 (C^{quat}), 147.19 (C^{quat}), 136.29 (d, J = 3.0 Hz, C^{quat}), 131.36 (C^{quat}), 131.04 (C^{quat}), 130.94 (C^{quat}), 130.72 (C^{quat}), 130.63 (C^{quat}), 129.83 (C^{Ar}), 129.78 (C^{Ar}), 129.67 (d, J = 6.6 Hz, C^{Ar}), 127.50 (d, J = 3.3 Hz, C^{Ar}), 126.38 (C^{Ar}), 125.36 (C^{Ar}), 125.14 (C^{Ar}), 123.08 (C^{Ar}), 122.66 (C^{Ar}), 122.19 (C^{Ar}), 121.45 (C^{Ar}), 118.29 (C^{Ar}), 115.33 (C^{Ar}), 70.50 (CH_2—O), 62.48 (CH_2—N), 62.30 (d, J = 7.0 Hz, CH_2—O—P), 49.37 (C^2, C^6 pip), 31.96 (d, J = 138.1 Hz, CH_2—P), 29.70 (C^3, C^5 pip), 15.25 (d, J = 5.9 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.42.^{19}F NMR (376 MHz, MeOD) δ −64.25. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{39}H_{42}F_3N_3O_5P$ 708.2811, found 708.2812.

4.1.14.3. Diethyl {[4-(2-{[1-({2-[3-(trifluoromethyl)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]oxy}phen-oxymethyl)phenyl] methyl}phosphonate (15c). The title compound was obtained following the general procedure 5 from benzimidazole **14c** (47 mg, 1 eq., 0.16 mmol), $SOCl_2$ (340 μL, 29 eq., 4.68 mmol) and then compound **11a** (70 mg, 1 eq., 0.16 mmol), diisopropylethylamine (144 μL, 5.3 eq., 0.85 mmol) in ACN (4 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **15c** was obtained as an amorphous creamy solid. (24 mg, 21%) ^{1}H NMR (400 MHz, MeOD) δ 8.41 (bs, 1H, H^{Ar}), 8.33 (d, J = 8.0 Hz, 1H, H^{Ar}), 7.71 (m, 5H, H^{Ar}), 7.32 (m, 4H, H^{Ar}), 6.94 (m, 4H, H^{Ar}), 5.01 (s, 2H, CH_2—O), 4.40 (s, 1H, H^4 pip), 3.96 (m, 4H, CH_2—O—P), 3.82 (s, 2H, CH_2—N), 3.19 (d, J = 23.3 Hz, 2H, CH_2—P), 2.97 (bs, 2H, H^2, H^6 pip), 2.68 (bs, 2H, H^2, H^6 pip), 1.95 (m, 4H, H^3, H^5 pip), 1.18 (t, J = 6.9 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 150.10 (C^{Ar}), 137.46 (d, J = 1.7 Hz, C^{Ar}), 136.93 (C^{Ar}), 136.27 (d, J = 3.7 Hz, C^{quat}), 129.87 (C^{Ar}), 129.82 (C^{Ar}), 129.68 (d, J = 6.7 Hz, C^{Ar}), 127.54 (d, J = 3.0 Hz, C^{Ar}), 123.85 (C^{Ar}), 122.34 (C^{Ar}), 121.45 (C^{Ar}), 118.42 (C^{Ar}), 115.29 (C^{Ar}), 70.51 (CH_2—O), 62.30 (d, J = 6.8 Hz, CH_2—O—P), 62.25 (CH_2—N), 49.20 (C^2, C^6 pip), 31.91 (d, J = 137.5 Hz, CH_2—P), 29.34 (C^3, C^5 pip), 15.21 (d, J = 5.9 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.44.^{19}F NMR (376 MHz, MeOD) δ −64.33. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{39}H_{42}F_3N_3O_5P$ 708.2811, found 708.2809.

4.1.14.4. Diethyl {[4-(2-{[1-({2-[4-(trifluoromethoxy)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]oxy}phen-oxymethyl)phenyl] methyl}phosphonate (15d). The title compound was obtained following the general procedure 5 from benzimidazole **14d** (36 mg, 1 eq., 0.12 mmol), $SOCl_2$ (244 μL, 29 eq., 3.35 mmol) and then compound **11a** (50 mg, 1 eq., 0.12 mmol), diisopropylethylamine (104 μL, 5.3 eq., 0.61 mmol) in ACN (2 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **15d** was obtained as an amorphous creamy solid. (17 mg, 23%) ^{1}H NMR (400 MHz, MeOD) δ 8.17 (d, J = 9.0 Hz, 2H, H^{Ar}), 7.61 (m, 2H, H^{Ar}), 7.46 (d, J = 8.7 Hz, 2H, H^{Ar}), 7.39 (d, J = 7.8 Hz, 2H, H^{Ar}), 7.30 (m, 3H, H^{Ar}), 6.99 (m, 2H, H^{Ar}), 6.89 (m, 2H, H^{Ar}), 5.02 (s, 2H, CH_2—O), 4.40 (s, 1H, H^4 pip), 3.99 (dqd, J = 9.8, 7.0, 1.6 Hz, 4H, CH_2—O—P), 3.80 (s, 2H, CH_2—N), 3.21 (d, J = 21.9 Hz, 2H, CH_2—P), 2.95 (bs, 2H, H^2, H^6 pip), 2.58 (bs, 2H, H^2, H^6 pip), 1.92 (m, 4H, H^3, H^5 pip), 1.21 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 151.27 (C^{quat}), 150.42 (C^{quat}), 150.08 (C^{quat}), 147.13 (C^{quat}), 136.27 (d, J = 4.0 Hz, C^{quat}), 131.00 (d, J = 9.2 Hz, C^{quat}), 129.68 (d, J = 6.7 Hz, C^{Ar}), 128.59 (C^{Ar}), 128.33 (C^{Ar}), 127.53 (d, J = 2.7 Hz, C^{Ar}), 125.04 (C^{Ar}), 122.29 (C^{Ar}), 121.74 (C^{Ar}), 121.45 (C^{Ar}), 121.18 (C^{Ar}), 118.37 (C^{Ar}), 115.30 (C^{Ar}), 70.50 (CH_2—O), 62.30 (d, J = 6.9 Hz, CH_2—O—P), 62.30 (CH_2—N), 49.20 (C^2, C^6 pip), 31.96 (d, J = 137.2 Hz, CH_2—P), 29.49 (C^3, C^5 pip), 15.24 (d, J = 6.0 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.43.^{19}F NMR (376 MHz, MeOD) δ −59.34. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{39}H_{42}F_3N_3O_6P$ 724.2760, found 724.2757.

4.1.14.5. Diethyl {[4-(2-{[1-({2-[3-(trifluoromethoxy)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]oxy}phen-oxymethyl)phenyl] methyl}phosphonate (15e). The title compound was obtained following the general procedure 5 from benzimidazole **14e** (36 mg, 1 eq., 0.12 mmol), $SOCl_2$ (244 μL, 29 eq., 3.35 mmol) and then compound **11a** (50 mg, 1 eq., 0.12 mmol), diisopropylethylamine (104 μL, 5.3 eq., 0.61 mmol) in ACN (2 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **15e** was obtained as an amorphous creamy solid. (18 mg, 25%) ^{1}H NMR (400 MHz, MeOD) δ 8.08 (d, J = 8.0 Hz, 1H, H^{Ar}), 8.03 (s, 1H, H^{Ar}), 7.62 (m, 3H, H^{Ar}), 7.41 (m, 3H, H^{Ar}), 7.31 (m, 3H, H^{Ar}), 6.99 (m, 2H, H^{Ar}), 6.88 (m, 2H, H^{Ar}), 5.02 (s, 2H, CH_2—O), 4.39 (s, 1H, H^4 pip), 3.99 (dqd, J = 8.0, 7.0, 1.6 Hz, 4H, CH_2—O—P), 3.77 (s, 2H, CH_2—N), 3.21 (d, J = 21.7 Hz, 2H, CH_2—P), 2.91 (s, 2H, H^2, H^6 pip), 2.54 (s, 2H, H^2, H^6 pip), 1.91 (s, 4H, H^3, H^5 pip), 1.21 (dt, J = 7.0, 0.4 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 150.88 (C^{quat}), 150.06 (C^{quat}), 149.71 (d, J = 2.0 Hz, 1H, C^{Ar}), 147.15 (C^{quat}), 136.29 (d, J = 3.9 Hz, C^{quat}), 131.78 (C^{Ar}), 131.00 (d, J = 9.5 Hz, C^{quat}), 130.69 (C^{quat}), 129.66 (d, J = 6.7 Hz, C^{Ar}), 127.51 (d, J = 3.2 Hz, C^{Ar}), 125.01 (C^{Ar}), 122.29 (C^{Ar}), 122.21 (C^{Ar}), 121.82 (C^{Ar}), 121.45 (C^{Ar}), 119.27 (C^{Ar}), 118.92 (C^{Ar}), 118.31 (C^{Ar}), 115.33 (C^{Ar}), 70.50 (CH_2—O), 62.43 (CH_2—N), 62.31 (d, J = 7.0 Hz, CH_2—O—P), 49.34 (C^2, C^6 pip), 32.02 (d, J = 138.0 Hz, CH_2—P), 29.67 (C^3, C^5 pip), 15.24 (d, J = 6.0 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.43.^{19}F NMR (376 MHz, MeOD) δ −59.35. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{39}H_{42}F_3N_3O_6P$ 724.2760, found 724.2759.

4.1.14.6. Diethyl ({3-[2-({1-[(2-phenyl-1H-1,3-benzodiazol-6-yl) methyl]piperidin-4-yl}oxy)phenoxymethyl]-phenyl}methyl)phosphonate (16a). The title compound was obtained following the general procedure 5 from benzimidazole **14a** (22 mg, 1 eq., 0.099 mmol), $SOCl_2$ (210 μL, 29 eq., 2.87 mmol) and then compound **11b** (43 mg, 1 eq., 0.099 mmol), diisopropylethylamine (90 μL, 5.3 eq., 0.53 mmol) in ACN (3 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **16a** was obtained as an amorphous creamy solid. (24 mg, 38%) ^{1}H NMR (400 MHz, MeOD) δ 8.09 (d, J = 7.2 Hz, 2H, H^{Ar}), 7.57 (m, 5H, H^{Ar}), 7.33 (m, 5H, H^{Ar}), 7.03 (m, 2H, H^{Ar}), 6.91 (m, 2H, H^{Ar}), 5.06 (s, 2H, CH_2—O), 4.43 (s, 1H, H^4 pip), 3.98 (ddq, J = 8.0, 7.0, 1.0 Hz, 4H, CH_2—O—P), 3.84 (s, 2H, CH_2—N), 3.23 (d, J = 21.7 Hz, 2H, CH_2—P), 2.99 (bs, 2H, H^2, H^6 pip), 2.62 (bs, 2H, H^2, H^6 pip), 1.97 (m, 4H, H^3, H^5

pip), 1.20 (td, $J = 7.0$, 1.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 152.28 (C^{quat}), 150.16 (C^{quat}), 147.08 (C^{quat}), 137.88 (d, $J = 3.3$ Hz, C^{quat}), 131.61 (d, $J = 9.4$ Hz, C^{quat}), 130.13 (C^{Ar}), 129.47 (C^{quat}), 129.13 (d, $J = 6.6$ Hz, C^{Ar}), 128.78 (C^{Ar}), 128.73 (d, $J = 7.0$ Hz, C^{Ar}), 128.32 (d, $J = 3.4$ Hz, C^{Ar}), 126.44 (C^{Ar}), 125.92 (d, $J = 3.7$ Hz, C^{Ar}), 122.36 (C^{Ar}), 121.44 (C^{Ar}), 118.51 (C^{Ar}), 115.19 (C^{Ar}), 70.54 (CH_2—O), 62.35 (d, $J = 6.9$ Hz, CH_2—O—P), 62.21 (CH_2—N), 49.29 (C^2, C^6 pip), 32.32 (d, $J = 137.7$Hz, CH_2—P), 29.41(C^3, C^5 pip), 15.25 (d, $J = 6.0$ Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.36 HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{37}H_{43}N_3O_5P$ 640.2941, found 640.2938.

4.1.14.7. Diethyl {[3-(2-{[1-({2-[4-(trifluoromethyl)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)-piperidin-4-yl]oxy}-phenoxymethyl) phenyl]methyl}phosphonate (16b). The title compound was obtained following the general procedure 5 from benzimidazole **14b** (34 mg, 1 eq., 0.115 mmol), $SOCl_2$ (243 μL, 29 eq., 3.35 mmol) and then compound **11b** (50 mg, 1 eq., 0.115 mmol), diisopropylethylamine (100 μL, 5.3 eq., 0.59 mmol) in ACN (3 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **16b** was obtained as an amorphous creamy solid. (19 mg, 22%) ^1H NMR (400 MHz, MeOD) δ 8.45 (bs, 1H, H^{Ar}), 8.36 (d, $J = 7.8$ Hz, 1H, H^{Ar}), 7.83 (m, 1H, H^{Ar}), 7.78 (m, 1H, H^{Ar}), 7.63 (m, 2H, H^{Ar}), 7.42 (bs, 1H, H^{Ar}), 7.32 (m, 4H, H^{Ar}), 7.02 (m, 2H, H^{Ar}), 6.91 (m, 2H, H^{Ar}), 5.06 (s, 2H, CH_2—O), 4.42 (d, $J = 7.4$ Hz, 1H, H^4 pip), 3.99 (m, 4H, CH_2—O—P), 3.80 (s, 2H, CH_2—N), 3.25 (d, $J = 21.7$ Hz, 2H, CH_2—P), 2.94 (bs, 2H, H^2, H^6 pip), 2.55 (bs, 2H, H^2, H^6 pip), 1.96 (m, 4H, H^3, H^5 pip), 1.22 (t, $J = 7.1$ Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 150.87 (C^{quat}), 150.11 (C^{quat}), 147.19 (C^{quat}), 137.94 (d, $J = 3.0$ Hz, C^{quat}), 131.58 (d, $J = 9.5$ Hz, C^{quat}), 131.39 (C^{quat}), 131.06 (C^{quat}), 130.63 (C^{quat}), 129.83 (C^{Ar}), 129.82 (C^{Ar}), 129.77 (C^{Ar}), 129.09 (d, $J = 6.8$ Hz, C^{Ar}), 128.71 (d, $J = 6.9$ Hz, C^{Ar}), 128.30 (d, $J = 2.8$ Hz, C^{Ar}), 126.40 (C^{Ar}), 125.89 (d, $J = 3.7$ Hz, C^{Ar}), 125.36 (C^{Ar}), 123.06 (C^{Ar}), 122.23 (C^{Ar}), 121.45 (C^{Ar}), 118.38 (C^{Ar}), 115.28 (C^{Ar}), 70.56 (CH_2—O), 62.38 (CH_2—N), 62.34 (d, $J = 6.9$ Hz, CH_2—O—P), 49.43 (C^2, C^6 pip), 32.28 (d, $J = 138.1$Hz, CH_2—P), 29.75 (C^3, C^5 pip), 15.26 (d, $J = 6.0$ Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.36 ^{19}F NMR (376 MHz, MeOD) δ −64.31. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{39}H_{42}F_3N_3O_5P$ 708.2811, found 708.2808.

4.1.14.8. Diethyl {[3-(2-{[1-({2-[3-(trifluoromethyl)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]-oxy}phenoxymethyl)phenyl] methyl}phosphonate (16c). The title compound was obtained following the general procedure 5 from benzimidazole **14c** (34 mg, 1 eq., 0.115 mmol), $SOCl_2$ (243 μL, 29 eq., 3.35 mmol) and then compound **11b** (50 mg, 1 eq., 0.115 mmol), diisopropylethylamine (100 μL, 5.3 eq., 0.59 mmol) in ACN (3 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **16c** was obtained as an amorphous creamy solid. (20 mg, 24%) ^1H NMR (400 MHz, MeOD) δ 8.40 (bs, 1H, H^{Ar}), 8.31 (d, $J = 7.9$ Hz, 1H, H^{Ar}), 7.67 (m, 4H, H^{Ar}), 7.28 (m, 5H, H^{Ar}), 6.90 (m, 4H, H^{Ar}), 5.01 (s, 2H, CH_2—O), 4.36 (s, 1H, H^4 pip), 3.94 (ddq, $J = 8.0$, 7.1, 1.0 Hz, 4H, CH_2—O—P), 3.71 (s, 2H, CH_2—N), 3.19 (d, $J = 21.7$ Hz, 2H, CH_2—P), 2.86 (s, 2H, H^2, H^6 pip), 2.44 (d, $J = 8.7$ Hz, 2H, H^2, H^6 pip), 1.91 (dd, $J = 12.9$, 8.4 Hz, 4H, H^3, H^5 pip), 1.16 (dt, $J = 7.1$, 1.0 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 150.79 (C^{quat}), 150.07 (C^{quat}), 147.25 (C^{quat}), 137.94 (d, $J = 3.2$ Hz, C^{quat}), 131.57 (d, $J = 9.4$ Hz, C^{quat}), 131.38 (C^{quat}), 131.06 (C^{quat}), 130.65 (C^{quat}), 129.81 (C^{Ar}), 129.79 (C^{Ar}), 129.07 (d, $J = 6.7$ Hz, C^{Ar}), 128.70 (d, $J = 6.7$ Hz, C^{Ar}), 128.29 (d, $J = 3.1$ Hz, C^{Ar}), 126.37 (C^{Ar}), 125.88 (d, $J = 3.6$ Hz, C^{Ar}), 123.08 (C^{Ar}), 122.15 (C^{Ar}), 121.45 (C^{Ar}), 118.31 (C^{Ar}), 115.31 (C^{Ar}), 70.57 (CH_2—O), 62.53(CH_2—N), 62.34 (d, $J = 7.0$ Hz, CH_2—O—P), 49.56(C^2, C^6 pip), 32.29 (d, $J = 137.6$Hz, CH_2—P), 29.89 (C^3, C^5 pip), 15.29, 15.26 (d, $J = 6.0$ Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.36.^{19}F NMR (376 MHz, MeOD) δ −64.31. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{39}H_{42}F_3N_3O_5P$ 708.2811, found 708.2807.

4.1.14.9. Diethyl {[3-(2-{[1-({2-[4-(trifluoromethoxy)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)-piperidin-4-yl]oxy}-phenoxymethyl) phenyl]methyl}phosphonate (16d). The title compound was obtained following the general procedure 5 from benzimidazole **14d** (37 mg, 1 eq., 0.12 mmol), $SOCl_2$ (246 μL, 29 eq., 3.34 mmol) and then compound **11b** (50 mg, 1 eq., 0.12 mmol), diisopropylethylamine (104 μL, 5.3 eq., 0.64 mmol) in ACN (3 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **16d** was obtained as an amorphous creamy solid. (19 mg, 31%) ^1H NMR (400 MHz, MeOD) δ 8.20 (d, $J = 8.8$ Hz, 2H, H^{Ar}), 7.63 (s, 2H, H^{Ar}), 7.39 (m, 7H, H^{Ar}), 7.03 (m, 2H, H^{Ar}), 6.92 (m, 2H, H^{Ar}), 5.07 (s, 2H, CH_2—O), 4.44 (s, 1H, H^4 pip), 3.99 (dqd, $J = 7.8$, 6.8, 3.0 Hz, 4H, CH_2—O—P), 3.83 (s, 2H, CH_2—N), 3.25 (d, $J = 21.6$ Hz, 2H, CH_2—P), 2.98 (bs, 2H, H^2, H^6 pip), 2.60 (bs, 2H, H^2, H^6 pip), 2.06−1.84 (m, 4H, H^3, H^5 pip), 1.22 (t, $J = 6.8$ Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 151.43 (C^{quat}), 151.25 (C^{quat}), 150.14 (C^{quat}), 147.14 (C^{quat}), 137.91 (d, $J = 3.3$ Hz, C^{quat}), 131.60 (d, $J = 9.1$ Hz, C^{quat}), 129.10 (d, $J = 6.4$ Hz, C^{Ar}), 128.72 (d, $J = 6.5$ Hz, C^{Ar}), 128.60 (C^{Ar}), 128.32 (C^{Ar}), 128.30 (d, $J = 2.7$ Hz, C^{Ar}), 125.90 (d, $J = 3.7$ Hz, C^{Ar}), 122.31 (C^{Ar}), 121.45 (C^{Ar}), 121.20 (C^{Ar}), 118.46 (C^{Ar}), 115.24 (C^{Ar}), 70.55 (CH_2—O), 62.35 (d, $J = 6.9$ Hz, CH_2—O—P), 62.32 (CH_2—N), 49.34 (C^2, C^6 pip), 32.32 (d, $J = 137.2$ Hz, CH_2—P), 29.57 (C^3, C^5 pip), 15.25 (d, $J = 6.1$ Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.36 ^{19}F NMR (376 MHz, MeOD) δ −57.38 HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{39}H_{42}F_3N_3O_6P$ 724.2760, found 724.2755.

4.1.14.10. Diethyl {[3-(2-{[1-({2-[3-(trifluoromethoxy)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]oxy}phenoxymethyl) phenyl]methyl}phosphonate (16e). The title compound was obtained following the general procedure 5 from benzimidazole **14e** (27 mg, 1 eq., 0.088 mmol), $SOCl_2$ (186 μL, 29 eq., 2.56 mmol) and then compound **11b** (38 mg, 1 eq., 0.088 mmol), diisopropylethylamine (79 μL, 5.3 eq., 0.47 mmol) in ACN (2 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **16e** was obtained as an amorphous creamy solid. (22 mg, 35%) ^1H NMR (400 MHz, MeOD) δ 8.06 (ddd, $J = 7.90$, 1.6, 1.0 Hz, 1H, H^{Ar}), 8.01 (s, 1H, H^{Ar}), 7.61 (q, $J = 8.2$ Hz, 3H, H^{Ar}), 7.33 (m, 6H, H^{Ar}), 6.98 (m, 2H, H^{Ar}), 6.88 (m, 2H, H^{Ar}), 5.01 (s, 2H, CH_2—O), 4.39 (s, 1H, H^4 pip), 3.94 (dqd, $J = 8.2$, 7.0, 1.1 Hz, 4H, CH_2—O—P), 3.80 (s, 2H, CH_2—N), 3.20 (d, $J = 21.7$ Hz, 2H, CH_2—P), 2.95 (s, 2H, H^2, H^6 pip), 2.57 (s, 2H, H^2, H^6 pip), 1.91 (s, 4H, H^3, H^5 pip), 1.21−1.10 (dt, $J = 7.0$, 0.4 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 150.93 (C^{quat}), 150.15 (C^{quat}), 149.73 (C^{quat}), 147.09 (C^{quat}), 137.88 (d, $J = 3.2$ Hz, C^{quat}), 131.73 (C^{Ar}), 131.61 (d, $J = 9.3$ Hz, C^{quat}), 130.71 (C^{Quat}), 129.10 (d, $J = 6.5$ Hz, C^{Ar}), 128.72 (d, $J = 6.7$ Hz, C^{Ar}), 128.31 (d, $J = 3.2$ Hz, C^{Ar}), 125.90 (d, $J = 3.6$ Hz, C^{Ar}), 125.01 (C^{Ar}), 122.34 (C^{Ar}), 121.44 (C^{Ar}), 118.93 (C^{Ar}), 118.48 (C^{Ar}), 115.20 (C^{Ar}), 73.12 (C^4 pip), 70.53 (CH_2—O), 62.34 (d, $J = 6.9$ Hz, CH_2—O—P), 62.17 (CH_2—N), 49.24 (C^2, C^6 pip), 32.33 (d, $J = 137.5$ Hz, CH_2—P), 29.45 (C^3, C^5 pip), 15.25 (d, $J = 6.0$ Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.36.^{19}F NMR (376 MHz, MeOD) δ −59.38. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{39}H_{42}F_3N_3O_6P$ 724.2760, found 724.2757.

4.1.15. 2-(2-Bromophenoxy)tetrahydropyran (18)

Under inert atmosphere, dihydropyran (3.96 mL, 1.5 eq., 43.35 mmol) was added to a solution of 2-bromophenol **17** (3.35 mL, 1 eq., 28.90 mmol) in dichloromethane (35 mL). A catalytic amount of pyridinium p-toluenesulfonate (726 mg, 0.1 eq., 2.89 mmol) was then inserted in the flask, and the mixture was stirred at room temperature for 12 h. The volatiles were then removed under reduced pressure, and the residue was purified by silica gel column chromatography, eluting pure PE, to afford compound **18** as a colorless oil. (7.43 g, quant.), [47]. CAS: 57999-46-9.

4.1.16. tert-Butyl 4-[hydroxy-(2-tetrahydropyran-2-yloxyphenyl) methyl] piperidine-1-carboxylate (19)

Under Ar atmosphere, compound **18** (1 g, 1 eq., 3.89 mmol) was dissolved in THF (8 mL) and cooled at −78 °C. After stirring for 5 min, *n*-BuLi (2.5 M in hexane, 1.56 mL, 1 eq., 3.89 mmol) was added dropwise to the mixture and stirred 30 min. at −78 °C. A solution of 1-(*tert*-Butoxycarbonyl)-4-piperidinecarboxaldehyde (830 mg, 1 eq., 3.89 mmol) in THF (8 mL) was then slowly introduced by cannulation into the flask. After 10 min at −78 °C, the solution was then allowed to stir at room temperature for 3 h and quenched by a slow addition of water (10 mL). The aqueous phase was subsequently extracted three times with ethyl acetate (3 x 30 mL) and the resulted organic layers dried over $MgSO_4$ and concentrated under reduced pressure. The resulting product was then loaded onto a silica gel column and purified eluting Petroleum ether/Ethyl Acetate 8:2 to afford desired piperidine derivative **19** as white solid. (914 mg, 60%) ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.18 (m, 3H, H^{Ar}), 6.97 (dt, J = 7.3, 1.3 Hz, 1H, H^{Ar}), 5.40 (m, 1H, O−CH−O), 4.62 (dt, J = 12.2, 6.8 Hz, 1H, CH−OH), 4.07 (m, 2H, H^2, H^6), 3.81 (m, 1H, CH_2−O), 3.59 (m, 1H, CH_2−O), 2.54 (m, 3H, OH, H^2, H^6), 1.79 (m, 9H, CH_2, H^3, H^4, H^5) 1.42 (s, 9H, CH_3 Boc), 1.21 (m, 2H, H^3, H^5). HRMS (ESI): *m/z* $[M+H]^+$ calcd for $C_{22}H_{34}NO_5$392.2438 found 392.2433.

4.1.17. 1-(Benzyloxy)-2-bromobenzene (20)

To a solution of 2-bromophenol (**17**) (1.34 mL, 1 eq., 11.6 mmol) in DMF (20 mL), benzyl chloride (1.46 mL, 1.1 eq., 12.7 mmol) and potassium carbonate (3.19 g, 2 eq., 23.1 mmol) were added. The resulting mixture was stirred for 12 h at 70 °C. After evaporation of all volatiles, the residue was dissolved in a mixture of EtOAc (80 mL) and water (40 mL). The aqueous phase was extracted thrice with EtOAc (3 x 80 mL), the organic phases were washed three times with water (3 x 40 mL), three times with brine (3 x 40 mL), dried over MgSO4 and concentrated under reduced pressure. After a filtration on silica gel, eluting petroleum ether/ethyl acetate 8:2, the desired product **20** was obtained as a colorless oil. (3.1 g, quant.), [48]. CAS: 31575-75-4.

4.1.18. tert-butyl 4-{[2-(benzyloxy)phenyl](hydroxy)methyl} piperidine-1-carboxylate (21)

Under Ar atmosphere, bromobenzene **20** (3.36 g, 1.1 eq., 12.7 mmol) was dissolved in THF (19 mL) and cooled at −78 °C. After stirring for 10 min, *n*-BuLi (2.5 M in hexane, 5.03 mL, 1.1 eq.12.7 mmol) was added dropwise to the solution and stirred 30 min. at −78 °C. A mixture of 1-(*tert*-Butoxycarbonyl)-4-piperidinecarboxaldehyde (2.47 g, 1 eq., 11.6 mmol) in THF (19 mL) was then slowly introduced by cannulation into the flask. After 10 min at −78 °C, the solution was then allowed to stir at room temperature for 3 h and quenched by the slow addition of water (20 mL). The aqueous phase was subsequently extracted three times with ethyl acetate (3 x 80 mL) and the resulted organic layers dried over $MgSO_4$ and concentrated under reduced pressure. The resulting product was then loaded onto a silica gel column and purified eluting Petroleum ether/Ethyl Acetate 8:2 to afford desired piperidine derivative **21** as colorless crystals. (2.94 g, 65%). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.36 (m, 5H, H^{Ar}), 7.22 (m, 2H, H^{Ar}), 6.94 (m, 2H, H^{Ar}), 5.08 (s, 2H, $CH_{2benzylic}$), 4.61 (bs, 1H, CH−OH), 4.01 (m, 2H, H^2, H^4, H^6 pip), 2.53 (m, 3H, H^2, H^4, H^6 pip),1.59 (m, 2H, H^3, H^5 pip), 1.43 (s, 9H, CH_3 Boc), 1.15 (m, 2H, H^3, H^5 pip). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.77 (C═O), 154.86 (C^{quat}), 136.66 (C^{quat}), 131.05 (C^{quat}), 128.71 (C^{Ar}), 128.46 (C^{Ar}), 128.29 (C^{Ar}), 128.15 (C^{Ar}), 127.32 (C^{Ar}), 121.02 (C^{Ar}), 112.01 (C^{Ar}), 79.24 (C^{quat} Boc), 75.05 (CH−OH), 70.24 ($CH_{2benzylic}$) 43.93 (C^4 pip), 42.56 (C^2, C^6 pip), 28.74 (C^3, C^5 pip), 28.47 (CH_3 Boc), 28.25 (C^3, C^5 pip). HRMS-ESI (*m/z*) $[M+H]^+$ calcd for $C_{24}H_{32}NO_4$ 398.2332, found 398.2326.

4.1.19. tert-Butyl 4-{[2-(benzyloxy)phenyl](hydroxy)methyl} piperidine-1-carboxylate (22)

Method A: In an autoclave, piperidine derivative **21** (1 g, 1 eq., 2.55 mmol) was dissolved in ethyl acetate (43 mL). The solution and the atmosphere in the apparatus were degassed with argon before the addition of 10% Pd/C (500 mg). The solution and the atmosphere were again saturated with argon, and then the autoclave was sealed and filled with H_2 until a pressure of 7 bars. The reaction mixture was stirred for 24 h at room temperature, filtrated onto Celite®.The filtrate was evaporated, giving pure product **22** as colorless crystals. (733 mg, quant.) Method B: Under inert atmosphere, compound **19** (200 mg, 1 eq., 0.51 mmol) was dissolved in dichloromethane (4 mL). Triethylsilane (408 μL, 5 eq., 2.55 mmol) and TFA (196 μL, 5 eq., 2.55 mmol) were successively added to this solution, and stirred at room temperature for 1 h. The reaction was quenched with a saturated aqueous solution of $NaHCO_3$ (5 mL), extracted with EtOAc (3 x 10 mL), washed with brine (10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The product was then purified by a flash column chromatography (PE/EtOAc 95:5), affording desired product **22** as colorless crystals. (30 mg, 21%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (m, 2H, H^{Ar}), 6.78 (m, 2H, H^{Ar}), 5.71 (s, 1H, OH), 4.01 (bd,J = 8.2 Hz, 2H, H^2, H^6 pip), 4.01 (bt,J = 11.9 Hz, 2H, H^2, H^6 pip), 2.53 (d,J = 7.1 Hz, 2H, CH_2),1.69 (m, 1H, H^4 pip),1.59 (bd,J = 14.4 Hz, 2H, H^3, H^5 pip), 1.43 (s, 9H, CH_3 Boc), 1.15 (bd,J = 11.9, 4.1 Hz, 2H, H^3, H^5 pip). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.04 (C═O), 154.06 (C^{quat} −CH_2), 131.23 (C^{Ar}), 127.21 (C^{Ar}), 126.51 (C^{quat} −OH), 120.22 (C^{Ar}), 115.26 (C^{Ar}), 79.45 (C^{quat} Boc), 44.10 (C^4 pip), 36.88 (C^2, C^6 pip), 36.80 (CH_2), 31.98 (C^3, C^5 pip), 28.50 (CH_3 Boc). HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{17}H_{26}NO_3$ 292.1913, found 292.1906.

4.1.20. tert-Butyl 4-{[2-({4-[(diethoxyphosphoryl)methyl]phenyl} methoxy)phenyl] methyl}piperidine-1-carboxylate (23a)

In a 10−20 mL microwave vial, phosphonate derivative **2a** (1.32 mg, 1.5 eq., 4.12 mmol) was dissolved in DMA (20 mL). To this mixture were sequentially added potassium carbonate (760 mg, 2 eq., 5.49 mol), phenol **22** (800 mg, 1 eq., 2.45 mmol) and finally a catalytic amount of sodium iodide (few crystals). This reaction was stirred for 30 min at 140 °C under microwave irradiation, and the obtained solution was dissolved in water (20 mL) and diethyl ether (100 mL). The aqueous phase was then extracted twice ethyl acetate (2 x 50 mL), and the organic phases washed with water (5 x 40 mL), once with brine (40 mL), dried over $MgSO_4$ and evaporated. The residue was consequently purified by column chromatography (DCM/MeOH 99:1) to afford the intermediate **23a** as a white solid, which was directly engaged in the next step.

4.1.21. tert-Butyl 4-{[2-({3-[(diethoxyphosphoryl)methyl]phenyl} methoxy)phenyl] methyl}piperidine-1-carboxylate (23b)

In a 2−5 mL microwave vial, phosphonate derivative **2b** (331 mg, 1.5 eq., 1.03 mmol) was dissolved in DMA (5 mL). To this mixture were sequentially added potassium carbonate (190 mg, 2 eq., 1.37 mol), phenol **22** (200 mg, 1 eq., 0.69 mmol) and finally a catalytic amount of sodium iodide (few crystals). This reaction was stirred for 30 min at 140 °C under microwave irradiation, and the obtained solution was dissolved in water (10 mL) and ethyl acetate (50 mL). The aqueous phase was then extracted with ethyl acetate (3 x 30 mL), and the organic phases washed with water (5 x 10 mL), once with brine (10 mL), dried over $MgSO_4$ and evaporated. The residue was consequently purified by column chromatography (DCM/MeOH 99:1) to afford **23b** compound as a colorless oil. (330 mg, 91%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (m, 4H, H^{Ar}), 7.07 (m, 2H, H^{Ar}), 6.82 (m, 2H, H^{Ar}), 5.01 (s, 2H, CH_2−O), 3.96 (m, 6H, CH_2−O−P, H^2, H^6 pip), 3.10 (d, J = 21.6 Hz, 2H, CH_2−P), 2.55 (d, J = 7.1 Hz, 4H, CH_2−CH,H^2, H^6 pip), 1.73 (m, 1H, H^4 pip), 1.56

(bd,J = 14.4 Hz, 2H, H^3, H^5 pip), 1.39 (s, 9H, CH_3 Boc), 1.17 (m, 8H, CH_3 Phosph, H^3, H^5 pip). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.58 (C^{quat} −CH_2), 154.86 (C=O), 137.79 (d, J = 3.0 Hz, C^{quat}), 131.90 (d, J = 9.0 Hz, C^{quat}), 131.01 (C^{Ar}), 129.17 (d, J = 6.7 Hz, C^{Ar}), 129.04 (C^{quat}), 128.73 (d, J = 3.4 Hz, C^{Ar}), 128.35 (d, J = 6.6 Hz, C^{Ar}), 127.18 (C^{Ar}), 125.51 (d, J = 3.6 Hz, C^{Ar}), 120.48 (C^{Ar}), 111.74 (C^{Ar}), 79.11 (C^{quat} Boc), 69.58 (CH_2−O), 62.15 (d, J = 6.9 Hz, CH_2−O−P), 44.28(C^2, C^6 pip), 37.34 (CH_2), 36.66 (C^4 pip), 33.82 (d, J = 138.0Hz, CH_2−P), 32.15 (C^3, C^5 pip), 28.46 (CH_3 Boc), 16.37 (d, J = 6.7 Hz, CH_3) ^{31}P NMR (162 MHz, MeOD) δ 26.22. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{29}H_{43}NO_6P$ 532.2828, found 532.2819.

4.1.21.1. Diethyl ({4-[2-(piperidin-4-ylmethyl)phenoxymethyl] phenyl} methyl)phosphonate (24a). Following general procedure 2, trifluoroacetic acid (18.9 mL, 100 eq., 245 mmol) was added to a solution of **23a** in DCM (15 mL). Pure compound **24a** was obtained after flash column chromatography (DCM/MeOH 95:5), affording desired product as a light brown solid. (356 mg, 88%) ^{1}H NMR (400 MHz, MeOD) δ 7.43 (d, J = 8.3 Hz, 2H, H^{Ar}), 7.34 (dd, J = 8.0, 2.4 Hz, 2H, H^{Ar}), 7.15 (m, 2H, H^{Ar}), 7.01 (dd, J = 8.0 Hz, 1H, H^{Ar}), 6.88 (d, J = 7.2 Hz, 1H, H^{Ar}), 5.10 (s, 2H, CH_2−O), 4.04 (dq, J = 7.3, 7.2 Hz, 4H, CH_2−O−P), 3.35 (under MeOD peak, m, 2H, H^2, H^6 pip), 3.26 (under MeOD peak, 2H, CH_2−P), 2.86 (dt, J = 12.5, 2.7 Hz, 2H, H^2, H^6 pip), 2.66 (d, J = 7.0 Hz, 2H, CH_2), 1.93 (m, 1H, H^4 pip), 1.82 (bd, J = 15.4 Hz, 2H, H^3, H^5 pip), 1.42 (dq, J = 12.4, 3.4 Hz, 2H, H^3, H^5 pip), 1.26 (t, J = 7.2 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 156.65(C^{quat} −CH_2), 136.36 (d, J = 3.9 Hz, C^{quat}), 131.03 (d, J = 9.4 Hz, C ^{quat}), 130.60(C^{Ar}), 129.76(d, J = 6.8 Hz, C^{Ar}), 127.62(C^{Ar}), 127.38(C^{Ar}), 127.32 (C^{Ar}), 120.44(C^{Ar}), 120.35(C^{Ar}), 111.87(C^{Ar}), 69.36(CH_2−O), 63.33 (d, J = 7.1 Hz, CH_2−O−P), 43.88 (C^2, C^6 pip), 36.09(CH_2), 34.35(C^4 pip), 31.96(d, J = 138.2 Hz, CH_2−P), 28.56 (C^3, C^5 pip), 15.24(d, J = 6.2 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.49. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{24}H_{35}NO_4P$ 432.2304, found 432.2306.

4.1.21.2. Diethyl ({3-[2-(piperidin-4-ylmethyl)phenoxymethyl] phenyl} methyl)phosphonate (24b). Following general procedure 2, trifluoroacetic acid (4.5 mL, 100 eq., 58.3 mmol) was added to a solution of compound **23b** (310 mg, 1 eq., 0.58 mmol) in DCM (9 mL). Pure compound **24b** was obtained after flash column chromatography (DCM/MeOH 95:5), affording desired product colorless oil. (235 mg, 94%) ^{1}H NMR (400 MHz, MeOD) δ 7.39 (s, 1H, H^{Ar}), 7.26 (m, 3H, H^{Ar}), 7.11 (dt, J = 7.6, 1.6 Hz, 1H, H^{Ar}), 7.06 (dd, J = 7.6, 1.6 Hz, 1H, H^{Ar}), 6.93 (d, J = 8.1 Hz, 1H, H^{Ar}), 6.83 (dt, J = 7.5, 0.7 Hz, 1H, H^{Ar}), 5.01 (s, 2H, CH_2−O), 3.96 (m, 4H, CH_2−O−P), 3.27 (m, 2H, H^2, H^6 pip), 3.21 (d, J = 21.6 Hz, 2H, CH_2−P), 2.80 (dt, J = 12.9, 2.3 Hz, 2H, H^2, H^6 pip), 2.61 (d, J = 7.1 Hz, 2H, CH_2), 1.86 (m, 1H, H^4 pip), 1.75 (bd, J = 14.1 Hz, 2H, H^3, H^5 pip), 1.45 (dq, J = 13.0, 10.9 Hz, 2H, H^3, H^5 pip), 1.17 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 157.91 (C^{quat} −CH_2), 139.20 (d, J = 3.2 Hz, C^{quat}), 133.95 (d, J = 9.3 Hz, C ^{quat}), 131.97(C^{Ar}), 130.47 (d, J = 6.7 Hz, C^{Ar}), 129.82 (d, J = 6.6 Hz, C^{Ar}), 129.74 (d, J = 3.1 Hz, C^{Ar}), 129.02(C^{quat}), 128.72(C^{Ar}), 127.05 (d, J = 3.7 Hz, C^{Ar}), 121.76(C^{Ar}), 113.04(C^{Ar}), 70.61(CH_2−O), 63.79 (d, J = 6.9 Hz, CH_2−O−P), 45.11(C^2, C^6 pip), 37.31 (CH_2), 35.84 (C^4 pip), 33.54 (d, J = 138.0Hz, CH_2−P), 29.73 (C^3, C^5 pip), 16.62 (d, J = 6.1 Hz, CH_3) ^{31}P NMR (162 MHz, MeOD) δ 27.01. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{24}H_{35}NO_4P$ 432.2304, found 432.2299.

4.1.21.3. Diethyl ({4-[2-({1-[(2-phenyl-1H-1,3-benzodiazol-6-yl) methyl]piperidin-4-yl}methyl)phenoxy-methyl]phenyl}-methyl)phosphonate (25a). The title compound **25a** was obtained following the general procedure 5 from benzimidazole **14a** (26 mg, 1 eq., 0.12 mmol), $SOCl_2$ (246 μL, 29 eq., 3.37 mmol) and then compound **24a** (50 mg, 1 eq., 0.12 mmol), diisopropylethylamine (105 μL, 5.3 eq., 0.61 mmol) in ACN (3 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **25a** was obtained as an amorphous creamy solid. (13 mg, 18%) ^{1}H NMR (400 MHz, MeOD) δ 8.11 (dd, J = 8.0, 2.0 Hz, 2H, H^{Ar}), 7.65 (m, 2H, H^{Ar}), 7.57 (m, 3H, H^{Ar}), 7.42 (d, J = 7.8 Hz, 2H, H^{Ar}), 7.33 (m, 3H, H^{Ar}), 7.16 (dt, J = 8.4, 1.6 Hz, 1H), 7.10 (dd, J = 7.3, 1.6 Hz, 1H, H^{Ar}), 6.98 (d, J = 8.4 Hz, 1H, H^{Ar}), 6.86 (t, J = 7.4 Hz, 1H, H^{Ar}), 5.08 (s, 2H, CH_2−O), 4.02 (m, 6H, CH_2−O−P, CH_2−N), 3.25 (d, J = 21.2 Hz, 2H, CH_2−P), 3.18 (bd, J = 12.1 Hz, 2H, H^2, H^6 pip), 2.64 (d, J = 6.7 Hz, 2H, CH_2), 2.47 (bs, 2H, H^2, H^6 pip), 1.82 (m, 1H, H^4 pip), 1.74 (bd, J = 14.5 Hz, 2H, H^3, H^5 pip), 1.45 (dq, J = 11.2, 2.7 Hz, 2H, H^3, H^5 pip), 1.24 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 156.62 (C^{quat} −CH_2), 153.11 (C^{quat}), 136.42 (d, J = 4.3 Hz, C^{quat}), 130.93 (d, J = 9.2 Hz, C^{quat}), 130.57 (C^{Ar}), 130.24 (C^{Ar}), 129.72 (d, J = 6.4 Hz, C^{Ar}), 129.38 (C^{Ar}), 128.81 (C^{Ar}), 128.30 (C^{Ar}), 127.26 (d, J = 3.2 Hz, C^{Ar}), 128.29 (C^{Ar}), 127.13 (C^{Ar}), 126.49 (C^{Ar}), 120.25 (C^{Ar}), 111.74 (C^{Ar}), 69.26 (CH_2−O), 62.30 (d, J = 7.3 Hz, CH_2−O−P), 61.89 (CH_2−N), 52.69 (C^2, C^6 pip), 36.24 (CH_2), 35.25 (C^4 pip), 31.88 (d, J = 138.1 Hz, CH_2−P), 30.13 (C^3, C^5 pip), 15.24 (d, J = 6.0 Hz, CH_3) ^{31}P NMR (162 MHz, MeOD) δ 27.27. HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{38}H_{45}N_3O_4P$ 638.3146, found 638.3144.

4.1.21.4. Diethyl ({3-[2-({1-[(2-phenyl-1H-1,3-benzodiazol-6-yl) methyl]piperidin-4-yl}methyl)phenoxymethyl]-phenyl}methyl)phosphonate (26a). The title compound **26a** was obtained following the general procedure 5 from benzimidazole **14a** (26 mg, 1 eq., 0.12 mmol), $SOCl_2$ (246 μL, 29 eq., 3.37 mmol) and then compound **24b** (50 mg, 1 eq., 0.12 mmol), diisopropylethylamine (105 μL, 5.3 eq., 0.61 mmol) in ACN (3 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **26a** was obtained as an amorphous creamy solid. (20 mg, 24%) ^{1}H NMR (400 MHz, MeOD) δ 8.10 (dd, J = 8.1, 2.7 Hz, 2H, H^{Ar}), 7.67 (s, 1H, H^{Ar}), 7.62 (d, J = 8.6 Hz, 1H, H^{Ar}), 7.54 (m, 3H, H^{Ar}), 7.42 (s, 1H, H^{Ar}), 7.32 (m, 3H, H^{Ar}), 7.24 (m, 1H, H^{Ar}), 7.15 (dt, J = 8.1, 1.6 Hz, 1H), 7.10 (dd, J = 7.4, 1.6 Hz, 1H, H^{Ar}), 6.98 (d, J = 7.8 Hz, 1H, H^{Ar}), 6.86 (td, J = 7.4, 0.8 Hz, 1H, H^{Ar}), 5.07 (s, 2H, CH_2−O), 3.96 (m, 6H, CH_2−O−P, CH_2−N), 3.24 (d, J = 21.6 Hz, 2H, CH_2−P), 3.18 (bd, J = 11.6 Hz, 2H, H^2, H^6 pip), 2.64 (d, J = 6.7 Hz, 2H, CH_2), 2.47 (s, 2H, H^2, H^6 pip), 1.80 (m, 1H, H^4 pip), 1.74 (bd, J = 14.9 Hz, 2H, H^3, H^5 pip), 1.45 (bq, J = 14.4 Hz, 2H, H^3, H^5 pip), 1.20 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 156.59 (C^{quat} −CH_2), 153.14 (C^{quat}), 149.72 (d, J = 2.0 Hz, C^{quat}), 138.00 (d, J = 3.4 Hz, C^{quat}), 131.64 (d, J = 9.2 Hz, C^{quat}), 130.59 (C^{Ar}), 130.25 (C^{Ar}), 129.37 (C^{Ar}), 129.04 (d, J = 6.7 Hz, C^{Ar}), 128.82 (C^{Ar}), 128.43 (d, J = 6.5 Hz, C^{Ar}), 128.34 (d, J = 3.2 Hz, C^{Ar}), 128.29 (C^{Ar}), 127.17 (C^{Ar}), 126.50 (C^{Ar}), 125.72 (C^{Ar}), 125.62 (d, J = 3.6 Hz, C^{Ar}), 120.31 (C^{Ar}), 111.65 (C^{Ar}), 69.23 (CH_2−O), 62.38 (d, J = 6.9 Hz, CH_2−O−P), 61.80 (CH_2−N), 52.70 (C^2, C^6 pip), 36.13 (CH_2), 35.38 (C^4 pip), 32.30 (d, J = 137.8 Hz, CH_2−P), 30.07 (C^3, C^5 pip), 15.26 (d, J = 6.0 Hz, CH_3) ^{31}P NMR (162 MHz, MeOD) δ 27.27. HRMS-ESI (m/z) $[M+H]^+$calcd for $C_{38}H_{45}N_3O_4P$ 638.3146, found 638.3141.

4.1.21.5. Diethyl {[3-(2-{[1-({2-[4-(trifluoromethyl)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]methyl}-phenoxymethyl) phenyl]methyl}phosphonate (26b). The title compound **26b** was obtained following the general procedure 5 from benzimidazole **14b** (60 mg, 1 eq., 0.20 mmol), $SOCl_2$ (452 μL, 29 eq., 6.20 mmol) and then compound **24b** (80 mg, 1 eq., 0.20 mmol), diisopropylethylamine (168 μL, 5.3 eq., 0.99 mmol) in ACN (4 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **26b** was obtained as an amorphous creamy solid. (24 mg, 19%) ^{1}H NMR (400 MHz, MeOD) δ 8.46 (s, 1H, H^{Ar}), 8.37 (d, J = 7.6 Hz, 1H, H^{Ar}), 7.75 (m, 4H, H^{Ar}), 7.33 (m, 5H, H^{Ar}), 7.14 (dd, J = 7.8, 1.8 Hz, 2H), 7.01 (dd, J = 8.3, 1.1 Hz, 1H, H^{Ar}), 6.88 (td, J = 7.4, 1.2 Hz, 1H, H^{Ar}), 5.08 (s, 2H, CH_2−O), 4.26 (s, 2H, CH_2−N), 3.99 (dqd,

J = 9.5, 7.1, 1.5 Hz, 4H, CH_2—O—P), 3.38 (under MeOD peak, m, 2H, H^2, H^6 pip), 3.22 (under MeOD peak, m, 2H, CH_2—P), 2.81 (t, J = 12.0 Hz, 2H, H^2, H^6 pip), 2.68 (d, J = 6.8 Hz, 2H, CH_2), 1.90 (m, 1H, H^4 pip),1.82 (bd, J = 13.5 Hz, 2H, H^3, H^5 pip), 1.55 (bt, J = 12.0 Hz, 2H, H^3, H^5 pip), 1.21 (td, J = 7.1, 0.6 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 156.60 (C^{quat} —CH_2), 151.84 (C^{quat}), 137.93 (d, J = 3.3 Hz, C^{quat}), 131.69 (d, J = 9.3 Hz, C^{quat}), 131.56 (C^{quat}), 130.57 (C^{Ar}), 130.36 (C^{Ar}), 129.94 (C^{Ar}), 129.90 (C^{quat}), 129.10 (d, J = 6.6 Hz, C^{Ar}), 128.45 (d, J = 6.5 Hz, C^{Ar}), 128.34 (d, J = 3.4 Hz, C^{Ar}), 127.88 (C^{Ar}), 127.35 (C^{Ar}), 125.68 (d, J = 3.9 Hz, C^{Ar}), 123.25 (C^{Ar}), 123.18 (C^{Ar}), 120.40 (C^{Ar}), 111.68 (C^{Ar}), 69.24 (CH_2—O), 62.41 (d, J = 6.9 Hz, CH_2—O—P), 60.93 (CH_2—N), 52.34 (C^2, C^6 pip), 35.70 (CH_2), 34.86 (C^4 pip),32.16 (d, J = 138.0Hz, CH_2—P), 29.22 (C^3, C^5 pip), 15.24 (d, J = 6.0 Hz, CH_3) ^{31}P NMR (162 MHz, MeOD) δ 27.22.^{19}F NMR (376 MHz, MeOD) δ −64.36. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{39}H_{44}F_3N_3O_4P$ 706.3022, found 706.3013.

4.1.21.6. Diethyl {[3-(2-{[1-({2-[3-(trifluoromethyl)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]methyl-}phenoxymethyl) phenyl]methyl}phosphonate (26c). The title compound **26c** was obtained following the general procedure 5 from benzimidazole **14c** (60 mg, 1 eq. 0.20 mmol), $SOCl_2$ (452 μL, 29 eq., 6.20 mmol) and then compound **24b** (80 mg, 1 eq., 0.20 mmol), diisopropylethylamine (168 μL, 5.3 eq., 0.99 mmol) in ACN (4 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **26c** was obtained as an amorphous creamy solid. (26 mg, 20%). ^{1}H NMR (400 MHz, MeOD) δ 8.46 (s, 1H, H^{Ar}), 8.37 (d, J = 7.8 Hz, 1H, H^{Ar}), 7.85 (d, J = 7.8 Hz, 1H, H^{Ar}), 7.79 (t, J = 7.8 Hz, 1H, H^{Ar}), 7.68 (m, 2H, H^{Ar}), 7.44 (s, 1H, H^{Ar}), 7.35 (m, 3H, H^{Ar}), 7.27 (m, 1H, H^{Ar}), 7.16 (dt, J = 8.3, 1.8 Hz, 1H, H^{Ar}), 7.11 (dd, J = 7.4, 1.5 Hz, 1H, H^{Ar}), 6.99 (d, J = 7.8 Hz, 1H, H^{Ar}), 6.87 (td, J = 7.5, 1.0 Hz, 1H, H^{Ar}), 5.09 (s, 2H, CH_2—O), 3.97 (m, 6H, CH_2—O—P, CH_2—N), 3.26 (d, J = 21.7 Hz, 2H, CH_2—P), 3.13 (bd, J = 11.3 Hz, 2H, H^2, H^6 pip), 2.65 (d, J = 6.8 Hz, 2H, CH_2), 2.38 (bs, 2H, H^2, H^6 pip), 1.78 (m, 1H, H^4 pip), 1.73 (bd, J = 13.4 Hz, 2H, H^3, H^5 pip), 1.44 (bq, J = 13.4 Hz, 2H, H^3, H^5 pip), 1.22 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 156.59 (C^{quat} —CH_2), 151.20 (C^{quat}), 138.03 (d, J = 3.4 Hz, C^{quat}), 131.63 (d, J = 9.1 Hz, C^{quat}), 131.42 (C^{quat}), 131.10 (C^{quat}), 130.59 (C^{Ar}), 130.55 (C^{Ar}), 129.87 (C^{Ar}), 129.83 (C^{Ar}), 129.02 (d, J = 6.4 Hz, C^{Ar}), 128.46 (C^{Ar}), 128.37 (d, J = 4.4 Hz, C^{Ar}), 128.33 (d, J = 3.2 Hz, C^{Ar}), 127.09 (C^{Ar}), 126.50 (C^{Ar}), 125.60 (d, J = 3.8 Hz, C^{Ar}), 125.34 (C^{Ar}), 123.12 (d, J = 4.1 Hz, C^{Ar}), 122.64 (C^{Ar}), 120.26 (C^{Ar}), 111.64 (C^{Ar}), 69.22 (CH_2—O), 62.36 (d, J = 7.0 Hz, CH_2—O—P), 62.12 (CH_2—N), 52.87 (C^2, C^6 pip), 36.33 (CH_2), 35.64 (C^4 pip), 32.30 (d, J = 137.2 Hz, CH_2—P), 30.46 (C^3, C^5 pip), 15.25 (d, J = 5.9 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.29.^{19}F NMR (376 MHz, MeOD) δ −64.34 HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{39}H_{44}F_3N_3O_4P$ 706.3022, found 706.3013.

4.1.21.7. Diethyl {[3-(2-{[1-({2-[4-(trifluoromethoxy)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]methyl}-phenoxymethyl) phenyl]methyl}phosphonate (26d). The title compound **26d** was obtained following the general procedure 5 from benzimidazole **14d** (60 mg, 1 eq., 0.20 mmol), $SOCl_2$ (452 μL, 29 eq., 6.20 mmol) and then compound **24b** (80 mg, 1 eq., 0.20 mmol), diisopropylethylamine (168 μL, 5.3 eq., 0.99 mmol) in ACN (4 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **26d** was obtained as an amorphous creamy solid. (31 mg, 24%) ^{1}H NMR (400 MHz, MeOD) δ 8.21 (d, J = 8.9 Hz, 2H, H^{Ar}), 7.67 (m, 2H, H^{Ar}), 7.49 (d, J = 9.0 Hz, 2H, H^{Ar}), 7.44 (s, 1H, H^{Ar}), 7.31 (m, 4H, H^{Ar}), 7.16 (dt, J = 8.3, 1.5 Hz, 1H), 7.12 (dd, J = 7.6, 1.6 Hz, 1H, H^{Ar}), 7.00 (d, J = 8.3 Hz, 1H, H^{Ar}), 6.88 (td, J = 7.5, 1.0 Hz, 1H, H^{Ar}), 5.10 (s, 2H, CH_2—O), 4.00 (m, 6H, CH_2—O—P, CH_2—N), 3.27 (d, J = 21.8 Hz, 2H, CH_2—P), 3.18 (bd, J = 11.8 Hz, 2H, H^2, H^6 pip), 2.67 (d, J = 6.8 Hz, 2H, CH_2), 2.47 (bs, 2H, H^2, H^6 pip), 1.81 (m, 1H, H^4 pip), 1.75 (bd, J = 15.4 Hz, 2H, H^3, H^5 pip), 1.46 (bq, J = 10.4 Hz, 2H, H^3, H^5 pip), 1.22 (t, J = 7.1 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 156.60 (C^{quat} —CH_2), 151.64 (C^{quat}), 150.52 (C^{quat}), 138.00 (d, J = 3.3 Hz, C^{quat}), 131.64 (d, J = 9.2 Hz, C^{quat}), 130.59 (C^{Ar}), 129.03 (d, J = 6.9 Hz, C^{Ar}), 128.50 (C^{Ar}), 128.43 (d, J = 6.6 Hz, C^{Ar}), 128.38 (C^{Ar}), 128.33 (d, J = 3.1 Hz, C^{Ar}), 127.15 (C^{Ar}), 125.62 (d, J = 3.7 Hz, C^{Ar}), 125.34 (C^{Ar}), 123.12 (d, J = 4.1 Hz, C^{Ar}), 121.74 (C^{Ar}), 121.25 (C^{Ar}), 120.29 (C^{Ar}), 119.20 (C^{Ar}), 111.65 (C^{Ar}), 69.23 (CH_2—O), 62.38 (d, J = 6.9 Hz, CH_2—O—P), 61.90 (CH_2—N), 52.87 (C^2, C^6 pip), 36.20 (CH_2), 35.45 (C^4 pip), 32.26 (d, J = 137.8 Hz, CH_2—P), 30.19 (C^3, C^5 pip), 15.25 (d, J = 6.0 Hz, CH_3) ^{31}P NMR (162 MHz, MeOD) δ 27.27.^{19}F NMR (376 MHz, MeOD) δ −59.39. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{39}H_{44}F_3N_3O_5P$ 722.2971, found 722.2965.

4.1.21.8. Diethyl {[3-(2-{[1-({2-[3-(trifluoromethoxy)phenyl]-1H-1,3-benzodiazol-6-yl}methyl)piperidin-4-yl]methyl}-phenoxymethyl) phenyl]methyl}phosphonate (26e). The title compound **26e** was obtained following the general procedure 5 from benzimidazole **14e** (60 mg, 1 eq., 0.20 mmol), $SOCl_2$ (452 μL, 29 eq., 6.20 mmol) and then compound **24b** (80 mg, 1 eq., 0.20 mmol), diisopropylethylamine (168 μL, 5.3 eq., 0.99 mmol) in ACN (4 mL). After purification by silica gel column chromatography, eluting DCM/MeOH 95:5, the clean compound **26e** was obtained as an amorphous creamy solid. (26 mg, 20%) ^{1}H NMR (400 MHz, MeOD) δ 8.10 (ddd, J = 7.8, 1.3, 0.6 Hz, 1H, H^{Ar}), 8.05 (s, 1H, H^{Ar}), 7.66 (m, 3H, H^{Ar}), 7.45 (m, 2H, H^{Ar}), 7.28 (m, 4H, H^{Ar}), 7.14 (dt, J = 7.4, 1.7 Hz, 1H), 7.10 (dd, J = 7.7, 1.7 Hz, 1H, H^{Ar}), 6.98 (d, J = 8.0 Hz, 1H, H^{Ar}), 6.86 (td, J = 7.4, 1.0 Hz, 1H, H^{Ar}), 5.07 (s, 2H, CH_2—O), 3.98 (m, 6H, CH_2—O—P, CH_2—N), 3.25 (d, J = 21.7 Hz, 2H, CH_2—P), 3.17 (bd, J = 10.2 Hz, 2H, H^2, H^6 pip), 2.64 (d, J = 6.7 Hz, 2H, CH_2), 2.47 (bt, J = 11.8 Hz, 2H, H^2, H^6 pip), 1.81 (m, 1H, H^4 pip), 1.74 (bd, J = 13.0 Hz, 2H, H^3, H^5 pip), 1.45 (bq, J = 13.3 Hz, 2H, H^3, H^5 pip), 1.20 (t, J = 6.8 Hz, 6H, CH_3). ^{13}C NMR (101 MHz, MeOD) δ 156.58 (C^{quat} —CH_2), 151.29 (C^{quat}), 149.72 (d, J = 2.0 Hz, C^{quat}), 137.99 (d, J = 3.3 Hz, C^{quat}), 131.63 (C^{quat}), 131.61 (d, J = 9.9 Hz, C^{quat}), 130.75 (C^{Ar}), 130.58 (C^{Ar}), 129.02 (d, J = 7.1 Hz, C^{Ar}), 128.41 (d, J = 6.9 Hz, C^{Ar}), 128.36 (C^{Ar}), 128.34 (d, J = 3.7 Hz, C^{Ar}), 127.15 (C^{Ar}), 125.61 (d, J = 3.7 Hz, C^{Ar}), 125.43 (C^{Ar}), 125.01 (C^{Ar}), 122.57 (C^{Ar}), 122.46 (C^{Ar}), 120.30 (C^{Ar}), 120.29 (C^{Ar}), 118.97 (C^{Ar}), 118.50 (C^{Ar}), 111.64 (C^{Ar}), 69.22 (CH_2—O), 62.36 (d, J = 6.9 Hz, CH_2—O—P), 61.80 (CH_2—N), 52.70 (C^2, C^6 pip), 36.14 (CH_2), 35.41 (C^4 pip), 32.23 (d, J = 137.7 Hz, CH_2—P), 30.12 (C^3, C^5 pip), 15.25 (d, J = 6.0 Hz, CH_3). ^{31}P NMR (162 MHz, MeOD) δ 27.27.^{19}F NMR (376 MHz, MeOD) δ −59.39. HRMS-ESI (*m/z*) $[M+H]^+$calcd for $C_{39}H_{44}F_3N_3O_5P$ 722.2971, found 722.2964.

4.2. Assays for anti-EBOV activity and cytotoxicity

All work with infectious virus was conducted in a BSL-4 laboratory at the Centers for Disease Control and Prevention. All laboratorians adhered to international practices appropriate for this biosafety level. Anti-EBOV activities were determined in Huh7 cells (Apath LLC), using recombinant virus expressing the fluorescent reporter protein ZsGreen (EBOV-ZsG), as described previously [49,50]. Briefly, 3000 Huh7 cells were seeded in each well of a 384-well plate. The following day, the cells were treated with varying concentrations of compound for 2 h, before infection with EBOV-ZsG virus at a multiplicity of infection of 0.3. The ZsGreen fluorescence was determined 3 days post-infection using a Synergy H1MD plate reader (BioTek). Cytotoxicity was determined on cells that received compound, but no virus, using CellTiter-Glo (Promega) according to the manufacturer's instructions. The 50% effective (EC_{50}) and 50% cytotoxic concentrations (CC_{50}) were determined using GraphPad Prism 7.0 (GraphPad Software) to fit a 4-parameter equation to semi-log plots of the concentration-response data. The Selectivity Index (SI) was calculated as the CC_{50}/EC_{50}.

Assays to test the inhibition of EBOV-GP-mediated entry were

performed with pseudotyped HIV particles as described [49]. Briefly, particles were generated by co-transfection of Lenti-X 293T cells with plasmids expressing EBOV-GP and a HIV luciferase reporter vector. Huh7 cells in 384-well plates were treated with varying concentrations of compound for 1 h at 37 °C, then the pseudotyped particles were added. Three days later, firefly luciferase activity was determined using the Bright-Glo luciferase assay system (Promega).

For filipin staining, HeLa cells were treated with the indicated concentrations of compounds, or the vehicle control at a final concentration of 0.1% (v/v). Test compounds were dissolved in DMSO, the positive control compound U18666A (Sigma-Aldrich) was dissolved in water. Twenty-four hours later, the cells were washed with Phosphate Buffered Saline (PBS) three times, then fixed with 3% paraformaldehyde for 1 h at room temperature. The cells were again washed three times with PBS, then incubated with 1.5 mg/mL of glycine for 10 min at room temperature. The cells were then stained with filipin (Sigma-Aldrich) at 0.5 mg/mL in PBS supplemented with 10% (v/v) fetal calf serum for 2 h at room temperature. The cells were washed another three times with PBS, then visualized on an Operetta High-Content Imaging System (PerkinElmer), using the DAPI filter set with a 20x objective.

Author contributions

LAA and VR initiated and headed the study. MB, EB synthesized all compounds. PC, MF and CFS generated the EBOV inhibition data. DW and AB generated the chemoinformatics data. PSR generated the filipin-staining data. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2021.113211.

Abbreviations used

BOC	Butyloxycarbonyl
Bs	Broad signal
DCM	Dichloromethane
DHP	3,4-Dihydropyran
DIPEA *N*	*N*-Diisoproylethylamine
DMA	Dimethylacetamide
DMF *N*	*N*-Dimethylformamide
DMSO	Dimethylsulfoxyde
EBOV	Ebola virus
FDA	US food and drug administration
HF	Hemorrhagic fever
GP	Glycoprotein
MW	Microwaves
NPC1	Niemann Pick C1 protein
PPTS	Pyridinium *p*-toluenesulfonate
RNA	Ribonucleic acid
r.t.	room temperature
SN	Nucleophilic substitution
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	thin layer chromatography

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