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Original article

Discovery of oxazole and triazole derivatives as potent and selective $S1P_1$ agonists through pharmacophore-guided design

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ABSTRACT

We have discovered a series of triazole/oxazole-containing 2-substituted 2-aminopropane-1,3-diol derivatives as potent and selective $S1P_1$ agonists (prodrugs) based on pharmacophore-guided rational design. Most compounds showed high affinity and selectivity for $S1P_1$ receptor. Compounds **19b**, **19d** and **19p** displayed clear dose responsiveness in the lymphocyte reduction model when administered orally at doses of 0.3, 1.0, 3.0 mg/kg with reduced effect on heart rate. These three compounds were also identified to have favorable pharmacokinetic properties.

1. Introduction

Sphingosine-1-phosphate (S1P, **1**, Fig. 1), a metabolite of sphingomyelin, is a bioactive lysophospholipid that regulates an array of physiological processes including angiogenesis, endothelial barrier enhancement, airway and blood vessel constriction, alveolar epithelial barrier disruption, lymphocyte trafficking, heart rate modulation, neurite extension, and bone homeostasis [1]. S1P affects these functions through interacting with a class A family of G-protein coupled receptors named $S1P_{1-5}$ receptors [2,3]. In recent years, the role of $S1P_1$ receptor in autoimmune diseases such as multiple sclerosis (MS) and lupus erythematosus has become the focus of intense research [4,5], partly inspired by the therapeutic development of FTY720 (fingolimod, **2**), which was approved by the FDA as the first orally active drug for the treatment of relapsing-remitting MS (RRMS) in 2010 [6,7]. FTY720 was identified as a prodrug, phosphorylated *in vivo* by sphingosine kinase 2 (SPHK2) to the active monophosphate ester (FTY720-P, **3**), which can activate four of five $S1P_1$ receptors ($S1P_{1,3-5}$) at nanomolar level [8,9]. FTY720-P was shown to elicit its immunosuppressive effect through activation of $S1P_1$ receptor, which leads to sequestration of lymphocytes in secondary lymphoid organs, preventing them from trafficking to lymphoid tissues and blocking the egress of mature thymocytes from the thymus [10].

On the other hand, the activation of $S1P_3$ receptor is thought to be responsible for the cardiovascular side effect, since treatment with FTY720 resulted in bradycardia in normal but not in $S1P_3$ knockout mice [11,12]. However, the intensive study of BAF312 (**4**), a dual $S1P_{1,5}$ agonist sparing $S1P_3$ activity, suggested species-dependent bradycardia and a dominant role of $S1P_1$ in mediating heart rate in humans via activation of the G protein-coupled inwardly-rectifying potassium (GIRK/IKAch) channel in cardiomyocytes [13–15]. Since there are other side-effects associated with $S1P_3$ agonism such as macular edema and decreased pulmonary function, numerous research groups still focus on discovering $S1P_3$-sparing $S1P_1$ agonists to improve the safety profile [16–19].

Pharmacophore modeling has been identified as a well-behaved approach to explore common chemical characteristics among a considerable number of structures with great diversity [20]. In this

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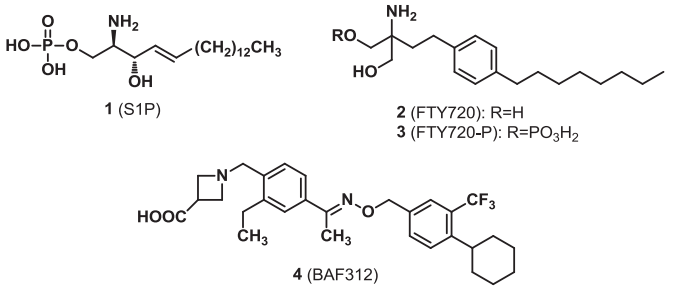


Fig. 1. Structures of S1P, FTY720, FTY720-P and BAF312.

study, a highly predictive 3D QSAR pharmacophore model based on a chemically diverse set of $S1P_1$ agonists was successfully developed, which could guide us to design desired molecules. On the basis of the best hypothesis, a series of 2-substituted 2-aminopropane-1,3-diol derivatives containing oxazole and triazole scaffold were designed and synthesized. In addition, the pharmacological effects such as $S1P_1$ and $S1P_3$ agonistic activity, peripheral blood lymphocyte lowering effects, influence on heart rate and pharmacokinetics (PK) profiles were also evaluated. Three compounds (**19b**, **19d** and **19p**) were found to have good *in vitro* and *in vivo* activities as well as favorable PK profiles, which had the potential for further development.

2. Results and discussion

2.1. Pharmacophore-guided molecular design

For the pharmacophore modeling studies, a set of 60 compounds was collected from the literature, which was divided into a training set of 36 compounds and a test set of 24 compounds [21]. The pharmacophore hypotheses were computed using HypoGen module implemented in Discovery Studio 3.0 and the top 10 hypotheses were generated (Table 1). The first hypothesis (Hypo1, Fig. 2) was the best pharmacophore hypothesis, characterized by the highest cost difference 61.601, lowest root mean square error 0.467 and the best correlation coefficient 0.975. The fixed cost, pharmacophore cost and null cost were 133.284, 137.367 and 198.968 respectively. The difference between null cost and fixed cost was large indicating that hypo1 has greater than 90% probability of correlating the data. An appropriate configuration cost value (11.069) was also obtained. Meanwhile, the predictive power and statistical significance of Hypo1 were validated using test set prediction and the Fischer randomization test [22]. The results demonstrated that we had successfully developed a reliable pharmacophore model with high predictivity.

Hypo1 consisted of spatial arrangement of five chemical features: one positive ionizable (PI), one negative ionizable (NI) and three hydrophobic (HY) features (Fig. 2). According to the known SAR, the structure of $S1P_1$ agonists can be divided into two parts: one is the "polar head group", consisting of a phosphate group (or carboxyl group), and an amino group, which can form salt bridge interaction with Arg120 and Glu121 in $S1P_1$ receptor; the other is the lipophilic chain which can form Van der Waals and π–π stacking interactions in a hydrophobic cavity of $S1P_1$ receptor. Our previous research revealed that structural rigidity of the lipophilic chain was required to increase $S1P_1$ agonistic potency and subtype selectivity [23]. Through analyzing the shape and composition of Hypo1, it was found that PI and NI features represented the "polar head group" and three linear aligned HY features represented the structural rigid lipophilic chain, which demonstrated that Hypo1 truly revealed the key characteristics of $S1P_1$ agonists.

On the basis of Hypo1, we designed a series of compounds containing the amino phosphate "polar head group" which mapped to the PI and NI features and the lipophilic chain with increased rigidity composed of aromatic rings which mapped to HY features (Fig. 3). A set of 1,2,3-triazole-containing 2-aminopropane-1,3-diol derivatives (prodrugs) were efficiently synthesized through Cu(I)-catalyzed 1,3-dipolar alkyne-azide cycloaddition (CuAAC), a key reaction in click chemistry which was widely used in drug design [24,25]. Triazole ring located in the middle of the lipophilic chain and served as a linker between two phenyl rings to enable lipophilic chain map well to HY features. Replacement of the triazole ring with oxazole ring provided another series of compounds which can also map Hypo1 well. For instance, compound **12a** and **20a** both had a high fitvalue (a measure of how well the ligand fits the pharmacophore) when mapped to Hypo1.

2.2. Chemistry

The preparation of 1,2,3-triazole-containing derivatives **11a**–**p** and their phosphates **12a**–**p** is shown in Scheme 1. Reacting 4-bromophenethyl bromide (**5**) with diethyl acetamidomalonate in the presence of sodium ethoxide produced **6**. Reduction of **6** with $NaBH_4/K_2HPO_4$ buffer gave diol **7**. Pd-catalyzed Sonogashira reaction was performed between **7** and trimethylsilylacetylene in the presence of $PdCl_2(PPh_3)_2$, CuI, PPh_3 and Et_3N to give **8** [26]. Treatment of **8** with K_2CO_3 provided terminal alkyne **9**. Anilines were converted to the corresponding phenyl azides using $TMSN_3$/*t*-BuONO and then reacted with **9** through a Cu(I)-catalyzed Huisgen azide–alkyne cycloaddition (CuAAC) using $CuSO_4$ and sodium ascorbate to afford triazoles **10a**–**j** [27]. In addition, CuAAC reaction of **9** with benzyl or aliphatic azides which were prepared from benzyl or aliphatic bromide with NaN_3 was carried out to furnish **10k**–**p** [28]. Hydrolysis of **10a**–**p** with NaOH followed by

Table 1
Results of pharmacophore hypotheses generated using training set for $S1P_1$ agonists.

Hypothesis	Total cost	Δcost[a]	RMS	Correlation	Features[b]
1	137.367	61.601	0.467	0.975	PI, NI, HY, HY, HY
2	138.383	60.585	0.520	0.968	PI, NI, HY, HY, HY
3	146.746	52.222	0.819	0.920	PI, NI, HY, HY
4	146.814	52.154	0.847	0.913	PI, NI, HY, HY
5	146.826	52.142	0.855	0.912	PI, NI, HY, HY
6	148.89	50.078	0.927	0.895	PI, NI, HY, HY, HY
7	149.097	49.871	0.920	0.897	PI, NI, HY, HY
8	149.488	49.48	0.903	0.901	PI, NI, HY, HY
9	150.466	48.502	0.948	0.890	PI, NI, HY, HY, HY
10	150.704	48.264	0.975	0.883	PI, NI, HY, HY, HY

[a] Cost difference = null cost-total cost. Null cost = 198.968. Fixed cost = 133.284. Configuration cost = 11.0694. All cost units are in bits.
[b] PI, positive ionizable; NI, negative ionizable; HY, hydrophobic.

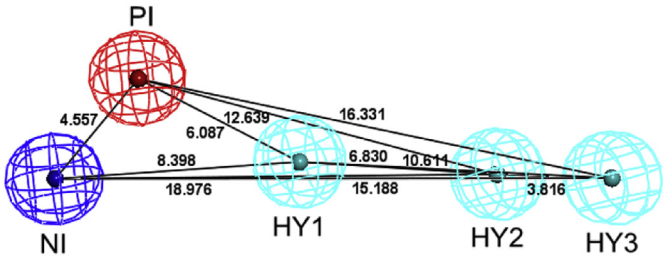


Fig. 2. Top scoring HypoGen pharmacophore Hypo1. The hypothesis features are color coded as follows: positive ionizable (PI), red; negative ionizable (NI), blue; hydrophobic (HY), light-blue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

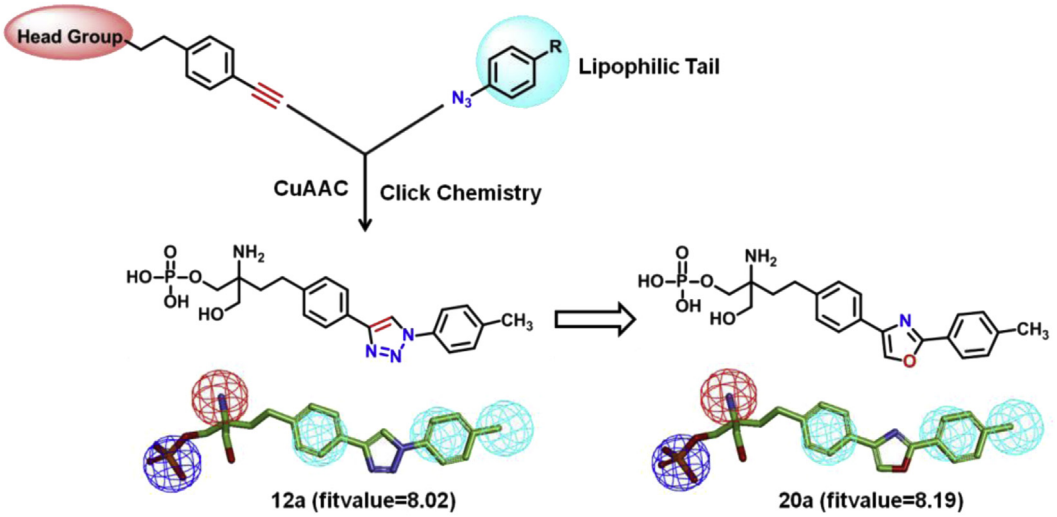
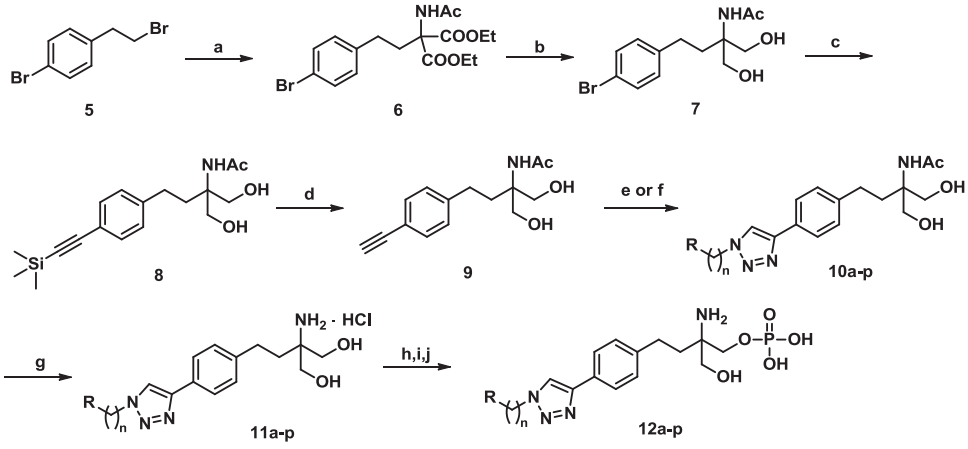


Fig. 3. Pharmacophore-guided design of $S1P_1$ agonists.



10-12a n=0, R=4-methylphenyl
10-12b n=0, R=4-methoxyphenyl
10-12c n=0, R=4-fluorophenyl
10-12d n=0, R=4-chlorophenyl
10-12e n=0, R=4-trifluoromethylphenyl
10-12f n=0, R=4-trifluoromethoxyphenyl
10-12g n=0, R=4-ethylphenyl
10-12h n=0, R=4-isopropylphenyl
10-12i n=0, R=4-*n*-propylphenyl
10-12j n=0, R=4-cyanophenyl
10-12k n=1, R=4-methylphenyl
10-12l n=1, R=4-fluorophenyl
10-12m n=1,R=4-chlorophenyl
10-12n n=1,R=4-trifluoromethylphenyl
10-12o n=1,R=4-ethylphenyl
10-12p n=0, R=*n*-pentyl

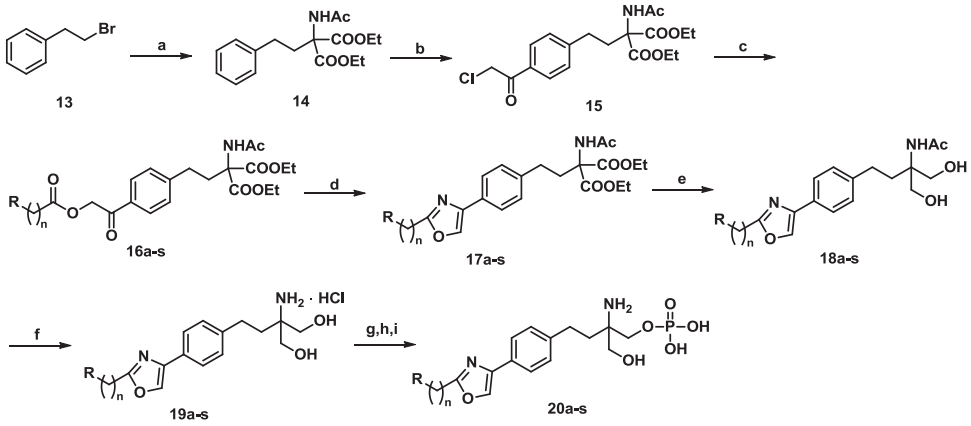
Scheme 1. Synthesis of 1,2,3-triazole derivatives. Reagents and conditions: (a) diethyl acetamidomalonate, sodium, absolute ethanol, 0 °C to reflux, 20 h; (b) $NaBH_4$, K_2HPO_4 buffer, EtOH, r.t., 12 h; (c) trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, CuI, PPh_3, Et_3N, THF, reflux, 12 h; (d) K_2CO_3, MeOH, r.t., 2 h; (e) aniline, $TMSN_3$, t-BuONO, $CuSO_4$, sodium ascorbate, Et_3N, CH_3CN, r.t.,12 h (compounds **10a**–**j**); (f) benzyl bromide, NaN_3, $CuSO_4$, sodium ascorbate, Et_3N, t-BuOH/H_2O, r.t.,12 h (compounds **10k**–**p**); (g) NaOH, MeOH, reflux, 8 h, then HCl–EtOH, r.t., 1 h; (h) CbzCl, $NaHCO_3$, ethyl acetate, r.t., 4 h; (i) TBPP, Ag_2O, Hex_4NI, CH_2Cl_2, r.t., 20 h; (j) TMSI, CH_2Cl_2, 0 °C, 1 h.

hydrochloric acid alcohol treatment afforded the hydrochloride of 2-aminopropane-1,3-diol prodrugs **11a**–**p**. The chemical synthesis of the phosphate was then carried out. After protection of the amino group of **11a**–**p** with CbzCl and $NaHCO_3$, phosphorylation using tetrabenzyl pyrophosphate (TBPP) was performed in the presence of Ag_2O and Hex_4NI to give dibenzyl phosphates [29]. Then both Cbz and benzyl groups were removed with TMSI to afford the desired phosphates (±) **12a**–**p**.

Scheme 2 illustrates the synthesis of oxazole-containing derivatives **19a**–**s** and their phosphates **20a**–**s**. Phenethyl bromide (**13**) was reacted with diethyl acetamidomalonate in the presence of sodium ethoxide to give **14**. Friedel–Crafts acylation of **14** with chloroacetyl chloride afforded **15**. Replacement of α-chloro of **15** with carboxylic acid gave esters **16a**–**s**. The oxazole ring was formed by the ring closing of **16a**–**s** with acetamide and $BF_3 \cdot Et_2O$ to furnish **17a**–**s** [30]. Reduction of **17a**–**s** with $NaBH_4/K_2HPO_4$ buffer provided diol **18a**–**s**. After hydrolysis and acidification, the hydrochloride of 2-aminopropane-1,3-diol prodrugs **19a**–**s** were obtained. The procedure of preparing phosphates (±) **20a**–**s** was the same as for the synthesis of phosphates (±) **12a**–**p**.

2.3. Biological evaluation

Since it is well accepted that the racemic phosphates can be used for *in vitro* evaluation [31], the agonistic activities of the target compounds **12a**–**p** and **20a**–**s** on human $S1P_1$ and $S1P_3$ receptors were evaluated using an HTRF-IP1 functional assay [32,33]. We first examined the SAR of triazole derivatives bearing a *p*-terphenyl type scaffold (n = 0) (Table 2). A series of electron-donating groups, electron-withdrawing groups and halogen atoms on the terminal



16-20a n=0, R=4-methylphenyl
16-20b n=0, R=4-methoxyphenyl
16-20c n=0, R=4-fluorophenyl
16-20d n=0, R=4-chlorophenyl
16-20e n=0, R=4-trifluoromethylphenyl
16-20f n=0, R=4-trifluoromethoxyphenyl
16-20g n=0, R=4-ethylphenyl
16-20h n=0, R=4-isopropylphenyl
16-20i n=0, R=4-*n*-propylphenyl
16-20j n=0, R=4-cyanophenyl
16-20k n=0, R=2-furanyl
16-20l n=0, R=2-thienyl
16-20m n=1, R=4-methylphenyl
16-20n n=1, R=4-methoxyphenyl
16-20o n=1,R=4-fluorophenyl
16-20p n=1,R=4-chlorophenyl
16-20q n=1,R=4-trifluoromethylphenyl
16-20r n=1, R=4-isopropylphenyl
16-20s n=0, R=*n*-pentyl

Scheme 2. Synthesis of oxazole derivatives. Reagents and conditions: (a) diethyl acetamidomalonate, sodium, absolute ethanol, 0 °C to reflux, 20 h; (b) chloroacetylchloride, $AlCl_3$, CH_2Cl_2, 0 °C to r.t., 5 h; (c) carboxylic acid, Et_3N, CH_3CN, reflux, 2 h; (d) acetamide, $BF_3{\cdot}Et_2O$, xylene, reflux, 40 h; (e) $NaBH_4$, K_2HPO_4 buffer, EtOH, r.t., 12 h; (f) NaOH, MeOH, reflux, 8 h, then HCl–EtOH, r.t., 1 h; (g) CbzCl, $NaHCO_3$, ethyl acetate, r.t., 4 h; (h) TBPP, Ag_2O, Hex_4NI, CH_2Cl_2, r.t., 20 h; (i)TMSI, CH_2Cl_2, 0 °C, 1 h.

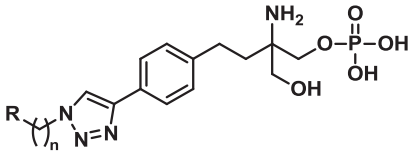
phenyl ring were investigated. Trifluoromethoxy substitution proved optimal in terms of potency with good $S1P_1$ selectivity over $S1P_3$ (**12f**). Compounds with chloro (**12d**), trifluoromethyl (**12e**), ethyl (**12g**), isopropyl (**12h**) and *n*-propyl (**12i**) substitutions also exhibited high $S1P_1$ agonistic activity. The 4-*n*-propylphenyl analogue (**12i**) showed best $S1P_1/S1P_3$ selectivity (247-fold). Decrease of $S1P_1$ agonistic activity was observed when methyl (**12a**), methoxy (**12b**), fluoro (**12c**) and cyano (**12j**) groups were introduced. Replacement of terminal phenyl ring with an aliphatic chain (**12p**) showed a moderate $S1P_1$ agonistic activity. In addition, a methylene group was inserted between the triazole ring and the terminal phenyl ring to explore the influence of molecular length and shape. As a result, most compounds showed a significant loss of $S1P_1$ potency (**12k**, **m**–**o**), presumably due to the change of molecular shape which could not fit the pharmacophore well. For example, **12k** just had a fitvalue of 5.58 and predicted EC_{50} of 1090 nM when mapped to Hypo1 (Fig. 4).

Replacement of the core triazole ring with an oxazole ring generally led to improved potency on the $S1P_1$ receptor (Table 3). We next studied the influence of substituents on the terminal phenyl ring of the oxazole derivatives. Methoxy substitution (**20b**) was optimal for $S1P_1$ potency and $S1P_1/S1P_3$ selectivity. Replacement of the methoxy group with methyl (**20a**), fluoro (**20c**), chloro (**20d**) and trifluoromethyl (**20e**) substitutions retained the potency. Compounds with alkyl substituents such as ethyl (**20g**), isopropyl (**20h**) and *n*-propyl (**20i**) also exhibited high $S1P_1$ agonistic activity. The cyano group (**20j**) reduced the potency. Replacement of the terminal phenyl ring with furanyl (**20k**), thienyl (**20l**) and *n*-pentyl group (**20s**) showed moderate $S1P_1$ agonistic activities. In contrast to the triazole derivatives, when a methylene group was introduced between the oxazole ring and the terminal phenyl ring ($n = 1$), only a slight decrease of $S1P_1$ agonistic activity was observed for methoxy (**20n**), fluoro (**20o**) and chloro (**20p**) substitutions, which indicated that the oxazole ring was a preferred linker in the lipophilic chain.

Based on these *in vitro* data, the prodrugs of three compounds (**19b**, **19d**, and **19p**) were evaluated in our pharmacodynamic (PD) lymphocyte reduction model. A clear dose response was established when the compounds were administered orally to SD rats at doses of 0.3, 1.0, and 3.0 mg/kg. Significant lymphocytes counts reduction was observed at 12 h postadministration (Fig. 5).

Furthermore, the pharmacokinetic (PK) profiles of **19b**, **19d**, **19p**, and their phosphates (**20b**, **20d**, **20p**) were evaluated after oral administration of prodrugs (**19b**, **19d**, and **19p**) at dose of 3 mg/kg, with FTY720 (**2**) and FTY720-P (**3**) as the positive control (Table 4). The phosphates were the predominant forms in blood, whose AUC and C_{max} were much larger than those of prodrugs. Compound **19b**

Table 2
SAR of triazole derivatives.[a]



No	*n*	R	EC_{50} (nM)	
			$hS1P_1$	$hS1P_3$
3	–	–	9.1	35.1
12a	0	4-Methylphenyl	111	1388
12b	0	4-Methoxyphenyl	126	3008
12c	0	4-Fluorophenyl	1626	>5000
12d	0	4-Chlorophenyl	42	1997
12e	0	4-Trifluoromethylphenyl	22	616
12f	0	4-Trifluoromethoxyphenyl	15	1219
12g	0	4-Ethylphenyl	53	1910
12h	0	4-Isopropylphenyl	29	496
12i	0	4-*n*-Propylphenyl	19	4689
12j	0	4-Cyanophenyl	257	1390
12k	1	4-Methylphenyl	1287	>5000
12l	1	4-Fluorophenyl	638	>5000
12m	1	4-Chlorophenyl	630	>5000
12n	1	4-Trifluoromethylphenyl	551	>5000
12o	1	4-Ethylphenyl	685	>5000
12p	0	*n*-Pentyl	93.9	1945

[a] EC_{50} is the mean of three experimental determinations.

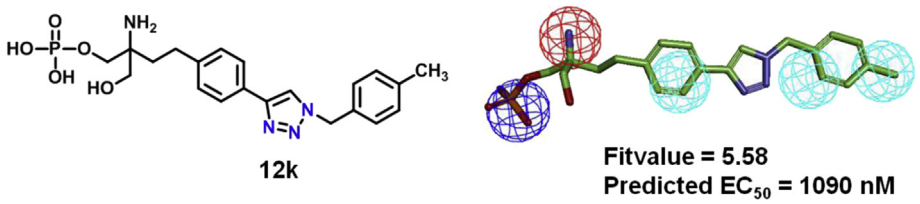


Fig. 4. Mapping of **12k** to Hypo1.

and its phosphate **20b** had relatively low blood concentration (C_{max}) and moderate half-life ($t_{1/2}$). Compounds **19d**, **19p**, and their phosphates (**20d**, **20p**) had longer half-life perhaps due to their low clearance. Incorporation with the lymphopenia activity, the PK/PD relationship of **19b** and its phosphate **20b** was analyzed (Fig. 6). After administration, the rapid increase in the compound concentration coincides with a remarkable decrease in lymphocyte counts. The time lymphocytes decreased maximumly (12 h) was later than the T_{max} (4–5 h). The PK profiles of **19b** and **20b** therefore showed a good correlation with the LC reductions in the rat. The result indicated that in spite of a low exposure, **19b** still exhibited excellent *in vivo* efficacy in reducing lymphocyte counts.

We next turned our attention to the effect of compounds **19b**, **19d**, and **19p** on heart rate following 10 mg/kg oral administration to SD rats. To our delight, unlike **2**, the compounds **19b**, **19d**, and **19p** did not show significant effect on heart rate, which demonstrated that the high $S1P_1/S1P_3$ selectivity helps to reduce the risk of cardiovascular side effects (Fig. 7).

3. Conclusion

In summary, a highly predictive 3D QSAR pharmacophore model based on chemically diverse set of $S1P_1$ agonists was successfully constructed. On the basis of the best hypothesis, a series of triazole-containing 2-substituted 2-aminopropane-1,3-diol derivatives (prodrugs) were designed and synthesized as potent and selective $S1P_1$ agonists. Replacement of triazole ring with oxazole ring provided another series of compounds with improved $S1P_1$ agonistic activities. The compounds **19b**, **19d**, and **19p** exhibited excellent dose response lymphopenia activities and reduced effect on heart rate. Ongoing work is to design and synthesize compounds with improved pharmacokinetic profiles while retaining $S1P_1$ potency and selectivity.

4. Experimental

4.1. Pharmacophore modeling

A set of 60 different compounds tested with the same assay (GTPγS binding assay) has been collected from different references considering their chemical structure diversity and wide coverage of activity range. The datasets are divided into a training set of 36 compounds and a test set of 24 compounds (Figs. S1, S2). The compounds were built using ChemBioDraw Ultra 12.0, and the conformational analysis of the molecules was performed using the poling algorithm within Discovery Studio 3.0. A maximum number of 255 conformations of each compound were selected with an energy constraint of 20 kcal/mol. The uncertainty value was set to 3.0, representing the ratio of the uncertainty range of measured biological activity against the actual activity for each compound. Default settings were used for other parameters. Taking into account the chemical nature of the compounds considered in this work, the following three features were selected to form the essential information in this hypothesis generation process: positive ionizable (PI), negative ionizable (NI), and hydrophobic (HY). Pharmacophores were then computed using HypoGen module implemented in Discovery Studio 3.0. The hypotheses generated were evaluated in terms of cost functions and other statistical parameters such as correlation coefficient and RMSD value. A meaningful pharmacophore hypothesis may result when the difference between null and fixed cost value is large: a value of 40–60 bits for a pharmacophore hypothesis may indicate that it has 75–90% probability of correlating the data. The configuration cost or entropy cost, which depends on the complexity of the pharmacophore hypothesis space, should have a value <17. The RMSD represents the quality of the correlation between the estimated and the actual activity data. The actual activity and estimated activity of each compound based on Hypo1 are listed in Table S1.

Test set prediction was used to validate the predictive power of Hypo1. Twenty-four test set compounds were mapped onto Hypo1 with a correlation coefficient of 0.951 (Table S2 and Fig. S3), suggesting that the Hypo1 not only fits for training set compounds but also for the external compounds. The statistical significance of Hypo1 was estimated using Fischer's randomization test. In this test, using CatScramble program, the experimental activities in the training set were scrambled randomly, and the resulting training set was used for a HypoGen run using the same features and parameters originated for Hypo1. This procedure was repeated 19 times. None of the outcome hypotheses has lower cost score than Hypo1 (Table S3 and Fig. S4). The result indicates that there is a 95% chance for the best hypothesis to represent a true correlation in the training set activity data.

4.2. Chemistry

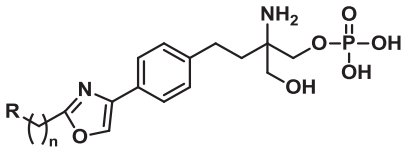
4.2.1. General experimental information

Melting points were determined on Yanaco MP-J3 microscope melting point apparatus. NMR spectra were recorded on Bruker AVIIIHD600, Bruker AVANCEIII400, Mercury-300 spectrometer. Chemical shifts are referenced to the residual solvent peak and reported in ppm (δ scale) and all coupling constant (J) values are given in Hz. The following multiplicity abbreviations are used: (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) multiplet, and (br) broad. ESI-HRMS data were measured on Thermo Exactive Orbitrap plus spectrometer. Flash column chromatography was performed on Biotage Isolera one. All the solvents and chemicals were obtained from commercial sources and used without further purification.

4.2.2. Preparation of diethyl 2-acetamido-2-(4-bromophenethyl) malonate (**6**)

Sodium (3.66 g, 159.1 mmol) was added to absolute EtOH (250 mL). After sodium dissolved completely, diethyl acetamidomalonate (36.2 g, 166.7 mmol) was added in portions at 0 °C. The solution was then allowed to return to room temperature and stirred for further 2 h. Then a solution of 1-bromo-4-(2-bromoethyl)benzene (**5**, 40 g, 151.5 mmol) in absolute EtOH (40 mL) was added. The mixture was heated at 80 °C for 20 h, then

Table 3
SAR of Oxazole Derivatives.[a]



No	*n*	R	EC_{50}(nM)	
			$hS1P_1$	$hS1P_3$
20a	0	4-Methylphenyl	12.8	1020
20b	0	4-Methoxyphenyl	7.6	1113
20c	0	4-Fluorophenyl	16.3	245
20d	0	4-Chlorophenyl	9.1	569
20e	0	4-Trifluoromethylphenyl	10.9	356
20f	0	4-Trifluoromethoxyphenyl	74	2110
20g	0	4-Ethylphenyl	25.4	416
20h	0	4-Isopropylphenyl	22.2	237
20i	0	4-*n*-Propylphenyl	42.4	844
20j	0	4-Cyanophenyl	147.6	2973
20k	0	2-Furanyl	69.5	>5000
20l	0	2-Thienyl	48.9	869
20m	1	4-Methylphenyl	108.2	>5000
20n	1	4-Methoxyphenyl	25.8	899.2
20o	1	4-Fluorophenyl	54.5	828
20p	1	4-Chlorophenyl	17.8	970
20q	1	4-Trifluoromethylphenyl	343	1391
20r	1	4-Isopropylphenyl	154	925
20s	0	*n*-Pentyl	64.1	1544

[a] EC_{50} is the mean of three experimental determinations.

filtered and concentrated. The residue was diluted with EtOAc (300 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (EtOAc/PE = 1:3) to afford compound **6** (20 g, 32.8% yield) as yellow solid. Mp: 35–38 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 8.4 Hz, 2H), 7.02 (d, J = 8.1 Hz, 2H), 6.76 (s, 1H), 4.25–4.16 (m, 4H), 2.69–2.63 (m, 2H), 2.46–2.41 (m, 2H), 1.99 (s, 3H), 1.25 (t, J = 6.9 Hz, 6H); MS (ESI) m/z 400.1 $(M+H)^+$.

4.2.3. Preparation of N-(4-(4-bromophenyl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl) acetamide (7)

To a solution of **6** (17 g, 42.5 mmol) in EtOH/THF (250 mL, 1:1) was added K_2HPO_4 (77 g, 339.8 mmol) buffer and $NaBH_4$ (9.7 g, 255 mmol), then stirred for 12 h at room temperature. The solution was poured slowly into a mixture of saturated aq. NH_4Cl and EtOAc

Table 4
Pharmacokinetic Parameters of **2**, **19b**, **19d**, **19p** and their phosphates (**3**, **20b**, **20d**, **20p**) after oral administration of 3 mg/kg of **2**, **19b**, **19d**, **19p** to SD rats.[a]

Compd	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0\text{-}t}$ (ng·h/mL)	CL/*F* (L/(h·kg))	V_{ss}/F (L/kg)
2	13.2	8.4	18.9	319	1630	208
3	384.6	6	25.2	4910	101	17.2
19b	9.51	4.7	5.2	73.4	41.5	300
20b	26.7	5.3	6.2	233	12.4	108
19d	28.5	6.7	18.8	900	2.2	60
20d	126	17.3	18.4	2940	0.7	19.7
19p	109	4	21.1	1700	1.5	44.1
20p	500	4	37.5	9780	0.2	9.6

[a] Data are expressed as mean ± SD of three animals.

(200 mL, 1:1). The aqueous phase was extracted with EtOAc (50 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 20:1) to afford compound **7** (10 g, 74.6% yield) as yellow solid. Mp: 113–114 °C; ^{1}H NMR (300 MHz, CD_3COCD_3) δ 7.40 (d, J = 8.4 Hz, 2H), 7.14 (d, J = 8.1 Hz, 2H), 4.60 (t, J = 6H, 2H), 3.72–3.57 (m, 4H), 2.64–2.58 (m, 2H), 2.03–1.92 (m, 2H); MS (ESI) m/z 316.1 $(M+H)^+$.

4.2.4. Preparation of N-(1-hydroxy-2-(hydroxymethyl)-4-(4-((trimethylsilyl)ethynyl) phenyl)butan-2-yl)acetamide (8)

To a solution of **7** (10 g, 31.6 mmol) in THF (160 mL) was added trimethylsilylacetylene (7.77 g, 79.1 mmol), $PdCl_2(PPh_3)_2$ (3.3 g, 4.74 mmol), CuI (0.9 g, 4.74 mmol), PPh_3 (1.66 g, 6.3 mmol) and Et_3N (158.1 mL, 1137.6 mmol). The mixture was heated at 70 °C under argon for 12 h, then filtered and concentrated. The residue was diluted with EtOAc (200 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 20:1) to afford compound **8** (7 g, 66.7% yield) as black solid. Mp: 115–117 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 8.1 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 5.93 (s, 1H), 3.84 (d, J = 11.4 Hz, 2H), 3.62 (d, J = 11.4 Hz, 2H), 2.66–2.61 (m, 2H), 2.00–1.92 (m, 5H), 0.24 (s, 9H); MS (ESI) m/z 334.2 $(M+H)^+$.

4.2.5. Preparation of N-(4-(4-ethynylphenyl)-1-hydroxy-2-(hydroxymethyl)butan-2-yl) acetamide (9)

To a solution of **8** (4.7 g, 14.1 mmol) in MeOH (60 mL) was added K_2CO_3 (1.5 g, 11.3 mmol). The mixture was stirred under argon at

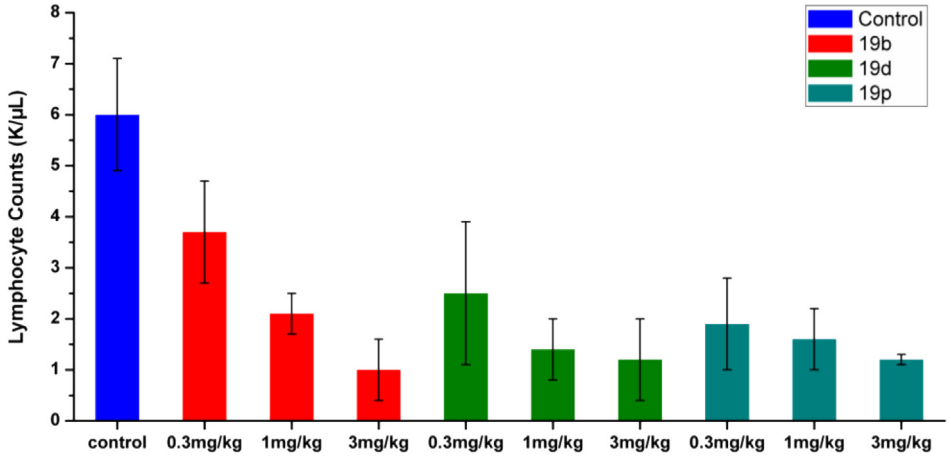


Fig. 5. Dose response lymphopenia of compounds **19b**, **19d** and **19p** relative to the vehicle at 12 h postadministration.

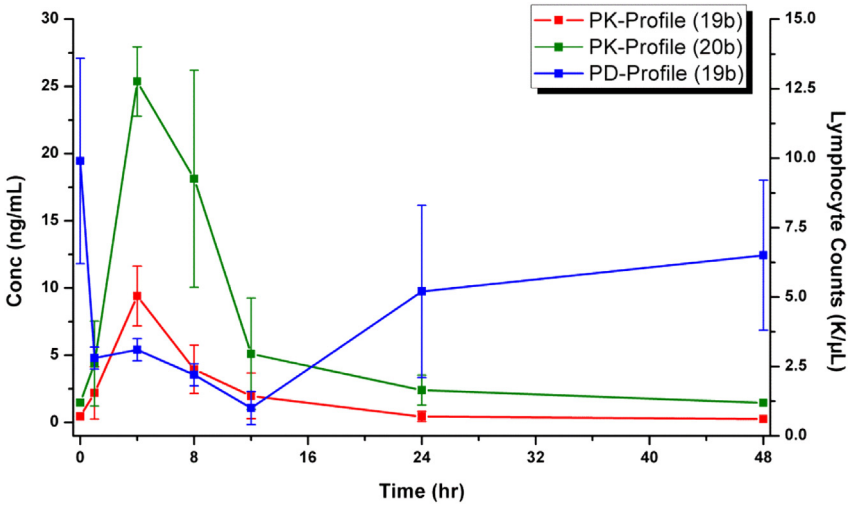


Fig. 6. PK/PD relationship of compounds **19b** and **20b** after 3 mg/kg oral administration of **19b** to SD rats.

room temperature for 2 h, then filtered and concentrated. The residue was diluted with CH_2Cl_2 (80 mL), washed with saturated aq. NH_4Cl and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 20:1) to afford compound **9** (3 g, 81.5% yield) as brown solid. Mp: 91–93 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.1 Hz, 2H), 5.98 (s, 1H), 3.84 (d, J = 11.7 Hz, 2H), 3.63 (d, J = 11.4 Hz, 2H), 3.04 (s, 1H), 2.67–2.62 (m, 2H), 2.00–1.93 (m, 5H); MS (ESI) m/z 262.1 $(M+H)^+$.

*4.2.6. General procedure for preparation of N-(1-hydroxy-2-(hydroxymethyl)-4-(4-(1-(substituted phenyl)-1H-1,2,3-triazol-4-yl)phenyl)butan-2-yl)acetamide (**10a**–**j**)*

To a solution of substituted aniline (1.45 mmol) in CH_3CN (10 mL) was added *t*-BuONO (2.2 mmol) and $TMSN_3$ (1.74 mmol). The mixture was stirred under argon at room temperature for 2 h. Then **9** (1.45 mmol) was added to the solution followed by aq. $CuSO_4$ (0.73 mmol), sodium ascorbate (0.73 mmol) and Et_3N (7.3 mmol). The mixture was stirred under argon at room temperature for 12 h, then filtered and concentrated. The residue was diluted with EtOAc (15 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 20:1) to afford compound **10a**–**j**. The analytical data was in the supporting information.

*4.2.7. General procedure for preparation of N-(1-hydroxy-2-(hydroxymethyl)-4-(4-(1- (substituted benzyl)-1H-1,2,3-triazol-4-yl)phenyl)butan-2-yl)acetamide (**10k**–**p**)*

To a solution of NaN_3 (1.6 mmol) in *t*-BuOH/H_2O (10 mL, 1:1) was added substituted benzyl bromide (1.45 mmol). The mixture was stirred under argon at room temperature for 2 h. Then **9** (1.45 mmol) was added to the solution followed by aq. $CuSO_4$ (0.73 mmol), sodium ascorbate (0.73 mmol) and Et_3N (7.3 mmol). The mixture was stirred under argon at room temperature for 12 h, then filtered and concentrated. The residue was diluted with EtOAc (15 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 20:1) to afford compound **10k**–**p**. The analytical data was in the supporting information.

*4.2.8. General procedure for preparation of 2-amino-2-(4-(1-substituted-1H-1,2,3-triazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**11a**–**p**)*

To a solution of **10** (0.46 mmol) in MeOH (5 mL) was added NaOH (0.46 mmol) and heated at 80 °C for 8 h. After cooled to room temperature, the mixture was added HCl–EtOH solution until pH = 2–3 and concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 8:1) to afford compound **11a**–**p**.

*4.2.8.1. 2-Amino-2-(4-(1-(p-tolyl)-1H-1,2,3-triazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**11a**).* Yield: 91.0%; Light yellow solid; Mp: 218–220 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.76 (s, 1H), 7.79 (d, J = 7.8 Hz, 2H), 7.71 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 8.1 Hz, 2H), 3.65 (s, 4H), 2.67–2.64 (m, 2H), 2.38 (s, 3H), 1.97–1.91 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.35, 143.00, 140.54, 136.14, 131.40, 129.99, 129.55, 127.05, 121.40, 120.07, 62.52, 62.08, 34.51, 29.92, 21.07; ESI-HRMS m/z calcd For $C_{20}H_{25}N_4O_2$ $[M+H]^+$ 353.1972, found 353.1972.

*4.2.8.2. 2-Amino-2-(4-(1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**11b**).* Yield: 58.3%; Light yellow solid; Mp: 225–228 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.69 (s, 1H), 7.77 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 7.06 (d, J = 8.7 Hz, 2H), 3.80 (s, 3H), 3.64 (s, 4H), 2.68–2.63 (m, 2H), 1.96–1.90 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.55, 149.27, 142.96, 131.73, 130.02, 129.59, 127.03, 123.15, 120.22, 115.94, 62.52, 62.08, 56.16, 34.51, 29.91; ESI-HRMS m/z calcd For $C_{20}H_{25}N_4O_3$ $[M+H]^+$ 369.1921, found 360.1922.

*4.2.8.3. 2-Amino-2-(4-(1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**11c**).* Yield: 81.2%; Light yellow solid; Mp: 238–240 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.85 (s, 1H), 7.90 (m, 2H), 7.81 (d, J = 7.2 Hz, 2H), 7.34–7.28 (m, 4H), 3.67 (s, 4H), 2.72–2.69 (m, 2H), 1.96 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.77, 149.56, 143.13, 130.02, 129.41, 127.06, 123.79, 123.70, 120.36, 117.84, 117.60, 62.52, 62.09, 34.50, 29.91; ESI-HRMS m/z calcd For $C_{19}H_{22}N_4O_2F$ $[M+H]^+$ 357.1721, found 357.1731.

*4.2.8.4. 2-Amino-2-(4-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**11d**).* Yield: 76.5%; White solid; Mp: 240–242 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.87 (s, 1H), 7.88 (d, J = 8.7 Hz, 2H), 7.80 (d, J = 7.8 Hz, 2H), 7.55 (d, J = 8.4 Hz,

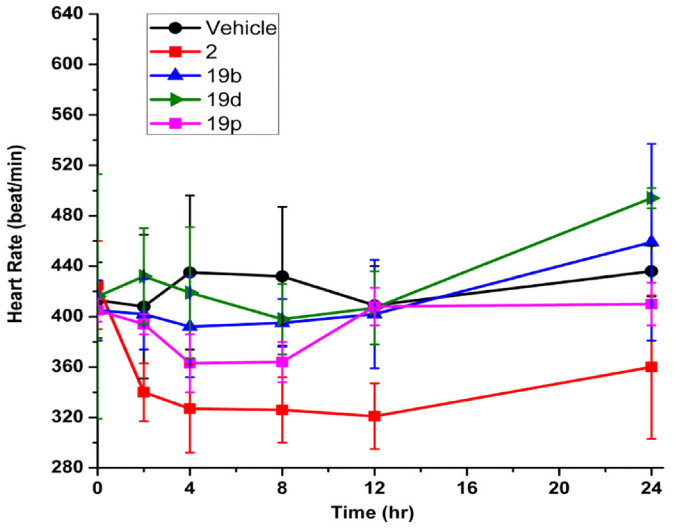


Fig. 7. Effect on heart rate after 10 mg/kg oral administration of **19b**, **19d** and **19p** to SD rats.

2H), 7.31 (d, J = 7.8 Hz, 2H), 3.66 (s, 4H), 2.70–2.65 (m, 2H), 1.97–1.91 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.65, 143.16, 137.10, 135.63, 131.07, 130.03, 129.36, 127.07, 122.91, 120.11, 62.51, 62.09, 34.50, 29.92; ESI-HRMS m/z calcd For $C_{19}H_{22}N_4O_2Cl$ $[M+H]^+$ 373.1426, found 373.1432.

*4.2.8.5. 2-Amino-2-(4-(1-(4-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**11e**).* Yield: 43.0%; Light yellow solid; Mp: 206–208 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.98 (s, 1H), 8.11 (d, J = 8.4 Hz, 2H), 7.87 (d, J = 8.7 Hz, 2H), 7.83 (d, J = 8.1 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 3.67 (s, 4H), 2.71–2.66 (m, 2H), 1.98–1.92 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 148.39, 141.80, 128.59, 127.81, 126.83, 126.79, 126.75, 125.73, 125.66, 120.24, 118.64, 61.06, 60.63, 33.04, 28.47; ESI-HRMS m/z calcd For $C_{20}H_{22}N_4O_2F_3$ $[M+H]^+$ 407.1689, found 407.1695.

*4.2.8.6. 2-Amino-2-(4-(1-(4-(trifluoromethoxy)phenyl)-1H-1,2,3-triazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**11f**).* Yield: 86.8%; White solid; Mp: 225–228 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.86 (s, 1H), 7.97 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 7.8 Hz, 2H), 7.46 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 3.64 (s, 4H), 2.69–2.63 (m, 2H), 1.96–1.90 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 150.33, 149.70, 143.17, 137.11, 130.02, 129.35, 127.09, 123.61, 123.18, 120.23, 62.52, 62.08, 34.50, 29.92; ESI-HRMS m/z calcd For $C_{20}H_{22}N_4O_3F_3$ $[M+H]^+$ 423.1639, found 423.1641.

*4.2.8.7. 2-Amino-2-(4-(1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**11g**).* Yield: 62.0%; Light yellow solid; Mp: 207–210 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.77 (s, 1H), 7.79 (d, J = 7.8 Hz, 2H), 7.73 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 3.65 (s, 4H), 2.72–2.65 (m, 4H), 1.96–1.91 (m, 2H), 1.22 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.36, 146.91, 142.99, 136.31, 130.29, 129.98, 129.57, 127.06, 121.53, 120.08, 62.53, 62.08, 34.52, 29.91, 29.42, 15.99; ESI-HRMS m/z calcd For $C_{21}H_{27}N_4O_2$ $[M+H]^+$ 367.2129, found 367.2134.

*4.2.8.8. 2-Amino-2-(4-(1-(4-isopropylphenyl)-1H-1,2,3-triazol-4-yl)phenethyl)propane-1,3- diol hydrochloride (**11h**).* Yield: 44.3%; Light yellow solid; Mp: 233–235 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.75 (s, 1H), 7.77 (d, J = 7.8 Hz, 2H), 7.71 (d, J = 7.8 Hz, 2H), 7.36 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 7.8 Hz, 2H), 3.64 (s, 4H), 2.93–2.89 (m, 1H), 2.67–2.62 (m, 2H), 1.95–1.90 (m, 2H), 1.20 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 151.36, 149.34, 143.00, 136.29, 129.99, 129.47, 128.84, 127.03, 121.47, 120.09, 62.51, 62.08, 35.06, 34.48, 29.90, 24.26; ESI-HRMS m/z calcd For $C_{22}H_{29}N_4O_2$ $[M+H]^+$ 381.2285, found 381.2291.

*4.2.8.9. 2-Amino-2-(4-(1-(4-propylphenyl)-1H-1,2,3-triazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**11i**).* Yield: 45.0%; Light yellow solid; Mp: 208–210 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.77 (s, 1H), 7.79 (d, J = 7.2 Hz, 2H), 7.73 (d, J = 6.9 Hz, 2H), 7.35 (d, J = 7.5 Hz, 2H), 7.30 (d, J = 7.5 Hz, 2H), 3.65 (s, 4H), 2.69–2.60 (m, 4H), 1.96–1.91 (m, 2H), 1.63 (q, J = 6.9 Hz, 2H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.36, 145.25, 143.00, 136.33, 130.90, 129.99, 129.55, 127.06, 121.42, 120.09, 62.53, 62.08, 38.48, 34.51, 29.92, 25.60, 14.00; ESI-HRMS m/z calcd For $C_{22}H_{29}N_4O_2$ $[M+H]^+$ 381.2285, found 381.2289.

*4.2.8.10. 4-(4-(4-(3-Amino-4-hydroxy-3-(hydroxymethyl)butyl)phenyl)-1H-1,2,3-triazol-1-yl) benzonitrile hydrochloride (**11j**).* Yield: 84.7%; White solid; Mp: 248–250 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.97 (s, 1H), 8.09 (d, J = 8.4 Hz, 2H), 7.91 (d, J = 8.7 Hz, 2H), 7.81 (d, J = 7.5 Hz, 2H), 7.31 (d, J = 7.8 Hz, 2H), 3.65 (s, 4H), 2.70–2.64 (m, 2H), 1.97–1.91 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.94, 143.31, 141.34, 135.24, 130.04, 129.18, 127.13, 121.74, 119.97, 118.85, 113.43, 62.52, 62.08, 34.48, 29.93; ESI-HRMS m/z calcd For $C_{20}H_{22}N_5O_2$ $[M+H]^+$ 364.1768, found 364.1771.

*4.2.8.11. 2-Amino-2-(4-(1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**11k**).* Yield: 85.0%; Brown solid; Mp: 163–167 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H), 7.65 (d, J = 8 Hz, 2H), 7.24 (d, J = 8 Hz, 2H), 7.19 (d, J = 7.6 Hz, 2H), 7.13 (d, J = 8 Hz, 2H), 5.51 (s, 2H), 3.63 (s, 4H), 2.65–2.61 (m, 2H), 2.25 (s, 3H), 1.92–1.88 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 149.03, 142.76, 139.67, 133.76, 130.63, 129.91, 129.71, 129.13, 126.90, 121.95, 62.50, 62.06, 54.86, 34.47, 29.86, 21.15; ESI-HRMS m/z calcd For $C_{21}H_{27}N_4O_2$ $[M+H]^+$ 367.2129, found 367.2128.

*4.2.8.12. 2-Amino-2-(4-(1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**11l**).* Yield: 88.2%; White solid; Mp: 198–200 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H), 7.65 (d, J = 7.6 Hz, 2H), 7.38–7.35 (m, 2H), 7.26 (d, J = 8 Hz, 2H), 7.06–7.02 (m, 2H), 5.57 (s, 2H), 3.62 (s, 4H), 2.65–2.61 (m, 2H), 1.92–1.87 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.05, 161.61, 147.26, 141.74, 131.21, 131.18, 130.05, 129.97, 128.57, 127.55, 125.60, 120.98, 115.49, 115.27, 61.05, 60.61, 53.10, 32.99, 28.43; ESI-HRMS m/z calcd For $C_{20}H_{24}N_4O_2F$ $[M+H]^+$ 371.1878 found 371.1879.

*4.2.8.13. 2-Amino-2-(4-(1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**11m**).* Yield: 62.1%; White solid; Mp: 200–202 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (s, 1H), 7.68 (d, J = 8 Hz, 2H), 7.36–7.30 (m, 2H), 7.26 (d, J = 8 Hz, 2H), 5.58 (s, 2H), 3.64 (s, 4H), 2.67–2.62 (m, 2H), 1.94–1.90 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 147.18, 141.90, 134.21, 133.82, 129.49, 128.74, 128.63, 127.31, 125.65, 121.28, 61.06, 60.63, 53.15, 32.98, 28.45; ESI-HRMS m/z calcd For $C_{20}H_{24}N_4O_2Cl$ $[M+H]^+$ 387.1582, found 387.1587.

*4.2.8.14. 2-Amino-2-(4-(1-(4-(trifluoromethyl)benzyl)-1H-1,2,3-triazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**11n**).* Yield: 84.7%; White solid; Mp: 191–193 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.38 (s, 1H), 7.70 (d, J = 8 Hz, 2H), 7.65 (d, J = 8 Hz, 2H), 7.50 (d, J = 8 Hz, 2H), 7.28 (d, J = 8 Hz, 2H), 5.72 (s, 2H), 3.65 (s, 4H), 2.68–2.64 (m, 2H), 1.95–1.90 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 147.21, 141.99, 139.43, 130.65, 130.30, 128.64, 128.35, 127.18, 125.68, 125.58, 125.55, 125.51, 125.47, 122.66, 121.57, 61.05, 60.63,

53.25, 32.98, 28.45; ESI-HRMS *m/z* calcd For $C_{21}H_{24}N_4O_2F_3$ $[M+H]^+$ 421.1846, found 421.1847.

*4.2.8.15. 2-Amino-2-(4-(1-(4-ethylbenzyl)-1H-1,2,3-triazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**11o**).* Yield: 58.0%; Light yellow solid; Mp: 214–216 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.25 (s, 1H), 7.66 (d, J = 7.5 Hz, 2H), 7.26–7.22 (m, 4H), 7.15 (d, J = 7.5 Hz, 2H), 5.53 (s, 2H), 3.63 (s, 4H), 2.64–2.60 (m, 2H), 2.57–2.55 (m, 2H), 1.92–1.88 (m, 2H), 1.16–1.11 (m, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 148.77, 146.18, 142.98, 133.83, 129.97, 129.52, 129.28, 126.99, 122.26, 62.51, 62.05, 55.10, 34.44, 29.87, 29.51, 16.06; ESI-HRMS *m/z* calcd For $C_{22}H_{29}N_4O_2$ $[M+H]^+$ 381.2285, found 381.2285.

*4.2.8.16. 2-Amino-2-(4-(1-pentyl-1H-1,2,3-triazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**11p**).* Yield: 86.8%; Light yellow solid; Mp: 177–179 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.36 (s, 1H), 7.74 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 8.1 Hz), 4.45 (t, J = 7.2 Hz, 2H), 3.69 (s, 4H), 2.73–2.67 (m 2H), 2.00–1.94 (m, 4H), 1.36 (m, 4H), 0.94–0.89 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 147.39, 143.93, 130.22, 127.69, 127.28, 123.37, 62.50, 62.06, 52.51, 34.39, 30.74, 29.92, 29.60, 23.11, 14.19; ESI-HRMS *m/z* calcd For $C_{18}H_{29}N_4O_2$ $[M+H]^+$ 333.2285, found 333.2289.

*4.2.9. General procedure for preparation of 2-amino-2-(hydroxymethyl)-4-(4-(1-substituted-1H-1,2,3-triazol-4-yl)phenyl) butyl dihydrogen phosphate (**12a**–**p**)*

To a solution of **11** (0.31 mmol) in saturated aq. $NaHCO_3$/EtOAc mixture (6 mL, 1:1) was added CbzCl (0.37 mmol). The mixture was stirred for 4 h at room temperature. The aqueous phase was extracted with EtOAc (2 mL × 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated. Purification of the residue by silica gel flash column chromatography (CH_2Cl_2/MeOH = 30:1) gave the amino-protected compound. To a solution of the amino-protected compound (0.21 mmol) in CH_2Cl_2 (5 mL) was added TBPP (0.25 mmol), Ag_2O (0.42 mmol) and Hex_4NI (0.42 mmol). After stirring under argon at room temperature for 20 h, the reaction mixture was filtered through celite to remove insoluble materials, and the filtrate was concentrated. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH = 30:1) to afford benzyl (1-((bis(benzyloxy) phosphoryl)oxy)-4-(4-(1-substituted-1*H*-1,2,3-triazol-4-yl)phenyl)-2-(hydroxymethyl)butan-2-yl)carbamate as oil. To a solution of the oil (0.08 mmol) in CH_2Cl_2 (5 mL) was added TMSI (0.8 mmol) slowly at 0 °C and stirred under argon at 0 °C for 1 h. The mixture was added MeOH (1 mL) to decompose the complex, then concentrated. The residue was precipitated from MeOH/10% aq. $Na_2S_2O_3$ (1:1) to give the title compound **12a**–**p**.

*4.2.9.1. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(p-tolyl)-1H-1,2,3-triazol-4-yl)phenyl)butyl dihydrogen phosphate (**12a**).* Yield: 72.5%; Light yellow solid; Mp: 259–261 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.21 (s, 1H), 7.87 (d, J = 7.7 Hz, 2H), 7.82 (d, J = 8.2 Hz, 2H), 7.43 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 7.7 Hz, 2H), 3.92 (m, 2H), 3.57 (s, 2H), 2.67 (m, 2H), 2.40 (s, 3H), 1.87 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 147.14, 141.40, 138.33, 134.40, 130.27, 128.81, 128.20, 125.46, 119.82, 119.17, 64.88, 61.01, 59.14, 33.08, 27.98, 20.59; ESI-HRMS *m/z* calcd For $C_{20}H_{26}N_4O_5P$ $[M+H]^+$ 433.1635, found 433.1626.

*4.2.9.2. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)phenyl) butyl dihydrogen phosphate (**12b**).* Yield: 72.4%; White solid; Mp: 263–265 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.14 (s, 1H), 7.87–7.83 (m, 4H), 7.34 (d, J = 8 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 3.91 (m, 2H), 3.85 (s, 3H), 3.57 (s, 2H), 2.67 (m, 2H), 1.88–1.86 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 159.26, 147.06, 141.52, 130.06, 128.78, 128.17, 125.39, 121.59, 119.24, 114.90, 64.44, 60.92, 59.35, 55.57, 33.15, 28.08; ESI-HRMS *m/z* calcd For $C_{20}H_{26}N_4O_6P$ $[M+H]^+$ 449.1585, found 449.1579.

*4.2.9.3. 2-Amino-4-(4-(1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl) phenyl)-2-(hydroxymethyl) butyl dihydrogen phosphate (**12c**).* Yield: 69.0%; White solid; Mp: 245–248 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.20 (s, 1H), 7.96 (dd, J = 8.6, 4.7 Hz, 2H), 7.84 (d, J = 7.5 Hz, 2H), 7.47 (t, J = 8.7 Hz, 2H), 7.33 (d, J = 7.7 Hz, 2H), 3.88 (m, 2H), 3.55 (s, 2H), 2.65 (m, 2H), 1.86 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 162.20, 160.57, 147.04, 141.36, 132.94, 128.58, 128.54, 127.76, 125.20, 122.07, 122.01, 119.30, 116.61, 116.46, 64.34, 60.71, 59.00, 32.85, 27.80; ESI-HRMS *m/z* calcd For $C_{19}H_{23}N_4O_5FP$ $[M+H]^+$ 437.1385, found 437.1376.

*4.2.9.4. 2-Amino-4-(4-(1-(4-chlorophenyl)-1H-1,2,3-triazol-4-yl) phenyl)-2-(hydroxymethyl) butyl dihydrogen phosphate (**12d**).* Yield: 65.0%; White solid; Mp: 265–267 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.28 (s, 1H), 7.99 (d, J = 8.6 Hz, 2H), 7.86 (d, J = 7.5 Hz, 2H), 7.71 (d, J = 8.9 Hz, 2H), 7.35 (d, J = 8.3 Hz, 2H), 3.90 (m, 2H), 3.57 (s, 2H), 2.67 (m, 2H), 1.86 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 147.87, 142.16, 135.89, 133.38, 130.39, 129.32, 128.38, 125.94, 122.06, 119.83, 65.04, 61.44, 59.76, 33.59, 28.54; ESI-HRMS *m/z* calcd For $C_{19}H_{23}N_4O_5ClP$ $[M+H]^+$ 453.1089, found 453.1082.

*4.2.9.5. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(4-(trifluoromethyl) phenyl)-1H-1,2,3-triazol-4-yl) phenyl)butyl dihydrogen phosphate (**12e**).* Yield: 50.2%; White solid; Mp: 271–272 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.97 (s, 1H), 8.10 (m, 2H), 7.86–7.81 (m, 4H), 7.33 (m, 2H), 3.97 (m, 2H), 3.67 (s, 2H), 2.68 (m, 2H), 1.95 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 160.90, 147.34, 141.50, 139.14, 131.15, 128.63, 127.54, 127.05, 127.03, 125.28, 124.48, 122.68, 120.06, 119.24, 117.28, 64.50, 60.74, 58.94, 32.82, 27.78; ESI-HRMS *m/z* calcd For $C_{20}H_{23}N_4O_5F_3P$ $[M+H]^+$ 487.1353, found 487.1346.

*4.2.9.6. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(4-(trifluoromethoxy) phenyl)-1H-1,2,3-triazol-4-yl)phenyl)butyl dihydrogen phosphate (**12f**).* Yield: 58.4%; White solid; Mp: >300 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 9.31 (s, 1H), 8.11–8.07 (m, 2H), 7.88 (d, J = 8 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8 Hz, 2H), 3.97–3.95 (m, 2H), 3.58 (s, 2H), 2.67 (m, 2H), 1.89–1.87 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 147.84, 147.44, 141.72, 135.50, 128.87, 128.83, 127.89, 125.48, 122.68, 121.88, 120.87, 119.56, 119.16, 64.58, 60.95, 59.25, 33.10, 28.15; ESI-HRMS *m/z* calcd For $C_{20}H_{23}N_4O_6F_3P$ $[M+H]^+$ 503.1304, found 503.1298.

*4.2.9.7. 2-Amino-4-(4-(1-(4-ethylphenyl)-1H-1,2,3-triazol-4-yl) phenyl)-2-(hydroxymethyl) butyl dihydrogen phosphate (**12g**).* Yield: 60.1%; Light yellow solid; Mp: 253–255 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.78 (s, 1H), 7.80 (d, J = 8 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8 Hz, 2H), 3.97–3.95 (m, 2H), 3.68 (s, 2H), 2.73–2.67 (m, 4H), 1.99–1.96 (m, 2H), 1.24 (t, J = 7.6 Hz, 3H); ^{13}C NMR (150 MHz, DMSO) δ 147.15, 144.52, 141.55, 134.56, 129.11, 128.80, 128.12, 125.42, 119.93, 119.16, 64.52, 60.96, 59.25, 33.14, 28.06, 27.67, 15.45; ESI-HRMS *m/z* calcd For $C_{21}H_{28}N_4O_5P$ $[M+H]^+$ 447.1792, found 447.1784.

*4.2.9.8. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(4-isopropylphenyl)-1H-1,2,3-triazol-4-yl)phenyl) butyl dihydrogen phosphate (**12h**).* Yield: 63.3%; Light yellow solid; Mp: 260–262 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.78 (s, 1H), 7.81–7.75 (m, 4H), 7.42 (d, J = 7.8 Hz, 2H), 7.33 (d, J = 7.8 Hz, 2H), 3.98 (m, 2H), 3.68 (s, 2H), 3.02 (m, 1H), 2.72 (m, 2H), 1.99 (m, 2H), 1.26 (d, J = 7.2 Hz, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 150.02, 141.53, 139.03, 134.97, 128.66, 127.47, 125.62, 120.13, 117.37, 117.23, 64.37, 61.08, 60.01, 33.72, 28.44, 25.08,

19.60.; ESI-HRMS *m/z* calcd For $C_{22}H_{30}N_4O_5P$ $[M+H]^+$ 461.1948, found 461.1935.

*4.2.9.9. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(4-propylphenyl)-1H-1,2,3-triazol-4-yl)phenyl) butyl dihydrogen phosphate (**12i**).* Yield: 63.0%; White solid; Mp: 255–257 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.78 (s, 1H), 7.80 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 8 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8 Hz, 2H), 3.98–3.95 (m, 2H), 3.68 (s, 2H), 2.73–2.62 (m, 4H), 1.99–1.96 (m, 2H), 1.68–1.63 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, DMSO) δ 147.13, 142.92, 141.46, 134.58, 129.67, 128.79, 128.15, 125.43, 119.86, 119.18, 64.64, 60.95, 59.24, 36.62, 33.09, 28.02, 23.93, 13.54; ESI-HRMS *m/z* calcd For $C_{22}H_{30}N_4O_5P$ $[M+H]^+$ 461.1948, found 461.1935.

*4.2.9.10. 2-Amino-4-(4-(1-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl) phenyl)-2-(hydroxymethyl) butyl dihydrogen phosphate (**12j**).* Yield: 40.5%; White solid; Mp: 258–260 °C; ^{1}H NMR (400 MHz, DMSO) δ 9.41 (s, 1H), 8.21–8.12 (m, 4H), 7.88 (d, *J* = 7.2 Hz, 2H), 7.36 (d, *J* = 7.6 Hz, 2H), 3.96 (m, 2H), 3.58 (s, 2H), 2.67 (m, 2H), 1.89 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 148.15, 142.30, 139.99, 134.84, 129.39, 128.17, 126.02, 120.77, 119.94, 118.61, 111.48, 65.24, 61.48, 59.65, 33.55, 28.51; ESI-HRMS *m/z* calcd For $C_{20}H_{23}N_5O_5P$ $[M+H]^+$ 444.1431, found 444.1420.

*4.2.9.11. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)phenyl) butyl dihydrogen phosphate (**12k**).* Yield: 71.2%; White solid; Mp: 242–244 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.54 (s, 1H), 7.76 (d, *J* = 8.1 Hz, 2H), 7.29–7.23 (m, 4H), 7.19 (d, *J* = 8.0 Hz, 2H), 5.57 (s, 2H), 3.89 (m, 2H), 3.55 (s, 2H), 2.64–2.60 (m, 2H), 2.28 (s, 3H), 1.86–1.83 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 146.58, 141.18, 137.50, 133.00, 129.31, 128.71, 128.49, 127.95, 125.25, 121.06, 64.51, 60.96, 59.24, 52.80, 33.15, 28.02, 20.68; ESI-HRMS *m/z* calcd For $C_{21}H_{28}N_4O_5P$ $[M+H]^+$ 447.1792, found 447.1776.

*4.2.9.12. 2-Amino-4-(4-(1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl) phenyl)-2-(hydroxymethyl) butyl dihydrogen phosphate (**12l**).* Yield: 42.9%; White solid; Mp: 252–254 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.23 (s, 1H), 7.67 (d, *J* = 8 Hz, 2H), 7.38–7.35 (m, 2H), 7.27 (d, *J* = 8 Hz, 2H), 7.09–7.04 (m, 2H), 5.56 (s, 2H), 3.95–3.92 (m, 2H), 3.65 (s, 2H), 2.68–2.65 (m, 2H), 1.95–1.92 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 163.62, 161.99, 147.56, 141.37, 131.53, 129.93, 129.87, 128.44, 128.20, 125.35, 120.65, 115.45, 115.30, 64.55, 61.26, 59.89, 52.83, 33.39, 28.27; ESI-HRMS *m/z* calcd For $C_{20}H_{25}N_4O_5FP$ $[M+H]^+$ 451.1541, found 451.1527.

*4.2.9.13. 2-Amino-4-(4-(1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl) phenyl)-2-(hydroxymethyl) butyl dihydrogen phosphate (**12m**).* Yield: 48.3%; Light yellow solid; Mp: 247–249 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.55 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 2H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.5 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 2H), 5.62 (s, 2H), 3.87 (m, 2H), 3.53 (s, 2H), 2.61–2.58 (m, 2H), 1.81 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 147.13, 141.71, 135.46, 133.34, 130.35, 129.27, 129.20, 125.76, 121.75, 118.01, 64.99, 61.43, 59.74, 52.66, 33.61, 28.50; ESI-HRMS *m/z* calcd For $C_{20}H_{25}N_4O_5ClP$ $[M+H]^+$ 467.1246, found 467.1238.

*4.2.9.14. 2-Amino-2-(hydroxymethyl)-4-(4-(1-(4-(trifluoromethyl) benzyl)-1H-1,2,3-triazol-4-yl)phenyl)butyl dihydrogen phosphate (**12n**).* Yield: 51.5%; Light yellow solid; Mp: 247–250 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.60 (s, 1H), 7.77–7.74 (m, 4H), 7.52 (d, *J* = 7.9 Hz, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 5.75 (s, 2H), 3.89 (m, 2H), 3.53 (s, 2H), 2.61–2.58 (m, 2H), 1.83 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 147.21, 141.79, 141.14, 129.21, 129.08, 128.83, 126.21, 126.19, 126.16, 125.78, 125.47, 123.67, 122.00, 64.98, 61.44, 59.73, 52.80, 33.63, 28.52; ESI-HRMS *m/z* calcd For $C_{21}H_{25}N_4O_5F_3P$ $[M+H]^+$ 501.1509, found 501.1498.

*4.2.9.15. 2-Amino-4-(4-(1-(4-ethylbenzyl)-1H-1,2,3-triazol-4-yl) phenyl)-2-(hydroxymethyl) butyl dihydrogen phosphate (**12o**).* Yield: 46.9%; White solid; Mp: 235–237 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.54 (s, 1H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.27–7.24 (m, 4H), 7.20 (d, *J* = 8.4 Hz, 2H), 5.56 (s, 2H), 3.88 (m, 2H), 3.53 (s, 2H), 2.57 (m, 4H), 1.82 (m, 2H), 1.13 (t, *J* = 7.8 Hz,3H); ^{13}C NMR (150 MHz, DMSO) δ 146.55, 143.82, 140.97, 133.27, 128.68, 128.57, 128.15, 127.99, 125.27, 121.10, 64.91, 60.99, 59.11, 52.81, 33.06, 27.80, 18.53, 15.56; ESI-HRMS *m/z* calcd For $C_{22}H_{30}N_4O_5P$ $[M+H]^+$ 461.1948, found 461.1938.

*4.2.9.16. 2-Amino-2-(hydroxymethyl)-4-(4-(1-pentyl-1H-1,2,3-triazol-4-yl)phenyl)butyl dihydrogen phosphate (**12p**).* Yield: 70.2%; White solid; Mp: 245–247 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.23 (s, 1H), 7.69 (d, *J* = 8.1 Hz, 2H), 7.28 (d, *J* = 8.4 Hz, 2H), 4.38 (t, *J* = 7.2 Hz, 2H), 3.95 (m, 2H), 3.66 (s, 2H), 2.66–2.62 (m, 2H), 1.93–1.91 (m, 4H), 1.31–1.23 (m, 4H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, DMSO) δ 146.64, 141.38, 129.17, 125.69, 121.40, 109.98, 61.50, 60.63, 59.66, 49.93, 33.57, 29.78, 28.49, 27.62, 22.00, 14.27; ESI-HRMS *m/z* calcd For $C_{18}H_{30}N_4O_5P$ $[M+H]^+$ 413.1948, found 413.1941.

*4.2.10. Preparation of diethyl 2-acetamido-2-phenethylmalonate (**14**)*

Sodium (4.1 g, 178.3 mmol) was added to absolute EtOH (250 mL). After sodium dissolved completely, diethyl acetamidomalonate (38.7 g, 178.3 mmol) was added in portions at 0 °C. The solution was then allowed to return to room temperature and stirred for further 2 h. Then a solution of (2-bromoethyl)benzene (**13**, 30 g, 162.1 mmol) in absolute EtOH (40 mL) was added. The mixture was heated at 80 °C for 20 h, then filtered and concentrated. The residue was diluted with EtOAc (300 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (EtOAc/PE = 1:3) to afford compound **14** (23 g, 44.2% yield) as white solid. Mp: 112–115 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29–7.24 (m, 2H), 7.19–1.13 (m, 3H), 6.76 (s, 1H), 4.24–4.17 (m, 4H), 2.70 (t, *J* = 7.2 Hz, 2H), 2.48 (t, *J* = 6.9 Hz, 2H), 1.98 (s, 3H), 1.25 (t, *J* = 6 Hz, 6H); MS (ESI) *m/z* 322.2 $(M+H)^+$.

*4.2.11. Preparation of diethyl 2-acetamido-2-(4-(2-chloroacetyl) phenethyl)malonate (**15**)*

Chloroacetyl chloride (3.87 g, 34.3 mmol) in CH_2Cl_2 (20 mL) was added dropwise to a cooled solution (0 °C) of **14** (10 g, 31.2 mmol) in CH_2Cl_2 (300 mL), then $AlCl_3$ (25 g, 187.2 mmol) was added in portions in 30 min. The solution was then allowed to return to room temperature and stirred for further 5 h. The mixture was poured slowly into 2NHCl-ice mixture (200 mL) and stirred for 1 h. The aqueous phase was extracted with CH_2Cl_2 (50 mL × 3). The combined organic layers were washed with saturated aq. $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (EtOAc/PE = 1:1) to afford compound **15** (8 g, 66.7% yield) as yellow solid. Mp: 78–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8 Hz, 2H), 7.28 (d, *J* = 8 Hz, 2H), 6.78 (s, 1H), 4.67 (s, 2H), 4.27–4.18 (m, 4H), 2.73–2.69 (m, 2H), 2.58–2.54 (m, 2H), 2.00 (s, 3H), 1.26 (t, *J* = 7.2 Hz, 6H); MS (ESI) *m/z* 398.1 $(M+H)^+$.

*4.2.12. General procedure for preparation of diethyl 2-acetamido-2-(4-(2-substituted acetyl)phenethyl)malonate (**16a–s**)*

To a solution of **15** (6.4 mmol) in CH_3CN (20 mL) was added carboxylic acid (14.7 mmol) and Et_3N (13.4 mmol). The mixture was heated to reflux for 2 h, then concentrated. The residue was diluted

with CH_2Cl_2 (30 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (EtOAc/PE = 1:1) to afford compound **16a**–**s**. The analytical data was in the supporting information.

*4.2.13. General procedure for preparation of diethyl 2-acetamido-2-(4-(2-substituted oxazol-4-yl)phenethyl) malonate (**17a**–**s**)*

To a solution of **16** (5.1 mmol) in xylene(30 mL) was added acetamide (25.5 mmol) and 47%$BF_3 \cdot Et_2O$(0.26 mL). The mixture was heated to reflux for 40 h, then concentrated. The residue was diluted with EtOAc (40 mL), washed with brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by silica gel flash column chromatography (EtOAc/PE = 1:1) to afford compound **17a**–**s**. The analytical data was in the supporting information.

*4.2.14. General procedure for preparation of N-(1-hydroxy-2-(hydroxymethyl)-(4-(4-(2-substituted oxazol-4-yl)phenyl)butan-2-yl) acetamide (**18a**–**s**)*

18a–**s** was prepared using the same procedure as that described for compound **7**. The analytical data was in the supporting information.

*4.2.15. General procedure for preparation of 2-amino-2-(4-(2-substituted oxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19a**–**s**)*

19a–**s** was prepared using the same procedure as that described for compound **11a**–**p**.

*4.2.15.1. 2-Amino-2-(4-(2-(p-tolyl)oxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19a**).* Yield: 96.9%; White solid; Mp: 224–227 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.18 (s, 1H), 7.90 (d, J = 8.1 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H), 7.27–7.25 (m, 4H), 3.65 (s, 4H), 2.68–2.62 (m, 2H), 2.34 (s, 3H), 1.96–1.90 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.73, 142.93, 142.59, 142.51, 135.22, 130.68, 130.44, 129.76, 127.48, 126.91, 125.78, 62.54, 62.07, 34.54, 29.91, 21.50; ESI-HRMS m/z calcd For $C_{21}H_{25}N_2O_3$ $[M+H]^+$ 353.1860, found 353.1861.

*4.2.15.2. 2-Amino-2-(4-(2-(4-methoxyphenyl)oxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19b**).* Yield: 85.1%; White solid; Mp: 200–202 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.15 (s, 1H), 7.95 (d, J = 9 Hz, 2H), 7.69 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 8.1 Hz, 2H), 6.99 (d, J = 9 Hz, 2H), 3.80 (s, 3H), 3.64 (s, 4H), 2.67–2.62 (m, 2H), 1.95–1.89 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.71, 163.36, 142.75, 142.55, 134.94, 130.44, 129.75, 129.23, 126.90, 120.99, 115.45, 62.53, 62.08, 55.96, 34.53, 29.91; ESI-HRMS m/z calcd For $C_{21}H_{25}N_2O_4$ $[M+H]^+$ 369.1809, found 369.1808.

*4.2.15.3. 2-Amino-2-(4-(2-(4-fluorophenyl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19c**).* Yield: 74.4%; White solid; Mp: 199–200 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.23 (s, 1H), 8.07 (dd, J = 5.4, 3.9 Hz, 2H), 7.72 (d, J = 8.1 Hz, 2H), 7.29–7.18 (m, 4H), 3.67 (s, 4H), 2.70–2.64 (m, 2H), 1.97–1.92 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.89, 164.41, 162.55, 143.13, 142.71, 135.58, 130.28, 129.88, 129.80, 126.88, 125.08, 117.18, 116.96, 62.51, 62.09, 34.50, 29.90; ESI-HRMS m/z calcd For $C_{20}H_{22}FN_2O_3$ $[M+H]^+$ 357.1609, found 357.1610.

*4.2.15.4. 2-Amino-2-(4-(2-(4-chlorophenyl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19d**).* Yield: 63.5%; White solid; Mp: 234–237 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.25 (s, 1H), 8.03 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 8.1 Hz, 2H), 7.49 (d, J = 8.7 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 3.65 (s, 4H), 2.69–2.63 (m, 2H), 1.96–1.90 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.42, 143.27, 142.72, 137.83, 135.78, 130.32, 130.28, 129.78, 128.96, 127.21, 126.92, 62.53, 62.07, 34.53, 29.92; ESI-HRMS m/z calcd For $C_{20}H_{22}ClN_2O_3$ $[M+H]^+$ 373.1313, found 373.1316.

*4.2.15.5. 2-Amino-2-(4-(2-(4-(trifluoromethyl)phenyl)oxazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**19e**).* Yield: 63.3%; White solid; Mp: 220–222 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.32 (s, 1H), 8.23 (d, J = 8.1 Hz, 2H), 7.79 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.1 Hz, 2H), 7.28 (d, J = 8.1 Hz, 2H), 3.65 (s, 4H), 2.69–2.64 (m, 2H), 1.96–1.91 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.90, 143.58, 142.83, 136.37, 133.33, 132.99, 131.98, 130.19, 129.81, 127.98, 127.10, 127.06, 126.95, 62.55, 62.06, 34.54, 29.93; ESI-HRMS m/z calcd For $C_{21}H_{22}F_3N_2O_3$ $[M+H]^+$ 407.1577, found 407.1578.

*4.2.15.6. 2-Amino-2-(4-(2-(4-(trifluoromethoxy)phenyl)oxazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**19f**).* Yield: 78.3%; White solid; Mp: 200–202 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (s, 1H), 8.12 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 7.6 Hz, 2H), 7.37 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 8 Hz, 2H), 3.65 (s, 4H), 2.67–2.63 (m, 2H), 1.95–1.91 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 160.66, 150.59, 141.87, 141.30, 134.48, 128.79, 128.34, 128.13, 127.94, 126.06, 125.46, 121.05, 61.07, 60.62, 33.06, 28.46; ESI-HRMS m/z calcd For $C_{21}H_{22}N_2O_4F_3$ $[M+H]^+$ 423.1526, found 423.1524.

*4.2.15.7. 2-Amino-2-(4-(2-(4-ethylphenyl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19g**).* Yield: 84.7%; Yellow solid; Mp: 182–184 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.18 (s, 1H), 7.92 (d, J = 8.1 Hz, 2H), 7.69 (d, J = 7.8 Hz, 2H), 7.30–7.24 (m, 4H), 3.63 (s, 4H), 2.68–2.61 (m, 4H), 1.95–1.89 (m, 2H), 1.19 (t, J = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.73, 148.87, 142.87, 142.61, 135.26, 130.36, 129.77, 129.54, 127.61, 126.92, 125.93, 62.53, 62.07, 34.51, 29.90, 29.79, 15.84; ESI-HRMS m/z calcd For $C_{22}H_{27}N_2O_3$ $[M+H]^+$ 367.2016, found 367.2022.

*4.2.15.8. 2-Amino-2-(4-(2-(4-isopropylphenyl)oxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19h**).* Yield: 50.6%; White solid; Mp: 235–238 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.17 (s, 1H), 7.92 (d, J = 8.1 Hz, 2H), 7.69 (d, J = 7.8 Hz, 2H), 7.31 (d, J = 8.1 Hz, 2H), 7.25 (d, J = 7.8 Hz, 2H), 3.64 (s, 4H), 2.92–2.87 (m, 1H), 2.67–2.61 (m, 2H), 1.95–1.89 (m, 2H), 1.20 (d, J = 6.9 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.69, 153.33, 142.93, 142.60, 135.23, 130.42, 129.77, 128.10, 127.62, 126.90, 126.12, 62.53, 62.08, 35.40, 34.52, 29.91, 24.15; ESI-HRMS m/z calcd For $C_{23}H_{29}N_2O_3$ $[M+H]^+$ 381.2173, found 381.2169.

*4.2.15.9. 2-Amino-2-(4-(2-(4-propylphenyl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19i**).* Yield: 59.2%; White solid; Mp: 204–208 °C; ^{1}H NMR (300 MHz, CD_3OD) δ8.20 (s, 1H), 7.94 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 7.8 Hz, 2H), 7.31–7.26 (m, 4H), 3.65 (s, 4H), 2.69–2.59 (m, 4H), 1.96–1.91 (m, 2H), 1.64 (q, J = 7.2 Hz, 2H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.77, 147.24, 142.94, 142.57, 135.25, 130.47, 130.18, 129.76, 127.50, 126.93, 126.04, 62.55, 62.07, 38.91, 34.54, 29.91, 25.51, 12.04; ESI-HRMS m/z calcd For $C_{23}H_{29}N_2O_3$ $[M+H]^+$ 381.2173, found 381.2174.

*4.2.15.10. 4-(4-(4-(3-Amino-4-hydroxy-3-(hydroxymethyl)butyl) phenyl)oxazol-2-yl) benzonitrile hydrochloride (**19j**).* Yield: 83.4%; White solid; Mp: 218–222 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.32 (s, 1H), 8.18 (d, J = 8.4 Hz, 2H), 7.82 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 8.1 Hz, 2H), 7.27 (d, J = 7.8 Hz, 2H), 3.64 (s, 4H), 2.68–2.62 (m, 2H), 1.95–1.89 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 161.49, 143.80, 142.91, 136.65, 134.00, 132.35, 130.04, 129.82, 127.98, 126.93, 119.21, 114.89, 62.53, 62.08, 34.50, 29.93; ESI-HRMS m/z calcd For $C_{21}H_{22}N_3O_3$ $[M+H]^+$ 364.1656, found 364.1657.

*4.2.15.11. 2-Amino-2-(4-(2-(furan-2-yl)oxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19k**).* Yield: 97.5%; Light yellow solid; Mp: 187–190 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.20 (s, 1H), 7.69–7.67 (m, 3H), 7.26 (d, J = 7.8 Hz, 2H), 7.10 (d, J = 3 Hz, 1H), 6.60 (dd, J = 1.8, 1.5 Hz, 1H), 3.65 (s, 4H), 2.68–2.62 (m, 2H), 1.95–1.90 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 156.33, 146.49, 143.81, 142.86, 142.78, 135.09, 129.89, 129.83, 126.94, 113.31, 113.12, 62.52, 62.08, 34.49, 29.90; ESI-HRMS m/z calcd For $C_{18}H_{21}N_2O_4$ $[M+H]^+$ 329.1496, found 329.1503.

*4.2.15.12. 2-Amino-2-(4-(2-(thiophen-2-yl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19l**).* Yield: 95.9%; Yellow solid; Mp: 212–214 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.17 (s, 1H), 7.70–7.67 (m, 3H), 7.58 (d, J = 4.2 Hz, 1H), 7.25 (d, J = 8.1 Hz, 2H), 7.13 (dd, J = 4.8, 3.9 Hz, 1H), 3.64 (s, 4H), 2.67–2.61 (m, 2H), 1.95–1.89 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 159.71, 142.97, 142.72, 134.95, 130.55, 130.23, 130.12, 129.78, 129.45, 129.22, 126.90, 62.52, 62.08, 34.51, 29.91; ESI-HRMS m/z calcd For $C_{18}H_{21}N_2O_3S$ $[M+H]^+$ 345.1267, found 345.1268.

*4.2.15.13. 2-Amino-2-(4-(2-(4-methylbenzyl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19m**).* Yield: 84.7%; Yellow solid; Mp: 158–161 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.06 (s, 1H), 7.56 (d, J = 7.5 Hz, 2H), 7.21 (d, J = 7.5 Hz, 2H), 7.11–7.02 (m, 4H), 4.04 (s, 2H), 3.61 (s, 4H), 2.63–2.58 (m, 2H), 2.20 (s, 3H), 1.91–1.85 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.79, 142.57, 141.48, 137.93, 135.71, 133.51, 130.37, 129.98, 129.79, 129.61, 126.75, 62.52, 62.04, 34.73, 34.46, 29.86, 21.06; ESI-HRMS m/z calcd For $C_{22}H_{27}N_2O_3$ $[M+H]^+$ 367.2016, found 367.2014.

*4.2.15.14. 2-Amino-2-(4-(2-(4-methoxybenzyl)oxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19n**).* Yield: 79.3%; Yellow solid; Mp: 179–182 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.22 (d, J = 8.1 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 6.8 (d, J = 8.7 Hz, 2H), 4.06 (s, 2H), 3.68 (s, 3H), 3.61 (s, 4H), 2.64–2.58 (m, 2H), 1.91–1.85 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.47, 160.49, 143.07, 140.58, 136.14, 130.93, 129.91, 128.98, 127.83, 126.90, 115.27, 62.50, 62.06, 55.72, 34.44, 34.16, 29.88; ESI-HRMS m/z calcd For $C_{22}H_{27}N_2O_4$ $[M+H]^+$ 383.1965, found 383.1962.

*4.2.15.15. 2-Amino-2-(4-(2-(4-fluorobenzyl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19o**).* Yield: 55.9%; Yellow solid; Mp: 145–148 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.06 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.28–7.20 (m, 4H), 7.11–7.01 (m, 2H), 4.15 (s, 1H), 3.62 (s, 1H), 2.64–2.58 (m, 2H), 1.92–1.87 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 162.99, 141.12, 140.44, 134.26, 130.86, 130.82, 129.11, 129.02, 128.78, 128.36, 125.37, 124.19, 124.16, 115.12, 114.91, 61.11, 60.67, 33.08, 28.47, 27.13; ESI-HRMS m/z calcd For $C_{21}H_{24}FN_2O_3$ $[M+H]^+$ 371.1765, found 371.1776.

*4.2.15.16. 2-Amino-2-(4-(2-(4-chlorobenzyl)oxazol-4-yl)phenethyl) propane-1,3-diol hydrochloride (**19p**).* Yield: 67.5%; Yellow solid; Mp: 185–188 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.08 (s, 1H), 7.58 (d, J = 8.4 Hz, 2H), 7.25–7.20 (m, 6H), 4.10 (s, 2H), 3.62 (s, 2H), 2.64–2.58 (m, 2H), 1.92–1.86 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 165.07, 142.66, 141.60, 135.85, 135.43, 134.14, 131.45, 129.92, 129.85, 129.79, 126.78, 62.50, 62.06, 34.47, 34.41, 29.87; ESI-HRMS m/z calcd For $C_{21}H_{24}N_2O_3Cl$ $[M+H]^+$ 384.1470, found 384.1489.

*4.2.15.17. 2-Amino-2-(4-(2-(4-(trifluoromethyl)benzyl)oxazol-4-yl) phenethyl)propane-1,3-diol hydrochloride (**19q**).* Yield: 61.5%; Yellow solid; Mp: 100–104 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.09 (s, 1H), 7.60–7.56 (m, 4H), 7.45 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 7.8 Hz, 2H), 4.21 (s, 2H), 3.62 (s, 4H), 2.64–2.59 (m, 2H), 1.92–1.86 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 164.50, 142.59, 141.89, 141.35, 135.86, 130.54, 130.12, 129.77, 126.76, 126.66, 126.62, 62.50, 62.06, 34.87, 34,48, 29.87; ESI-HRMS m/z calcd For $C_{22}H_{24}F_3N_2O_3$ $[M+H]^+$ 421.1734, found 421.1739.

*4.2.15.18. 2-Amino-2-(4-(2-(4-isopropylbenzyl)oxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19r**).* Yield: 51.1%; Yellow solid; Mp: 145–148 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.10 (s, 1H), 7.54 (d, J = 8 Hz, 2H), 7.20 (d, J = 8 Hz, 2H), 7.11 (d, J = 8 Hz, 2H), 7.07 (d, J = 8 Hz, 2H), 4.07 (s, 2H), 3.56 (s, 4H), 2.75–2.71 (m, 1H), 2.61–2.57 (m, 2H), 1.88–1.84 (m, 2H), 1.08 (d, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.30, 149.28, 143.14, 140.43, 136.20, 133.18, 129.93, 129.82, 128.75, 127.87, 126.89, 62.46, 62.05, 35.00, 34.56, 34.39, 29.86, 24.38; ESI-HRMS m/z calcd For $C_{24}H_{31}N_2O_3$ $[M+H]^+$ 395.2329, found 195.2332.

*4.2.15.19. 2-Amino-2-(4-(2-pentyloxazol-4-yl)phenethyl)propane-1,3-diol hydrochloride (**19s**).* Yield: 80.0%; Yellow solid; Mp: 112–115 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.26 (s, 1H), 7.56 (d, J = 7.8 Hz, 2H), 7.25 (d, J = 7.8 Hz, 2H), 3.59 (s, 4H), 2.87 (t, J = 7.8 Hz, 2H), 2.64–2.58 (m, 2H), 1.90–1.84 (m, 2H), 1.74 (t, J = 6.9 Hz, 2H), 1.29–1.27 (m, 2H), 0.82 (t, J = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 168.52, 143.59, 139.23, 136.28, 130.07, 127.79, 127.01, 62.48, 62.05, 34.38, 32.27, 29.88, 28.55, 27.33, 23.22, 14.19; ESI-HRMS m/z calcd For $C_{19}H_{29}N_2O_3$ $[M+H]^+$ 333.2173, found 333.2164.

*4.2.16. General procedure for 2-amino-4-(4-(2-substituted oxazol-4-yl)phenyl)-2-(hydroxyl methyl)butyl dihydrogen phosphate (**20a**–**s**)*

20a–**s** was prepared using the same procedure as that described for compound **12a**–**p**.

*4.2.16.1. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(p-tolyl)oxazol-4-yl) phenyl)butyl dihydrogen phosphate (**20a**).* Yield: 62.5%; White solid; Mp: 246–248 °C; ^{1}H NMR (300 MHz, CD_3OD) δ8.20 (s, 1H), 7.93 (d, J = 7.8 Hz, 2H), 7.71 (d, J = 7.5 Hz, 2H), 7.29 (d, J = 7.8 Hz, 4H), 3.97 (m, 2H), 3.66 (s, 2H), 2.68 (m, 2H), 2.37 (s, 3H), 1.98 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 160.87, 141.00, 140.66, 140.28, 134.32, 129.32, 128.44, 128.23, 125.73, 125.13, 123.98, 64.619, 60.90, 59.10, 32.83, 27.74, 20.63; ESI-HRMS m/z calcd For $C_{21}H_{26}N_2O_6P$ $[M+H]^+$ 433.1523, found 433.1519.

*4.2.16.2. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-methoxyphenyl) oxazol-4-yl) phenyl)butyl dihydrogen phosphate (**20b**).* Yield: 47.6%; White solid; Mp: 238–240 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.15 (s, 1H), 7.96 (d, J = 8.1 Hz, 2H), 7.68 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.1 Hz, 2H), 3.94 (m, 2H), 3.80 (s, 3H), 3.66 (s, 2H), 2.65 (m, 2H), 1.94 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 165.81, 163.64, 143.15, 142.78, 135.23, 130.79, 130.13, 129.53, 127.18, 121.39, 115.74, 66.06, 62.82, 61.72, 56.26, 35.02, 30.15; ESI-HRMS m/z calcd For $C_{21}H_{26}N_2O_7P$ $[M+H]^+$ 449.1472, found 449.1476.

*4.2.16.3. 2-Amino-4-(4-(2-(4-fluorophenyl)oxazol-4-yl)phenyl)-2-(hydroxymethyl)butyl dihydrogen phosphate (**20c**).* Yield: 88.2%; White solid; Mp: 246–249 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.65 (s, 1H), 8.07 (m, 2H), 7.77 (d, J = 7.8 Hz, 2H), 7.42–7.36 (m, 2H), 7.30 (d, J = 7.5 Hz, 2H), 3.86 (m, 2H), 3.55 (s, 2H), 2.64 (m, 2H), 1.85 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 164.29, 162.64, 160.14, 141.45, 140.97, 135.26, 128.67, 128.60, 128.54, 128.46, 125.38, 123.46, 116.43, 116.29, 64.66, 60.97, 59.21, 33.10, 28.04; ESI-HRMS m/z calcd For $C_{20}H_{23}FN_2O_6P$ $[M+H]^+$ 437.1272, found 437.1267.

*4.2.16.4. 2-Amino-4-(4-(2-(4-chlorophenyl)oxazol-4-yl)phenyl)-2-(hydroxylmethyl)butyl dihydrogen phosphate (**20d**).* Yield: 58.8%; White solid; Mp: 249–252 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.69 (s,

1H), 8.03 (d, J = 7.8 Hz, 2H), 7.78 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 8.1 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 3.92 (m, 2H), 3.55 (s, 2H), 2.65 (m, 2H), 1.85 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 159.79, 141.14, 140.95, 135.17, 135.01, 128.96, 128.27, 127.50, 125.40, 125.18, 64.69, 60.90, 59.03, 32.79, 27.73; ESI-HRMS m/z calcd For $C_{20}H_{23}ClN_2O_6P$ $[M+H]^+$ 453.0977, found 453.0975.

*4.2.16.5. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-(trifluoromethyl) phenyl)oxazol-4-yl)phenyl) butyl dihydrogen phosphate (**20e**).* Yield: 83.3%; White solid; Mp: 247–249 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.77 (s, 1H), 8.24 (d, J = 7.2 Hz, 2H), 7.93 (d, J = 7.8 Hz, 2H), 7.81 (d, J = 7.5 Hz, 2H), 7.31 (d, J = 7.5 Hz, 2H), 3.94 (m, 2H), 3.56 (s, 2H), 2.64 (m, 2H), 1.86 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 159.51, 141.47, 141.29, 136.08, 130.42, 130.21, 130.16, 130.01, 128.64, 128.17, 126.70, 126.15, 126.13, 125.37, 124.73, 122.93, 64.79, 60.90, 59.02, 32.95, 27.89; ESI-HRMS m/z calcd For $C_{21}H_{23}F_3N_2O_6P$ $[M+H]^+$ 487.1240, found 487.1238.

*4.2.16.6. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-(trifluoromethoxy) phenyl)oxazol-4-yl)phenyl) butyl dihydrogen phosphate (**20f**).* Yield: 63.2%; White solid; Mp: 244–246 °C; ^{1}H NMR (400 MHz, DMSO) δ 8.72 (s, 1H), 8.17 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.32 (d, J = 7.8 Hz, 2H), 3.91 (m, 2H), 3.57 (s, 2H), 2.67 (m, 2H), 1.86 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 159.77, 149.77, 141.44, 141.14, 135.68, 128.69, 128.39, 128.23, 125.87, 125.42, 121.65, 120.83, 119.12, 64.89, 60.99, 59.12, 33.05, 27.98; ESI-HRMS m/z calcd For $C_{21}H_{23}F_3N_2O_7P$ $[M+H]^+$ 503.1190, found 503.1194.

*4.2.16.7. 2-Amino-4-(4-(2-(4-ethylphenyl)oxazol-4-yl)phenyl)-2-(hydroxylmethyl)butyl dihydrogen phosphate (**20g**).* Yield: 68.2%; White solid; Mp: 235–238 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.62 (s, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.1 Hz, 2H), 7.39 (d, J = 8.1 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 3.86 (m, 2H), 3.55 (s, 2H), 2.71–2.66 (m, 4H), 1.86 (m, 2H), 1.21 (t, J = 7.5 Hz, 3H); ^{13}C NMR (150 MHz, DMSO) δ 161.182, 146.90, 141.41, 140.93, 134.98, 128.75, 128.67, 126.22, 125.47, 124.46, 64.83, 61.07, 59.33, 33.19, 29.04, 28.16, 15.33; ESI-HRMS m/z calcd For $C_{22}H_{28}N_2O_6P$ $[M+H]^+$ 447.1679, found 447.1681.

*4.2.16.8. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-isopropylphenyl) oxazol-4-yl)phenyl)butyl dihydrogen phosphate (**20h**).* Yield: 38.5%; White solid; Mp: 236–239 °C; ^{1}H NMR (300 MHz, DMSO) δ 8.20 (s, 1H), 7.96 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 7.5 Hz, 2H), 7.35 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 8.1 Hz, 2H), 3.96 (m, 2H), 3.67 (s, 2H), 2.93 (m, 1H), 2.70–2.67 (m, 2H), 1.97 (m, 2H), 1.25–1.23 (m, 6H); ^{13}C NMR (150 MHz, CD_3OD) δ 153.64, 143.27, 142.82, 135.54, 130.14, 129.15, 128.42, 128.10, 127.93, 127.19, 126.46, 66.05, 62.79, 61.66, 35.72, 35.00, 30.15, 24.46; ESI-HRMS m/z calcd For $C_{23}H_{30}N_2O_6P$ $[M+H]^+$ 461.1836, found 461.1835.

*4.2.16.9. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-propylphenyl)oxazol-4-yl)phenyl)butyl dihydrogen phosphate (**20h**).* Yield: 62.5%; White solid; Mp: 235–237 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.20 (s, 1H), 7.94 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.5 Hz, 2H), 7.28 (d, J = 7.2 Hz, 4H), 3.95 (m, 2H), 3.66 (s, 2H), 2.63–2.61 (m, 4H), 1.97 (m, 2H), 1.65–1.62 (m, 2H), 0.91 (t, J = 6.9 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 162.32, 145.78, 141.57, 141.12, 133.84, 129.02, 128.76, 128.43, 126.09, 125.49, 124.65, 64.38, 61.09, 59.95, 37.50, 33.29, 28.43, 24.12, 16.95; ESI-HRMS m/z calcd For $C_{23}H_{30}N_2O_6P$ $[M+H]^+$ 461.1836, found 461.1823.

*4.2.16.10. 2-Amino-4-(4-(2-(4-cyanophenyl)oxazol-4-yl)phenyl)-2-(hydroxylmethyl)butyl dihydrogen phosphate (**20j**).* Yield: 58.8%; White solid; Mp: 260–263 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.28 (s, 1H), 8.12 (d, J = 7.8 Hz, 2H), 7.95 (d, J = 8.1 Hz, 2H), 7.73 (d, J = 7.2 Hz, 2H), 7.28 (d, J = 7.5 Hz, 2H), 3.94 (m, 2H), 3.66 (s, 2H), 2.66 (m, 2H), 1.94 (m, 2H); ^{13}C NMR (150 MHz, DMSO) δ 167.10, 160.27, 141.75, 141.28, 135.90, 135.64, 128.91, 128.71, 128.33, 128.28, 125.85, 125.37, 64.39, 60.99, 59.26, 33.23, 28.16; ESI-HRMS m/z calcd For $C_{21}H_{23}N_3O_6P$ $[M+H]^+$ 444.1319, found 444.1312.

*4.2.16.11. 2-Amino-4-(4-(2-(furan-2-yl)oxazol-4-yl)phenyl)-2-(hydroxymethyl)butyl dihydrogen phosphate (**20k**).* Yield: 71.4%; White solid; Mp: 235–237 °C; ^{1}H NMR (300 MHz, CD_3OD) δ8.24 (s, 1H), 7.72 (m, 3H), 7.30 (d, J = 8.1 Hz, 2H), 7.13 (s, 1H), 6.62 (s, 1H), 3.98 (m, 2H), 3.69 (s, 2H), 2.68 (m, 2H), 1.97 (m, 2H); ^{13}C NMR (151 MHz, CD_3OD) δ 154.93, 145.08, 142.43, 141.39, 133.68, 128.49, 125.51, 111.89, 111.71, 110.00, 64.37, 61.08, 59.94, 33.26, 28.43; ESI-HRMS m/z calcd For $C_{18}H_{22}N_2O_7P$ $[M+H]^+$ 409.1159, found 409.1152.

*4.2.16.12. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(thiophen-2-yl)oxazol-4-yl)phenyl)butyl dihydrogen phosphate (**20l**).* Yield: 58.5%; White solid; Mp: 240–242 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.18 (s, 1H), 7.71–7.68 (m, 3H), 7.59 (d, J = 4.5 Hz, 1H), 7.28 (d, J = 8.1 Hz, 2H), 7.14 (m, 1H), 3.96 (m, 2H), 3.67 (m, 2H), 2.70–2.67 (m, 2H), 1.97–1.95 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 158.29, 141.61, 141.29, 133.87, 133.54, 128.79, 128.67, 128.45, 128.02, 127.81, 125.47, 64.40, 61.15, 59.93, 33.33, 28.44; ESI-HRMS m/z calcd For $C_{18}H_{22}N_2O_6PS$ $[M+H]^+$ 425.0931, found 425.0927.

*4.2.16.13. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-methylbenzyl) oxazol-4-yl)phenyl)butyl dihydrogen phosphate (**20m**).* Yield: 40.0%; White solid; Mp: 221–223 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.06 (s, 1H), 7.60 (d, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 7.13 (d, J = 8.1 Hz, 2H), 7.08 (d, J = 8.4 Hz, 2H), 4.06 (s, 2H), 3.94 (m, 2H), 3.66 (s, 2H), 2.67–2.64 (m, 2H), 2.25 (s, 3H), 1.95 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 166.03, 142.72, 142.01, 138.22, 125.89, 133.99, 130.67, 130.52, 130.13, 129.92, 127.03, 66.08, 62.78, 61.70, 35.06, 34.97, 30.10, 21.36; ESI-HRMS m/z calcd For $C_{22}H_{28}N_2O_6P$ $[M+H]^+$ 447.1680, found 447.1669.

*4.2.16.14. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-methoxybenzyl) oxazol-4-yl)phenyl)butyl dihydrogen phosphate (**20n**).* Yield: 55.6%; White solid; Mp: 223–225 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.06 (s, 1H), 7.60 (d, J = 7.8 Hz, 2H), 7.25 (d, J = 7.8 Hz, 2H), 7.18 (d, J = 8.1 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 4.04 (s, 2H), 3.95 (m, 2H), 3.71 (s, 3H), 3.66 (s, 2H), 2.64 (m, 2H), 1.95 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 166.21, 160.64, 142.72, 142.00, 135.87, 131.09, 130.55, 130.13, 128.97, 127.03, 115.48, 66.09, 62.79, 61.71, 55.99, 34.97, 34.64, 30.11; ESI-HRMS m/z calcd For $C_{22}H_{28}N_2O_7P$ $[M+H]^+$ 463.1629, found 463.1618.

*4.2.16.15. 2-Amino-4-(4-(2-(4-fluorobenzyl)oxazol-4-yl)phenyl)-2-(hydroxylmethyl)butyl dihydrogen phosphate (**20o**).* Yield: 43.5%; White solid; Mp: 225–228 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.07 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.31–7.7.23 (m, 4H), 7.12–7.04 (m, 2H), 4.16 (s, 2H), 3.96–3.93 (m, 2H), 3.66 (s, 2H), 2.69–2.62 (m, 2H), 1.96–1.93 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 164.69, 163.57, 161.62, 142.74, 142.17, 135.96, 132.53, 130.72, 130.49, 130.11, 127.05, 125.89, 116.80, 116.63, 66.08, 62.78, 61.69, 34.96, 30.10, 28.82; ESI-HRMS m/z calcd For $C_{21}H_{25}N_2O_6FP$ $[M+H]^+$ 451.1410, found 451.1412.

*4.2.16.16. 2-Amino-4-(4-(2-(4-chlorobenzyl)oxazol-4-yl)phenyl)-2-(hydroxylmethyl)butyl dihydrogen phosphate (**20p**).* Yield: 43.7%; White solid; Mp: 220–222 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.09 (s, 1H), 7.61 (d, J = 7.8 Hz, 2H), 7.28–7.24 (m, 6H), 4.12 (s, 2H), 3.95 (m, 2H), 3.66 (s, 2H), 2.68–2.65 (m, 2H), 1.96–1.94 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 165.25, 142.78, 142.15, 136.04, 135.91, 134.40,

131.73, 130.46, 130.14, 127.03, 66.05, 62.79, 61.65, 34.97, 34.75, 30.11; ESI-HRMS *m/z* calcd For $C_{21}H_{25}N_2O_6ClP$ $[M+H]^+$ 467.1133, found 467.1132.

*4.2.16.17. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-(trifluoromethyl) benzyl)oxazol-4-yl)phenyl) butyl dihydrogen phosphate (**20q**).* Yield: 41.7%; White solid; Mp: 213–215 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.10 (s, 1H), 7.59 (m, 4H), 7.48 (m, 2H), 7.24 (d, J = 7.5 Hz, 2H), 4.22 (s, 2H), 3.94 (m, 2H), 3.65 (s, 2H), 2.64 (m, 2H), 1.93 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD) δ 164.79, 142.83, 142.26, 141.85, 136.15, 130.85, 130.45, 130.15, 127.05, 126.95, 66.06, 62.82, 61.65, 35.19, 35.00, 30.12; ESI-HRMS *m/z* calcd For $C_{22}H_{25}N_2O_6F_3P$ $[M+H]^+$ 501.1397, found 501.1404.

*4.2.16.18. 2-Amino-2-(hydroxymethyl)-4-(4-(2-(4-isopropylbenzyl) oxazol-4-yl)phenyl)butyl dihydrogen phosphate (**20r**).* Yield: 48.3%; Light yellow solid; Mp: 215–217 °C; ^{1}H NMR (400 MHz, CD_3OD) δ 8.44 (s, 1H), 7.68 (d, J = 7.6 Hz, 2H), 7.27–7.19 (m, 6H), 4.13 (s, 2H), 3.89 (m, 2H), 3.55 (s, 2H), 2.87 (m, 1H), 2.65–2.61 (m, 2H), 1.84–1.83 (m, 2H), 1.18 (d, J = 6.8 Hz, 6H); ^{13}C NMR (150 MHz, DMSO) δ 163.68, 147.46, 141.54, 140.12, 135.14, 133.58, 129.23, 129.14, 129.05, 127.00, 125.68, 65.10, 61.41, 59.74, 33.81, 33.58, 33.53, 28.48, 24.35; ESI-HRMS *m/z* calcd For $C_{24}H_{32}N_2O_6P$ $[M+H]^+$ 475.1993, found 475.2003.

*4.2.16.19. 2-Amino-2-(hydroxymethyl)-4-(4-(2-pentyloxazol-4-yl) phenyl)butyl dihydrogen phosphate (**20s**).* Yield: 45.5%; White solid; Mp: 228–230 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.06(S, 1H), 7.60 (d, J = 7.2 Hz, 2H), 7.26 (d, J = 6.9 Hz, 2H), 3.96 (m, 2H), 3.67 (s, 2H), 2.77 (m, 2H), 2.69 (m, 2H), 1.98 (m, 2H), 1.75 (m, 2H), 1.33 (m, 4H), 0.88 (m, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 167.66, 142.65, 141.77, 135.50, 130.65, 130.12, 127.02, 66.06, 62.80, 58.63, 34.99, 32.67, 30.11, 29.14, 28.18, 23.61, 18.67; ESI-HRMS *m/z* calcd For $C_{19}H_{30}N_2O_6P$ $[M+H]^+$ 413.1836, found 413.1839.

4.3. Biological evaluation

4.3.1. IP_1 functional assay

The CHO–$S1P_1$ and CHO–$S1P_3$ cells (purchased from Multispan) were plated into 384-well plates at 7×10^4 cells/well in Stimulation Buffer (containing LiCl). Then different concentrations of test agonists were added into each well and incubated at 37 °C in 5% CO_2 for 2 h. Then D2-labeled IP_1 and Ab-Cryp were subsequently added into each well in lysis buffer and incubated for 1 h at room temperature according to the manufacturer's instructions. The plates were read in EnVision reader (PE company), with data expressed as the ratio of 665 nm/615 nm fluorescence.

4.3.2. Determination of in vivo lymphopenia activity

For the assessment of lymphopenia activities of agonists in conscious rats, male Sprague–Dawley rats (200–220 g) were purchased from Beijing Vital River Laboratory Animal Technology Co, Ltd. The rats (three per group) were dosed through intragastric administration with different doses of test compound dissolved in DMSO. 10 μL of blood was withdrawn via tail vein and the peripheral blood lymphocyte counts at the time 0 h, 1 h, 4 h, 8 h, 12 h, 24 h and 48 h after administration were assessed using MEK-7222K hematology analyzer. The lymphopenia of selected compounds to the vehicle at 12 h postadministration was calculated.

4.3.3. Determination of heart rate

Male Sprague–Dawley rats (200–220 g) were purchased from Beijing Vital River Laboratory Animal Technology Co, Ltd. The conscious rats (three per group) were dosed through intragastric administration with 10 mg/kg of test compound dissolved in DMSO and vehicle. The heart rate at the time 0 h, 1 h, 4 h, 8 h, 12 h and 24 h after administration were assessed using intelligent non-invasive blood pressure measurement meter (Softron, Japan).

4.3.4. Pharmacokinetic study

Pharmacokinetics of test compounds in male SD rats was evaluated using an aliquot of blood collected at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h postdose. The blood was centrifuged to prepare plasma and the plasma concentration was measured using LC-MS/MS system consisted of an Agilent 1260 Series high performance liquid chromatography and an API 4000 triple quadruple mass spectrometer (AB SCIEX, USA). The column used was a Zorbax SB-C18 analytical column (3.5 μm, 100 mm × 2.1 mm, Agilent, Santa Clara, USA). Data fitting and pharmacokinetic parameter estimates were carried out using WinNonLin Software (Version 6.1, Pharsight Corporation).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.07.081.

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