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Design and Synthesis of Marine Phidianidine Derivatives as Potential Immunosuppressive Agents

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ABSTRACT: A series of novel marine phidianidine derivatives were designed, synthesized, and evaluated for their immunosuppressive activities during our search of potential immunosuppressive agents with high efficacy and low toxicity from marine sources. These compounds were tested for their inhibitory activity on Con A-induced T cells and LPS-induced B cells proliferation. Compounds **14a** and **18c**, displaying the most promising inhibitory effects and

low toxicities, were further found to possess immune-regulatory activities upon cross-linking of T cell receptor (TCR) and B cell receptor (BCR) on purified T and B cells, respectively.

■ INTRODUCTION

Over the past decades, immune disorders have been affecting a large population worldwide. Those patients are commonly subjected to abnormal immune responses and inflammation, manifesting with disturbance of immune cells populations, immune cells activation, upregulation of inflammatory mediators, and tissue damage.[1] Therapeutic immunosuppressants, *e.g.* cyclosporin A (CsA), mycophenolate mofetil (MMF), rapamycin, tacrolimus, are available for treating diverse autoimmune diseases, inflammation, and organ transplant, including rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, acute and chronic inflammation.[1,2] However, despite the undeniable clinical advantages, the aforementioned common immunosuppressive drugs, exemplified by CsA and MMF, were found to cause considerably serious side effects, such as renal or liver toxicity, infection, malignancy, and other adverse effects.[3-7] Thus, the discovery of novel immunosuppressants with high efficacy and low adverse effects remains a challengeable task.

In our continuous search of new potential immunosuppressive agents from marine sources, a screening of our compound library of marine natural products (MNPs) and derivatives was conducted, leading to the discovery of a series of novel immunologically active MNPs and their synthetic derivatives, such as xishacorenes A−C,[8] sarinfacetamide A,[9] and phidianidine analogs. Phidianidines A and B (**1** and **2**, Figure 1) are two novel indole alkaloids, bearing an uncommon 1,2,4-oxadiazole ring and a guanidine side chain, isolated from the marine opisthobranch mollusk *Phidiana militaris* in 2011.[10] Interestingly, these metabolites and their derivatives were

found to exhibit widespread biological activities, such as cytotoxic, neuroprotective, dopamine transporter (DAT) inhibitory, and protein tyrosine phosphatase-1B (PTP1B) inhibitory activities,[10-13] which are probably due to the unique combination of structure features, including the indole ring (A), the oxadiazole ring (B), and the guanidine side chain (C) (Figure 1). In fact, the 1,2,4-oxadiazole ring was proved to be responsible for the biological properties of some previously reported immunologically active compounds, such as **4a** and **4b** (Figure 1).[14,15] Therefore, in this study, phidianidine derivatives containing the 1,2,4-oxadiazole ring have been designed and synthesized for the evaluation of their immunosuppressive activity. Through the initial screening, compound **3a** (Figure 1), bearing a modification at the C-moiety of phidianidine B, was found to exhibit considerable inhibitory activity on lipopolysaccharide (LPS)-induced B cell proliferation with low toxicity. The intriguing activity of **3a** stimulated us for the further structure modification and biological study of related compounds toward immunosuppressive agents. In order to obtain simplified structures with improved activities, a structure-activity relationship (SAR)-based synthetic strategy was employed for the synthesis of phidianidine analogs towards novel and potential MNP-derived immunosuppressive agents. Herein, we report the design and synthesis of immunosuppressive phidianidine derivatives and their immune-regulatory activities.

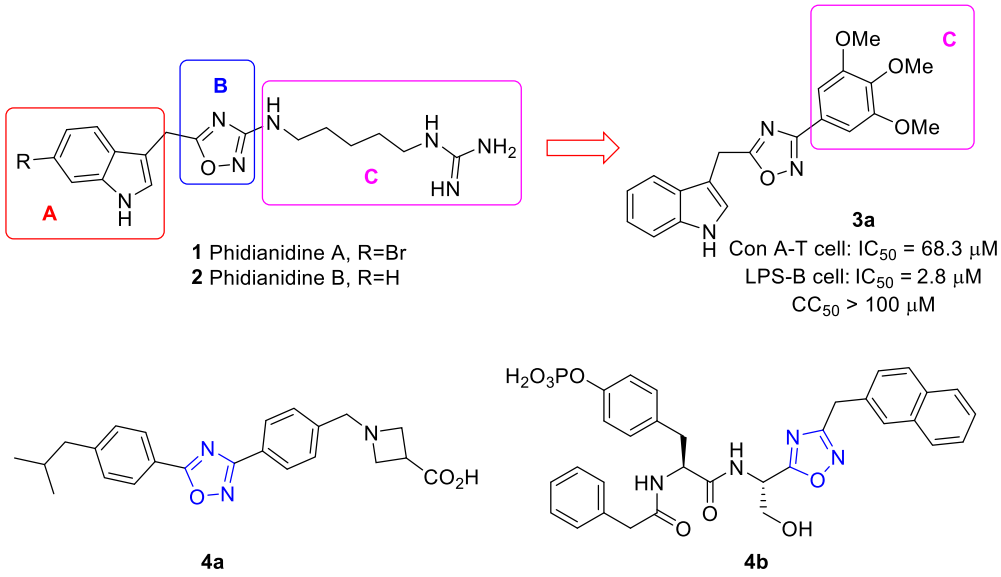


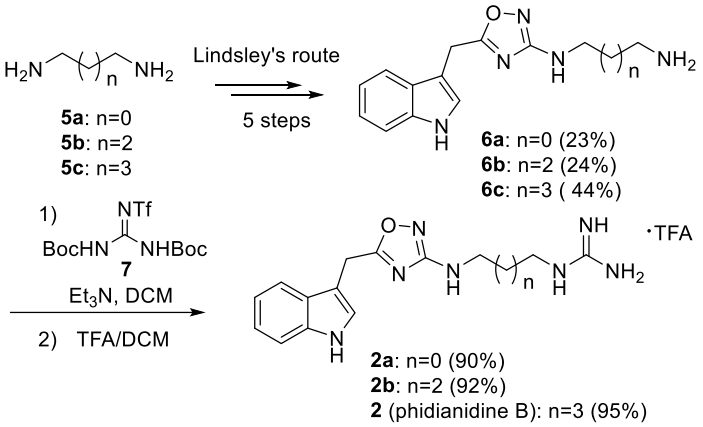
Figure 1 Structures of phidianidines, immunosuppressive analog **3a**, and structurally related immunosuppressive compounds (**4a** and **4b**).

■ RESULTS AND DISCUSSION

Chemistry. Four round syntheses have been carried out. The first round concerned the synthesis of the natural product and its analogs (Scheme 1) with different guanidine chain lengths, as initially to test their influence on the targeted activity. The second round was designed to simplify the synthesis by replacing the guanidine chain to various aryl rings (Scheme 2), to increase the hydrophobicity and to evaluate the importance of the guanidine moiety in the biological activity. These two-round syntheses were achieved before the screening, resulting in the discovery of **3a** as the lead compound for further structure modification. The third round was aiming to greatly shorten the synthetic steps for easily obtaining more analogs for biological evaluation. The last round was to replace the indole A-ring by a 3,4,5-trimethoxyphenyl ring since the latter was proved to be crucial in the activity during our biological activity evaluation.

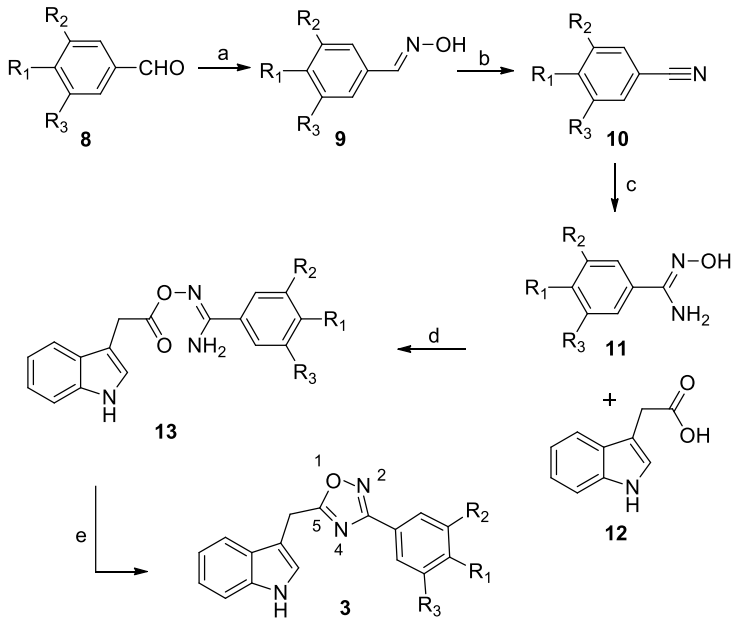
The TFA salts of the natural product phidianidine B (**2**) and its analogs **2a** and **2b** with shorter guanidine side chains, were initially synthesized according to Lindsley's synthetic route (Scheme

1).[11] The only difference was the use of more easily accessible 1,3-di-Boc-2-(trifluoromethylsulf onyl)guanidine (**7**) for the final guanidation in the last two steps.



Scheme 1 Synthetic route to phidianidine B and its analogs with different guanidine chain lengths.

In the second round, other phidianidine derivatives were designed to simplify the C moiety (Figure 1) by replacing the guanidine side chain to different aryl rings as shown in Scheme 2. The synthesis was similar as our previous reported route.[16] The aryl aldehyde **8** (**8a**–**8f**) first reacted with hydroxylamine hydrochloride ($NH_2OH·HCl$), in the presence of NaOH in EtOH/H_2O (1:1), to afford the oxime **9**. It was then dehydrated by dichloro(*p*-cymene)ruthenium(II) dimer in acetonitrile (CH_3CN), yielding the nitrile **10**,[17] which was further treated by $NH_2OH·HCl$ and $NaHCO_3$ in EtOH, to afford amidoxime **11**. Esterification of **11** with 3-indoleacetic acid (**12**) by using 2-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA) in CH_2Cl_2 gave compound **13**. Finally, an intramolecular cyclization of **13** in the presence of sodium acetate (NaOAc) in 30% EtOH (in H_2O) under reflux led to the oxadiazole product **3**.[18]

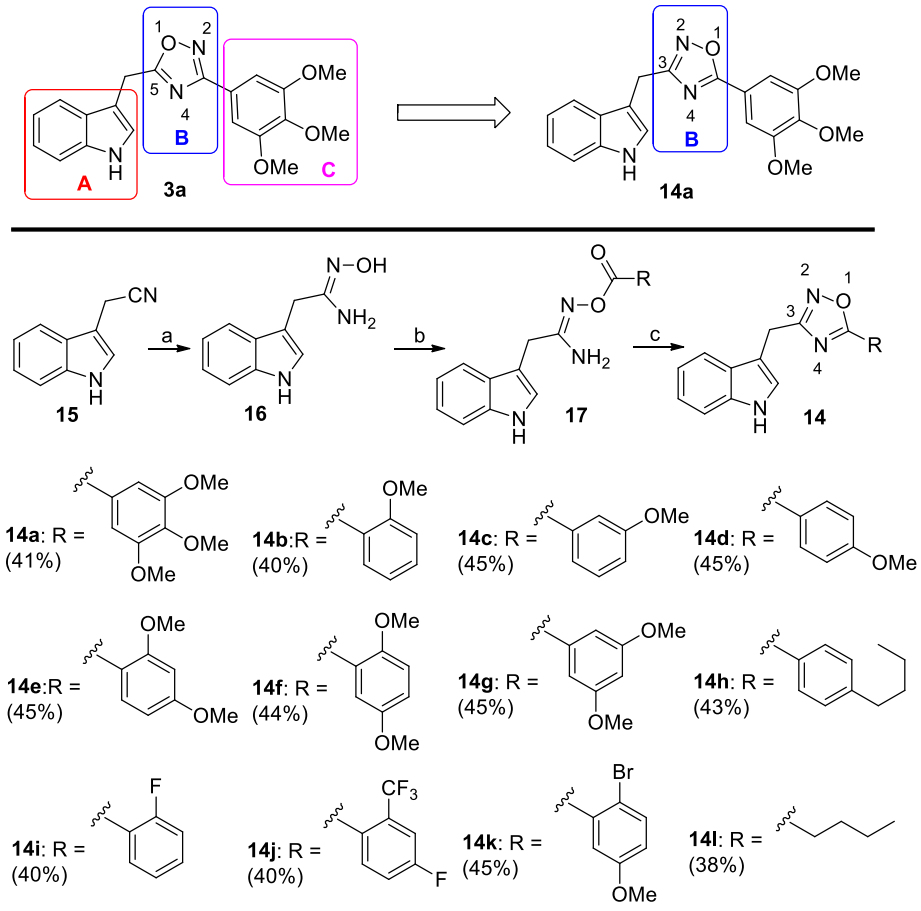


3a: $R_1 = R_2 = R_3 = OMe$, (31%); **3b**: $R_1 = R_2 = R_3 = H$, (35%); **3c**: $R_1 = F$, $R_2 = R_3 = H$, (28%); **3d**: $R_1 = Cl$, $R_2 = R_3 = H$, (28%); **3e**: $R_1 = NO_2$, $R_2 = R_3 = H$, (30%); **3f**: $R_1 = Et$, $R_2 = R_3 = H$, (30%)

Scheme 2 Reagent and conditions: (a) $NH_2OH \cdot HCl$, NaOH, 50% EtOH, rt., overnight; (b) Dichloro(p-cymene)ruthenium (II) dimer, CH_3CN, 80 °C, 4h; (c) $NH_2OH \cdot HCl$, $NaHCO_3$, EtOH, rt., 4h; (d) HATU, DIPEA, CH_2Cl_2, rt., 2h; (e) NaOAc, 30% EtOH, reflux, overnight.

As will be discussed in the biological part, compound **3a** displayed the strongest inhibitory activity against LPS-induced B cell proliferation, with no cytotoxicity over murine splenocytes. In order to rapidly obtain more analogs of **3a**, a simplified synthetic strategy was applied to flip the 1,2,4-oxadiazole ring of compound **3a**, placing the indole ring at the C-3 position in compound **14a**, as displayed in Scheme 3. Consequently, the synthetic sequence could be shortened to three steps, avoiding the costly ruthenium catalyst, and the yield could be greatly improved. Based on this new synthetic strategy, we initially synthesized compound **14a** as the isomer of **3a**. The similar immunosuppressive effects on LPS-induced B cell proliferation of both compounds encouraged us to design and synthesize a series of **14a** analogs. As for the synthetic

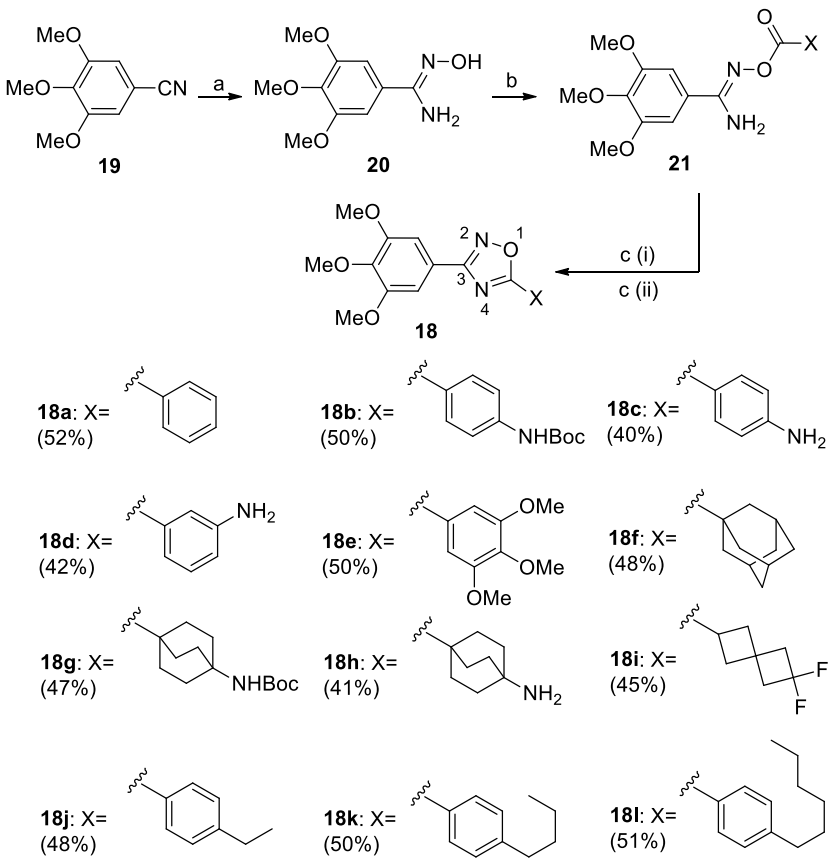
route, by using the similar method shown in Scheme 2, 3-indoleacetonitrile (**15**) was transformed to the amidoxime **16**, which then underwent an esterification with various carboxylic acids, followed by an immediate 1,2,4-oxadiazole ring closing under reflux, yielding a number of **14a** analogs **14b**–**14l** (Scheme 3).



Scheme 3 Reagent and conditions: (**a**) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH, 65 °C, 4h; (**b**) carboxylic acid, HATU, DIPEA, CH_2Cl_2, r.t., 2h; (**c**) NaOAc, 30% EtOH, reflux, overnight.

The last round synthesis was designed on the basis of the biological results of the previous synthesized compounds **14a**–**14l**, of which the 3,4,5-trimethoxyphenyl ring was associated to the best biological activity, and thus was used to replace the indole ring of phidianidine B for the A-moiety, whereas structural variations were introduced on the C-moiety, through aryl,

bicycloalkyl, adamantyl or spiroalkyl substituents. The synthetic steps (Scheme 4) towards **18a**–**18l** were similar as those shown in Scheme 3, with only the replacement of the starting material from 3-indoleacetonitrile (**15**) to 3,4,5-trimethoxybenzonitrile (**19**).



Scheme 4 Reagent and conditions: (**a**) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH, 65 °C, 4h; (**b**) carboxylic acid, HATU, DIPEA, CH_2Cl_2, r.t., h; (**c**) i, NaOAc, 30% EtOH, reflux, overnight. (**c**) ii, for **18c, 18d**, **18h**, TFA/CH_2Cl_2, (1:10 v/v), rt, 8h.

Biological Activity. The spleen functions like a large lymph node, which plays a critical role in the immune system. The proliferation of murine splenocytes, predominantly including T and B cells, could be triggered by concanavalin A (Con A) and LPS, respectively, which can be applied to evaluate the immunosuppressive activity of compounds *in vitro*. Ten phidianidine

derivatives, including phidianidine B (**2**), **2a**, **2b**, **3a**–**3f**, and **13a**, were initially assayed on the proliferation of T and B cells. Among them, **2**, **2a** and **2b**, the three compounds with guanidine side chains, showed no inhibitory activity at a concentration of 50 μM. However, among those phenyl substituted derivatives, compound **3a** exhibited remarkable inhibitory activity against LPS-induced B cells proliferation with IC_{50} value of 2.8 μM, but only low activity on Con A-induced T cells proliferation, and low cytotoxicity on murine splenocytes, at a concentration of 50 μM (Table 1). The above results indicated that the guanidine is not the optimal group for the activity. In addition, when comparing the activity of **3a** and its precursor **13a**, the results shown in Table 1 suggested that the opening of the oxadiazole ring would increase the cytotoxicity of the compounds, which is not compatible with the development of an immunosuppressive activity. Therefore, from these preliminary results, we deduced that the oxadiazole ring is needed for the immunosuppressive activity, while the guanidine group is not necessary.

Table 1 Immunosuppressive activity of compounds **3a**–**3f**, and **13a**.

No.	R	Cytotoxicity	Con A stimulation	LPS stimulation
		CC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
3a	OMe	>50	>50	2.8
3b	H	>50	>50	>50
3c	F	13.6	5.4	13.6
3d	Cl	42.8	22.6	>50
3e	NO_2	>50	>50	>50
3f	C_2H_5	24.4	7.5	13.9
13a	OMe	37.3	19.5	10.4
CsA[a]	–	1.2	0.04	0.4

[a] positive control, with the problem of high toxicity.

As discussed in the chemistry part, the simplified structural modification of compound **3a** led to its isomer **14a**, with only different connection patterns of the oxadiazole, while the synthetic

steps were greatly decreased and the yield was increased. It is worth noting that **14a** exhibited the similar effect against LPS-induced B cells proliferation as **3a**, with IC_{50} value of 3.1 μM (Table 2), which indicated that the linkage position of the 1,2,4-oxadiazole has no obvious influence on the activity. Moreover, **14a** also exhibited moderate inhibitory activity on the Con A-induced T cells proliferation with IC_{50} value of 11.0 μM. In addition, **17a**, the oxadiazole ring opening precursor of **14a**, was found to exhibit high cytotoxicity, which futher confirmed that the oxadiazole ring is an important group for the optimization of the biological profile (Table 2).

Table 2 Immunosuppressive activity of compounds **14a**–**14l**.

No.	Cytotoxicity CC_{50} (μM)	Con A stimulation IC_{50} (μM)	LPS stimulation IC_{50} (μM)
14a	>50	11.0	3.1
14b	42.2	16.7	11.1
14c	18.4	18.5	11.1
14d	>50	>50	>50
14e	9.3	8.6	4.7
14f	>50	>50	8.4
14g	>50	>50	13.9
14h	8.2	11.2	9.8
14i	>50	>50	>50
14j	40.6	15.7	9.7
14k	33.6	13.3	6.9
14l	>50	>50	>50
17a	10.1	11.9	6.8

Based on the above results, eleven **14a** analogs **14b**–**14l** were rapidly synthesized by replacing the 3,4,5-trimethoxyphenyl ring by other aromatic rings with either electron-donating groups (*e.g.* **14b**–**14h**) or electron withdrawing groups (*e.g.* **14i**–**14j**), or the combination of both groups (*e.g.* **14k**), or aliphatic group (*e.g.* **14l**). However, as shown in Table 2, none of them showed better activity than **14a**. Therefore, 3,4,5-trimethoxyphenyl ring was suggested to be the optimal group,

leading to the last round synthesis towards **18a**–**18l**. Among them, compound **18c** with a *p*-aminophenyl group at the C-moiety, showed significant inhibitory activities on both Con A-induced T cells proliferation and LPS-induced B cells proliferation, with IC_{50} values of 0.8 and 1.7 μM, respectively, and without significant cytotoxicity at a concentration of 50 μM. All the other compounds either showed no immunosuppressive activity or high cytotoxicity. Interestingly, for compound **18c**, when protecting its amino group to be –NHBoc group towards **18b** or changing the position of the amino group to be –*meta* towards **18d**, the cytotoxicity would be greatly increased (Table 3). It is also worth mentioning that, although the bicyclo[2,2,2]octane, adamantane, and spiro[3.3]heptane were generally considered to be good replacing candidates of aromatic rings in some bioactive molecules for increasing their druggability,[19,20] the molecules designed with these functionalities (*e.g.* **18f**–**18i**) showed high cytotoxicity in our case, which limited them for immunosuppressant applications.

Table 3 Immunosuppressive activity of compounds **18a**–**18l**.

No.	Cytotoxicity CC_{50} (μM)	Con A stimulation IC_{50} (μM)	LPS stimulation IC_{50} (μM)
18a	>50	>50	>50
18b	9.2	1.6	2.2
18c	>50	0.8	1.7
18d	8.2	7.0	5.5
18e	>50	5.5	>50
18f	26.5	2.7	18.8
18g	12.7	2.5	3.6
18h	25.8	4.8	4.1
18i	28.5	5.2	5.6
18j	31.3	>50	>50
18k	21.2	>50	36.2
18l	25.0	23.8	22.9

The synthesis of phidianidine derivatives, on the basis of our SAR analysis, resulted into the discovery of two easily synthesized immunosuppressive compounds **14a** and **18c** with low

cytotoxicity. In order to deeply understand the mechanism of the immunosuppressive activity of the two compounds, further investigation of **14a** and **18c** on T cell receptor (TCR) and B cell receptor (BCR)-mediated immune responses were evaluated upon cross-linking of TCR and BCR on purified murine $CD4^+$ T cells and $CD19^+$ B cells, stimulated by anti-CD3/CD28 Abs and anti-IgM/CD40 Abs, respectively. Abnormal T cells and B cells activation have been implicated in mediating multiple aspects of inflammatory and autoimmune diseases and cytokines play a central role in the initiation and regulation of immune responses which may involve one or more biological effect including cell division, proliferation, migration and further effector functions. *In vitro* stimulation with anti-CD3/CD28 mAbs could mimic the physiologic cross-linking of TCR and induce the proliferation and activation of T cells. Similarly, cross-linking of BCR plus CD40L lead to proliferation and activation of B cells.[21,22]

The biological results showed that both compounds **14a** and **18c** dramatically suppressed primary $CD4^+$ T cells proliferation induced by anti-CD3/CD28 (Figure 2A). Inflammatory cytokines IL-2 and IFN-γ have been proven to promote inflammation and tissue injury in multiple diseases. Therefore, we also determined IL-2 and IFN-γ production upon T cells activation by ELISA. Both **14a** and **18c** showed remarkable suppression effects on IL-2 and IFN-γ production stimulated with anti-CD3/CD28 for 24 h (Figure 2B and 2C). CD25, also known as IL-2RA, is generally regarded as the activation surface marker of T cells. Interestingly, compounds **14a** and **18c** significantly downregulated the percentage of CD25 expression (Figure 2D), suggesting that both compounds exhibited potent suppressive activity on T cell functions. As demonstrated in Figure 2D, compound **18c** exhibited better effects than compound **14a**, which is in agreement with *in vitro* results for immunosuppressive activity (IC_{50} 11.0 μM and 0.8 μM for **14a** and **18c**, respectively).[21]

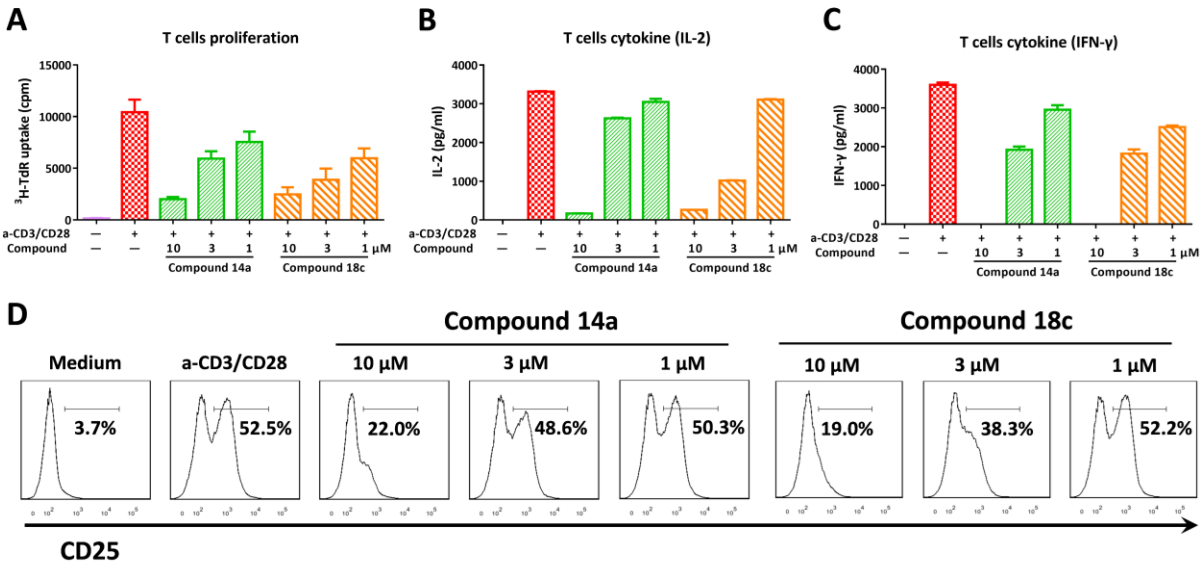


Figure 2. The inhibitory effects of compounds **14a** and **18c** on anti-CD3/CD28-induced $CD4^+$ T cells proliferation, activation and cytokines production. **A**: Primary $CD4^+$ T cells (4×10^5/well) were cultured with plate-coated anti-CD3 (5 μg/ml) anti-CD28 mAb (2 μg/ml) and in the absence or presence of compounds (10, 3, and 1 μM) in 96-well plates for 48 h to determine T cells proliferation. **B** and **C**: After stimulation with anti-CD3/CD28 mAbs for 24 h, the culture supernatants were collected and assayed for release of inflammatory cytokines. D: Flow cytometry analysis was performed to determine the percentage of T cells activation marker CD25.

Ligation of BCR induces signals that are critical for activation of the quiescent B cells to proliferate and differentiate into plasma cells. For B cells function, compounds **14a** and **18c** strikingly inhibited $CD19^+$ B cells proliferation induced by anti-IgM/CD40 Abs (Figure 3A) and also showed regulatory effects on cytokines production, manifesting decreasing IL-6 and increasing IL-10 secretion, in which IL-6 serves as an inflammatory cytokine, while IL-10 bearing an anti-inflammatory property (Figure 3B and 3C). In B cells, membrane-bound IgD

serves as a fundamental activation marker and when B cells were activated, the expression of IgD would decrease or disappear. Consistently, we found that the level of IgD was downregulated after stimulation with anti-IgM/CD40 mAbs, while **14a** and **18c** could reverse this effect (Figure 3D).[22] Therefore, the above biological investigation indicated that compounds **14a** and **18c** exhibited the potent immunosuppressive effects *in vitro*, including T and B cells proliferation, activation and cytokines production.

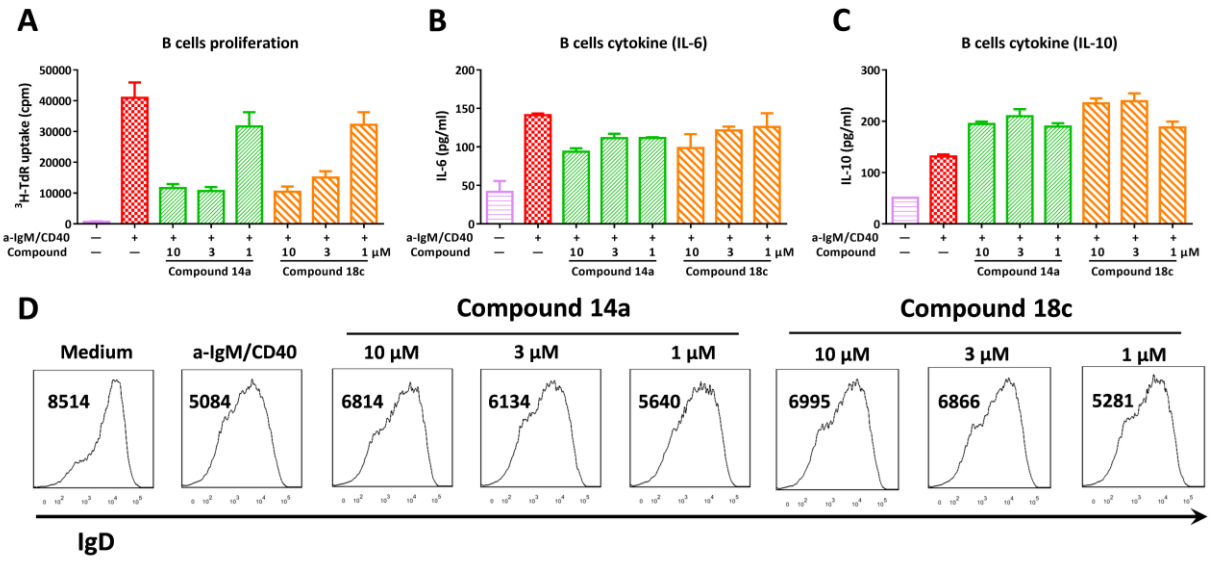


Figure 3. The regulatory effects of compounds **14a** and **18c** on the proliferation, activation and cytokines production from anti-IgM/CD40-primed CD19$^+$ B cells. **A**: Primary CD19$^+$ B cells were cultured with F(ab')2 anti-mouse IgM (10 μg/ml) and purified hamster anti-mouse CD40 mAb (1 μg/ml) in the absence or presence of compounds (10, 3, and 1 μM) in 96-well plates for 48 h to determine B cells proliferation. **B** and **C**: After 24 h incubation, the culture supernatants were collected and assayed for release of IL-6 and IL-10. D: The cells were collected and determined the expression of IgD and data were shown as the mean fluorescence intensity (MFI).

■ CONCLUSION

In summary, a series of novel phidianidine derivatives, together with some previously reported ones, were designed and synthesized on the basis of several rounds of SAR analysis towards potential immunosuppressive agents. The synthetic steps of the target compounds were greatly reduced and the overall yields were improved to be 40%−50%, compared to the synthesis of the original natural products. Their inhibitory activity on Con A-induced T cells and LPS-induced B cells proliferation, as well as their cytotoxicity on murine splenocytes, were evaluated in comparison with the positive control CsA *in vitro*. Among them, **14a** and **18c** exhibited the most remarkable inhibitory activity on T and B cells proliferation, with very low cytotoxicity. The SAR analysis indicated that the 1,2,4-oxadiazole ring and the 3,4,5-trimethoxyphenyl ring were important for the activity. It is worth mentioning that, although CsA has been used as an immunosuppressive drug, the high cytotoxicity (CC_{50} = 1.2 μM) limited its medicinal application.[3,4] Comparing to CsA, the toxicity of our compounds **14a** and **18c** are much lower (CC_{50} > 50 μM), and their selectivity index (SI = CC_{50}/IC_{50}) are even higher than CsA, especially on the LPS-induced B cells proliferation ($SI_{\mathbf{CsA}}$ = 3.0; $SI_{\mathbf{14a}}$ > 16.1; $SI_{\mathbf{18c}}$ > 29.4). The SI of **18c** on Con A-induced T cells proliferation is also higher than the positive control CsA ($SI_{\mathbf{CsA}}$ = 30.0; $SI_{\mathbf{18c}}$ > 62.5). All these data indicate the promising immunosuppressive activity of both compounds. In the light of such intriguing activity of **14a** and **18c**, they were further investigated for their regulatory effects on TCR and BCR-mediated immune responses, which demonstrated that both **14a** and **18c** showed dramatic effects on proliferation, activation and cytokines release of T and B cells (Figure 4). Since redundancy activation of immune system and inflammation have been consistently regarded as the jokers in immune disorders, our findings may provide an alternative strategy for the discovery of novel immunosuppressants in clinical application. Compounds **14a** and **18c**, as two lead compounds for the treatment of immune disorders, will be further studied *in vivo* for their targeted immunological diseases.

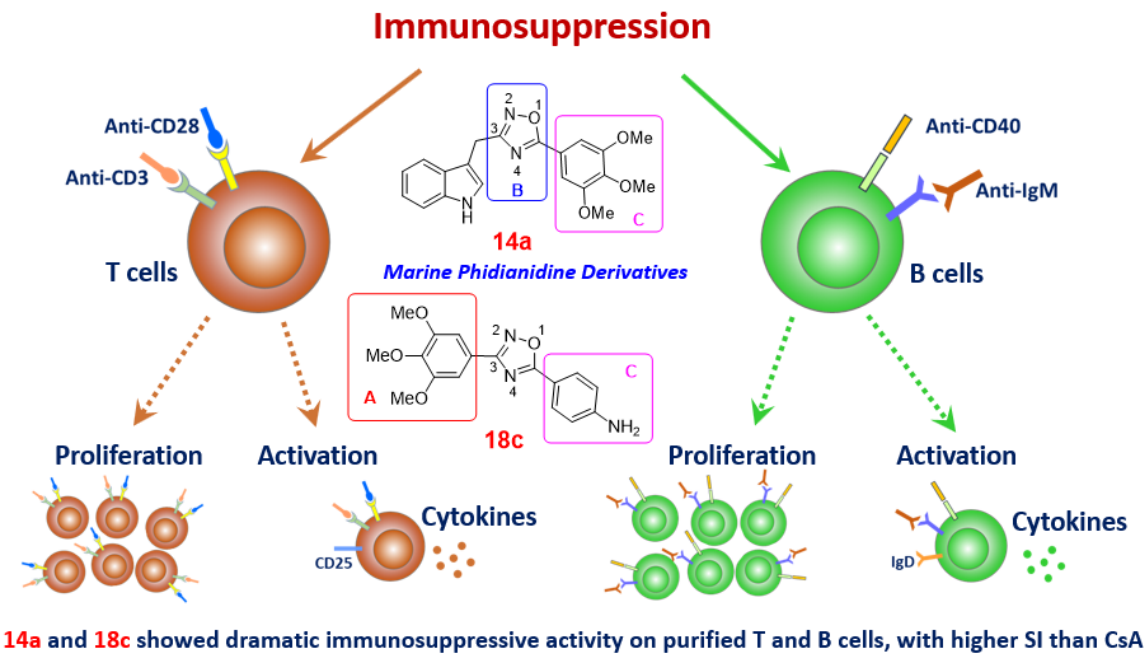


Figure 4. Marine phidianidine derived **14a** and **18c** with promising regulatory effects on TCR and BCR-mediated immune responses.

■ EXPERIMENTAL SECTION

1. Chemistry. All reagents and solvents were purchased from common commercial suppliers and were used without further purification. ^{1}H NMR spectra were recorded at 400 MHz, and ^{13}C NMR were recorded at 125 MHz on a Bruker NMR spectrometer instrument (Avance 400 for ^{1}H, Avance 500 for ^{13}C, Bruker Biospin AG, Uster, Switzerland). All ^{1}H and ^{13}C NMR shifts are reported in δ units (ppm) relative to the signals for $CHCl_3$ (δ_H 7.26, δ_C 77.16), and MeOH (δ_H 3.31, δ_C 49.00). All coupling constants (J values) are reported in hertz (Hz). NMR abbreviations are as follows: bs, broadened singlet; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and dd, doublet of doublets. Thin-layer chromatography (TLC) was performed on pre-coated silica-gel plates (HSGF254, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). ESI-MS spectra were recorded on a Q-TOF Micromass spectrometer (1290-6545 UHPLC-QTOF, Micromass, Wythenshawe, UK). Column chromatography was performed using a 200−300 mesh

silica gel (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). All final compounds were determined to have purities of >95% by one of the aforementioned methods, in combination with the high performance liquid chromatography (HPLC).

1.1. General Procedure for the Synthesis of 2, 2a and 2b. Compounds **6a**−**6c** were synthesized from the diamines **5a**−**5c** in 5 steps, according to Lindsley's synthetic route.[11] To a CH_2Cl_2 solution of compounds **6a**−**6c** (1.0 mmol) was successively added triethylamine (2.0 mmol) and 1,3-di-Boc-2-(trifluoromethylsulfonyl)guanidine (**7**, 1.2 mmol). The reaction mixture was stirred at room temperature overnight, which was then stopped and washed by sat. aq. $NaHCO_3$ (10 mL x 2) and water (10 mL x 1), dried over anhydrous $MgSO_4$ and concentrated. The residue was subjected to silica gel chromatography with petroleum ether (PE)/ CH_2Cl_2 (1:1) to give the Boc protected guanidine compounds, which was then deprotected by dissolving in 10 mL of a 9:1 (v/v) solution of CH_2Cl_2 and trifluoroacetic acid (TFA). The solution was stirred at ambient temperature for 8 h, and the reaction mixture was diluted with 8 mL of MeOH and concentrated *in vacuo* to afford compound **2**, **2a** and **2b**, respectively. Phidianidine B (**2**) has been previously described by Guo and Gavagnin.[10]

1-(2-((5-((1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)ethyl)guanidine (**2a**). Brown solid; yield 21% (Start from compound **5a** over 7 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.53 (d, *J* = 7.9 Hz, 1H), 7.36 (d, *J* = 8.2 Hz,1H), 7.22 (s, 1H), 7.11 (t, *J* = 7.6 Hz,1H), 7.02 (t, *J* = 7.5 Hz, 1H), 4.24 (s, 2H), 3.35 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD): δ 179.8, 170.1, 158.9, 138.1, 128.1, 124.7, 122.7, 120.1, 119.2, 112.4, 108.1, 42.9, 41.4, 24.1; HR-ESI: $[M+H]^+$ calcd for $C_{14}H_{18}N_7O$ 300.1567, found: 300.1567.

1-(4-((5-((1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)butyl)guanidine (2b). Brown solid; yield 22% (Start from compound **5b** over 7 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.53 (d, *J* = 8.0 Hz, 1H), 7.36 (d, *J* = 7.9 Hz,1H), 7.22 (s, 1H), 7.11 (t, *J* = 7.3 Hz,1H), 7.01 (t, *J* = 7.4 Hz, 1H), 4.22 (s, 2H), 3.18 (m, 4H), 1.63 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD): δ 179.4, 170.0,

158.6, 138.1, 128.1, 124.7, 122.7, 120.1, 119.2, 112.4, 108.1, 43.4, 42.1, 27.3, 27.2, 24.1; HR-ESI: $[M+H]^+$ calcd for $C_{16}H_{22}N_7O$ 328.1880, found: 328.1871.

1.2. General Procedure for the Synthesis of compounds 13a−13f. To a solution of aldehyde **8** (1.0 mmol) in EtOH (5 mL) was added hydroxylamine hydrochloride (1.2 mmol) and sodium acetate (2.0 mmol). The mixture was stirred at room temperature overnight. EtOH was removed *in vacuo*. The residue was added water (5 mL) and extracted with ethyl acetate (3×5ml), washed with brine (3×5ml), dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was subjected to silica gel chromatography with petroleum ether (PE)/CH_2Cl_2 (3:2) to give oxime **9**. To a solution of oxime **9** (1.0 mmol) in acetonitrile (5 mL) was added $[Ru_2(p\text{-}PriC_6H_4Me)_2(\mu\text{-}Cl)Cl]_2$ (0.05 mmol) and refluxed for 4 h. The mixture was filtered and the filtrate was concentrated in *vacuo*. The residue was subjected to silica gel chromatography with PE/CH_2Cl_2 (2:1) to give nitrile **10**. To a solution of nitrile **10** (1.0 mmol) in EtOH (5 mL) was added hydroxylamine hydrochloride (1.2 mmol) and $NaHCO_3$ (2.0 mmol). The mixture was refluxed for 4 h. The reaction mixture was diluted with EtOAc, filtered and concentrated *in vacuo*. The residue was added water (5 mL) and extracted with ethyl acetate (3×5 mL), washed with brine (1×5 mL), dried over anhydrous $MgSO_4$ and concentrated. The residue was subjected to silica gel chromatography with EtOAc/MeOH (9:1) to give amidoxime **11**. To a solution of 3-indoleacetic acid (1.0 mmol) in CH_2Cl_2 (5 mL) was added DIPEA (1.3 mmol) and HATU (1.0 mmol) and stirred for 30 min, then amidoxime **11** (1.0 mmol) in CH_2Cl_2 (2 mL) was added and stirred for 2 h. The mixture was filtered and the residue was washed with CH_2Cl_2, the solution was combined and concentrated. The residue was subjected to silica gel chromatography with PE/EtOAc (2:1) to give **13**. The spectroscopic data of **13b**−**13f** has been previously reported.[16]

((2-(1H-indol-3-yl)acetoxy)amino)(3,4,5-trimethoxyphenyl)methanamine (13a). White solid; yield 34% (Start from compound **8a** over 4 steps);^{1}H NMR (400 MHz, CD_3OD): δ 7.65 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 8.1 Hz, 1H), 7.25 (s, 2H), 7.12 (t, 1H), 7.05 (m, 3H), 3.96 (s, 2H), 3.86 (s, 6H), 3.77 (s, 3H); ^{13}C NMR (400 MHz, CD_3OD): δ 172.2, 159.6, 154.5, 141.3, 138.1,

128.6, 128.1, 124.8, 122.6, 120.0, 119.4, 112.4, 108.4, 105.7, 61.1, 56.7, 31.0; HR-ESI: $[M+H]^+$ calcd for $C_{20}H_{22}N_3O_5$ 384.1554, found: 384.1550.

1.3. General Procedure for the Synthesis of compounds 3a−3f. A solution of **13** (1.0 mmol) and sodium acetate (2.0 mmol) in 30% EtOH/H_2O (5 mL) was refluxed overnight. The EtOH was then removed *in vacuo*, the residue was added water (5 mL) and extracted with ethyl acetate (3×5 mL), washed with brine (1×5 mL), dried over anhydrous $MgSO_4$ and concentrated. The residue was subjected to silica gel chromatography with PE/EtOAc (5:1) to give compound **3**. The spectroscopic data of **3b**−**3f** has been previously reported.[16]

5-((1H-indol-3-yl)methyl)-3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole 3a. White solid; yield 90% ; ^{1}H NMR (400 MHz, CD_3OD): δ 7.57 (d, *J* = 7.8 Hz, *1*H), 7.37 (d, *J* = 8.2 Hz, 1H), 7.35 (s, 2H), 7.29 (s, 1H), 7.13 (t, *J* = 7.5 Hz, 1H), 7.04 (t, *J* = 7.5 Hz, 1H), 4.47 (s, 2H), 3.89 (s, 6H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 181.2, 169.4, 155.0, 141.8, 138.1, 128.2, 124.8, 123.5, 122.8, 120.2, 119.1, 112.5, 108.1, 105.8, 61.2, 56.7, 24.1; HR-ESI: $[M-H]^-$ calcd for $C_{20}H_{18}N_3O_4$ 364.1303, found: 364.1294.

1.4. General Procedure for the Synthesis of compounds 14a−14l and 18a−18l. To a stirred solution of the nitrile **15** or **19** (1.0 mmol) in EtOH (5 mL) were added hydroxylamine hydrochloride (1.5 mmol) and $NaHCO_3$ (3.0 mmol). The mixture was stirred for 4 h at 65℃. The reaction mixture was diluted with EtOAc, filtered and concentrated in vacuo. The residue was added water (5 mL) and extracted with ethyl acetate (3×5 mL), washed with brine (1×5 mL), dried over anhydrous $MgSO_4$ and concentrated. The residue was subjected to silica gel chromatography with EtOAc /MeOH (9:1) to give amidoxime **16** or **20**. To a solution of carboxylic acid (1.0 mmol) in CH_2Cl_2 (5 mL), were added DIPEA (1.3 mmol) and HATU (1.0 mmol), the reaction mixture was stirred for 30 min, after which amidoxime **16** or **20** (1.0 mmol) in CH_2Cl_2 (2 mL) was added and stirred for 2 h. The mixture was filtered and the residue was washed with CH_2Cl_2, the solution was combined and concentrated. The residue was subjected to silica gel chromatography with PE/EtOAc (2:1) to give compound **17** or **21**. A solution of

compound **17** or **21** (1.0 mmol) and sodium acetate (2.0 mmol) in 30% EtOH/H_2O (5 mL), was refluxed overnight. The EtOH was removed *in vacuo*, and the residue was added water (5 mL) and extracted with ethyl acetate (3×5 mL), washed with brine (1×5 mL), dried over anhydrous $MgSO_4$ and concentrated. The residue was subjected to silica gel chromatographic with PE/EtOAc (5:1) to give compound **14** or **18a**, **18b**, **18e**−**18g**, **18i**−**18l**. For **18c**, **18d**, and **18h**: Compound **18b**, **NBoc-18d**, or **18g** (1.0 mmol) was dissolved in 5 mL of a 9:1 (v/v) solution of CH_2Cl_2 and trifluoroacetic acid, and the solution was stirred at ambient temperature for 8 h. Afterwards, the black-colored reaction mixture was diluted with 8 mL of MeOH and concentrated *in vacuo* to afford compound **18c**, **18d**, and **18h**, respectively. The spectroscopic data of **14d**,[16] **14h**,[16] **14i**,[16] **14l,**[16] **18e**[23] **and 18j**[24] has been previously reported.

3-((1H-indol-3-yl)methyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (14a). White solid; yield 41% (Start from compound **15a** over 3 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.57 (d, *J* = 8.0 Hz, 1H), 7.40 (s, 2H), 7.35 (d, *J* = 8.2 Hz,1H), 7.23 (s, 1H), 7.10 (td, *J* = 7.5, 1.0 Hz, 1H), 7.00 (td, *J* = 7.5, 1.0 Hz,1H), 4.25 (s, 2H), 3.90 (s, 6H), 3.83 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.9, 171.9, 155.1, 143.4, 138.2, 128.4, 124.6, 122.6, 120.4, 119.9, 119.3, 112.4, 109.7, 106.5, 61.2, 56.8, 23.4; HR-ESI: $[M+H]^+$ calcd for $C_{20}H_{20}N_3O_4$ 366.1376, found: 366.1449.

3-((1H-indol-3-yl)methyl)-5-(2-methoxyphenyl)-1,2,4-oxadiazole (**14b).** White solid; yield 40% (Start from compound **15b** over 3 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.95 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.58 (td, *J* = 7.8, 1.8 Hz, 1H), 7.34 (d, *J* = 8.1 Hz,1H), 7.21 (s, 1H), 7.20 (d, *J* = 9.6 Hz, 1H), 7.08 (m, 2H), 7.01 (td, *J* = 7.0, 1.0 Hz, 1H), 4.27 (s, 2H), 3.94 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.4, 171.2, 160.0, 138.2, 135.6, 132.3, 128.4, 124.5, 122.6, 121.8, 119.9, 119.4, 114.2, 113.5, 112.3, 109.9, 56.4, 23.4; HR-ESI: $[M+H]^+$ calcd for $C_{18}H_{16}N_3O_2$ 306.1164, found: 306.1241.

3-((1H-indol-3-yl)methyl)-5-(3-methoxyphenyl)-1,2,4-oxadiazole (**14c).** White solid; yield 45% (Start from compound **15c** over 3 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.65 (d, *J* = 7.7

Hz, 1H), 7.59 (m, 2H), 7.42 (t, *J* = 8.0 Hz, 1H), 7.34 (d, *J* = 8.1 Hz,1H), 7.22 (s, 1H), 7.15 (ddd, *J* = 8.4, 2.6, 1.0 Hz, 1H), 7.10 (td, *J* = 7.6, 1.0 Hz, 1H), 7.01 (td, *J* = 7.5, 1.0 Hz, 1H), 4.25 (s, 2H), 3.83 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 177.0, 172.0, 161.6, 138.2, 131.5, 128.4, 126.4, 124.6, 122.6, 121.3, 120.2, 119.9, 119.4, 113.7, 112.3, 109.8, 56.0, 23.4; HR-ESI: $[M+H]^+$ calcd for $C_{18}H_{16}N_3O_2$ 306.1164, found: 306.1241.

3-((1H-indol-3-yl)methyl)-5-(2,4-dimethoxyphenyl)-1,2,4-oxadiazole (14e). White solid; yield 45% (Start from compound **15e** over 3 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.90 (d, *J* = 8.7 Hz, 1H), 7.59 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.20 (s, 1H), 7.09 (td, *J* = 7.0, 1.0 Hz, 1H), 7.00 (td, *J* = 7.5, 1.0 Hz, 1H), 6.63 (m, 2H), 4.22 (s, 2H), 3.91 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.3, 171.0, 166.5, 161.7, 138.1, 133.7, 128.4, 124.5, 122.6, 119.9, 119.4, 112.3, 110.0, 107.3, 106.8, 99.7, 56.4, 56.2, 23.4; HR-ESI: $[M+H]^+$ calcd for $C_{19}H_{18}N_3O_3$ 336.1270, found: 336.1350.

3-((1H-indol-3-yl)methyl)-5-(2,5-dimethoxyphenyl)-1,2,4-oxadiazole (**14f).** White solid; yield 44% (Start from compound **15f** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 3.1 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.20 (m, 2H), 7.13 (td, *J* = 7.4, 1.0 Hz, 1H), 7.08 (dd, *J* = 9.1, 3.2 Hz, 1H), 6.98 (d, *J* = 9.2 Hz, 1H), 4.32 (s, 2H), 3.90 (s, 3H), 3.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.8, 168.9, 153.5, 153.0, 136.4, 127.4, 123.2, 122.4, 120.6, 119.8, 119.3, 115.4, 114.0, 111.3, 110.3, 56.9, 56.1, 23.0; HR-ESI: $[M+H]^+$ calcd for $C_{19}H_{18}N_3O_3$ 336.1270, found: 336.1347.

3-((1H-indol-3-yl)methyl)-5-(3,5-dimethoxyphenyl)-1,2,4-oxadiazole (14g). White solid; yield 45% (Start from compound **15g** over 3 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.58 (d, *J* = 7.9 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.22 (m, 3H), 7.09 (td, *J* = 7.6, 1.0 Hz, 1H), 7.01 (td, *J* = 7.5, 1.0 Hz, 1H), 6.71 (t, *J* = 2.3 Hz, 1H), 4.25 (s, 2H), 3.82 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ 177.0, 172.0, 162.9, 138.2, 128.4, 126.8, 124.6, 122.6, 119.9, 119.3, 112.3, 109.8, 106.7, 106.2, 56.1, 23.4; HR-ESI: $[M+H]^+$ calcd for $C_{19}H_{18}N_3O_3$ 336.1270, found: 336.1350.

3-((1H-indol-3-yl)methyl)-5-(4-fluoro-2-(trifluoromethyl)phenyl)-1,2,4-oxadiazole (14j). White solid; yield 40% (Start from compound **15j** over 3 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 8.03(dd, *J* = 8.7, 5.3 Hz, 1H), 7.73 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.54 (td, *J* = 8.3, 2.6 Hz, 1H), 7.34 (d, *J* = 8.1 Hz, 2H), 7.21 (s, 1H), 7.10 (td, *J* = 7.1, 1.0 Hz, 1H), 7.01 (td, *J* = 7.1, 1.0 Hz, 1H), 4.29 (s, 2H); ^{13}C NMR (125 MHz, CD_3OD): δ 174.8, 172.0, 165.7, 138.1, 136.1, 132.7, 128.4, 124.9, 124.5, 122.6, 120.7, 119.9, 119.4, 116.3, 112.3, 109.7, 23.3; HR-ESI: $[M+H]^+$ calcd for $C_{18}H_{12}F_4N_3O$ 362.0911, found: 362.0919.

3-((1H-indol-3-yl)methyl)-5-(2-bromo-5-methoxyphenyl)-1,2,4-oxadiazole (14k). White solid; yield 45% (Start from compound **15k** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, *J* = 7.8 Hz, 1H), 7.60 (d, *J* = 8.9 Hz, 1H), 7.46 (d, *J* = 3.1 Hz, 1H), 7.36 (d, *J* = 8.0 Hz, 1H), 7.21 (m, 2H), 7.15 (td, *J* = 6.9, 1.0 Hz, 1H), 6.94 (dd, *J* = 3.1, 8.9 Hz, 1H), 4.34 (s, 2H), 3.82 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.8, 170.2, 158.9, 136.4, 135.6, 127.3, 126.4, 123.1, 122.5, 120.0, 119.8, 119.2, 116.7, 112.5, 111.3, 110.0, 55.9, 22.9; HR-ESI: $[M+H]^+$ calcd for $C_{18}H_{15}BrN_3O_2$ 384.0342, found: 384.0338.

5-Phenyl-3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (18a). White solid; yield 52% (Start from compound **19a** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, *J* = 7.0 Hz, 2H), 7.62 (t, *J* = 7.3 Hz, 1H), 7.56 (t, *J* = 7.3 Hz, 2H), 7.43 (s, 2H), 3.98 (s, 6H), 3.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.8, 168.9, 153.7, 132.9, 129.3, 128.3, 124.4, 122.3, 104.7, 61.1, 56.5; HR-ESI: $[M+H]^+$ calcd for $C_{17}H_{17}N_2O_4$ 313.1110, found: 313.1182.

Tert-butyl (4-(3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazol-5-yl)phenyl)carbamate (18b). White solid; yield 50% (Start from compound **19b** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (d, *J* = 8.8 Hz, 2H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.41 (s, 2H), 3.97 (s, 6H), 3.92 (s, 3H) , 1.54 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.5, 168.8, 153.7, 152.3, 142.8, 140.6, 129.6, 122.5, 118.6, 118.2, 104.8, 81.6, 61.1, 56.5, 28.4; HR-ESI: $[M+H]^+$ calcd for $C_{22}H_{26}N_3O_6$ 428.1743, found: 428.1820.

4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-oxadiazol-5-yl)aniline (18c). White solid; yield 40% (Start from compound **19b** over 4 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.91 (d, *J* = 8.7 Hz, 2H), 7.41 (s, 2H), 6.79 (d, *J* = 8.8 Hz, 2H), 3.93 (s, 6H), 3.84 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 177.9, 169.5, 155.0, 154.4, 141.7, 130.9, 123.9, 115.3, 112.8, 105.7, 61.2, 56.7; HR-ESI: $[M+H]^+$ calcd for $C_{17}H_{18}N_3O_4$ 328.1219, found: 328.1301.

3-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-oxadiazol-5-yl)aniline (18d). White solid; yield 42% (Start from compound **19d** over 4 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.95 (d, *J* = 7.9 Hz, 1H), 7.92 (s, 1H), 7.59 (t, *J* = 7.9 Hz, 1H), 7.44 (s, 2H), 7.38 (d, *J* = 7.2 Hz, 1H), 3.94 (s, 6H), 3.84 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD): δ 176.9, 170.0, 155.1, 142.8, 142.1, 131.7, 126.5, 124.1, 123.4, 123.1, 118.7, 105.9, 61.2, 56.8; HR-ESI: $[M+H]^+$ calcd for $C_{17}H_{18}N_3O_4$ 328.1219, found: 328.1301.

5-(Adamantan-1-yl)-3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (18f). White solid; yield 48% (Start from compound **19f** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (s, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 2.13 (m, 9H), 1.80 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 185.6, 168.0, 153.5, 140.3, 122.6, 104.6, 61.0, 56.4, 40.1, 36.3, 35.7, 27.9; HR-ESI: $[M+H]^+$ calcd for $C_{21}H_{27}N_2O_4$ 371.1893, found: 371.1970.

Tert-butyl (4-(3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazol-5-yl)bicyclo[2.2.2]octan-1-yl)carbamate (18g). White solid; yield 47% (Start from compound **19g** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (s, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 2.13 (m, 6H), 1.99 (m, 6H), 1.43 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 184. 8, 168.1, 153.7, 140.6, 122.4, 104.8, 61.1, 56.4, 49.9, 33.3, 30.3, 30.1, 28.6; HR-ESI: $[M+H]^+$ calcd for $C_{24}H_{34}N_3O_6$ 460.2369, found: 460.2370.

4-(3-(3,4,5-Trimethoxyphenyl)-1,2,4-oxadiazol-5-yl)bicyclo[2.2.2]octan-1-amine (18h). White solid; yield 41% (Start from compound **19g** over 4 steps); ^{1}H NMR (400 MHz, CD_3OD): δ 7.31 (s, 2H), 3.89 (s, 6H), 3.81 (s, 3H), 2.22 (m, 6H), 1.97 (m, 6H); ^{13}C NMR (125 MHz, CD_3OD): δ 185.1, 169.2, 154.9, 141.8, 123.4, 105.6, 61.2, 56.7, 52.2, 34.2, 30.2, 30.0, 29.9; HR-ESI: $[M+H]^+$ calcd for $C_{19}H_{26}N_3O_4$ 360.1845, found: 360.1923.

5-(6,6-Difluorospiro[3.3]heptan-2-yl)-3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (18i). White solid; yield 45% (Start from compound **19i** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30 (s, 2H), 3.93 (s, 6H), 3.90 (s, 3H), 3.75 (m, 1H), 2.66 (m, 8H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 181.4, 168.4, 153.7, 140.7, 122.2, 119.2, 104.7, 61.1, 56.4, 47.6, 47.1, 38.8, 29.7, 26.9; HR-ESI: $[M+H]^+$ calcd for $C_{18}H_{21}F_2N_2O_4$ 367.1391, found: 367.1462.

5-(4-Butylphenyl)-3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (18k). White solid; yield 50% (Start from compound **19k** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (d, *J* = 8.3 Hz, 2H), 7.41 (s, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 3.96 (s, 6H), 3.92 (s, 3H), 2.69 (m, 2H), 1.63 (m, 2H), 1.37 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.9, 168.8, 153.6, 148.6, 140.5, 129.3, 128.3, 122.4, 121.7, 104.6, 61.2, 56.4, 35.9, 33.4, 22.4, 14.0; HR-ESI: $[M+H]^+$ calcd for $C_{21}H_{25}N_2O_4$ 369.1736, found: 369.1814.

5-(4-Hexylphenyl)-3-(3,4,5-trimethoxyphenyl)-1,2,4-oxadiazole (18l). White solid; yield 51% (Start from compound **19l** over 3 steps); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (d, *J* = 8.3 Hz, 2H), 7.42 (s, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 3.97 (s, 6H), 3.92 (s, 3H), 2.69 (m, 2H), 1.65 (m, 2H), 1.31 (m, 6H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.0, 168.8, 153.6, 148.7, 140.5, 129.3, 128.3, 122.4, 121.8, 104.7, 61.1, 56.4, 36.2, 31.8, 31.2, 29.0, 22.7, 14.2; HR-ESI: $[M+H]^+$ calcd for $C_{23}H_{29}N_2O_4$ 397.2049, found: 397.2127.

2. Biological assay.

2.1. Materials. Compounds were dissolved with 100% dimethylsulfoxide (DMSO, Sinopharm, China) and then diluted with RPMI 1640 medium (Hyclone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS, Hyclone). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo (Kumamoto, Japan). Concanavalin A (Con A), lipopolysaccharide (LPS, *Escherichia coli* 055:B5) were purchased from Sigma (St Louis, MO, USA). Anti-CD3/CD28 antibodies, F(ab')2 anti-mouse IgM antibodies were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Purified hamster anti-mouse CD40 was purchased from BD Pharmingen (San Diego, CA, USA).

2.2. Animals. Inbred 6-8-week-old female BALB/c mice were purchased from Shanghai Lingchang Biotechnology Co., Ltd. (Certificate No.2013-0018, China). The mice were housed under specific pathogen-free conditions with a controlled environment (12 h of light/12 h of dark cycle, 22 ± 1 °C, 55 ± 5% relative humidity). All mice were fed standard laboratory chow and water *ad libitum* and allowed to acclimatize in our facility for one week before any experiments started. All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of the Shanghai Institute of Materia Medica.

2.3. Splenocytes Preparation from Mice. Female BALB/c mice were sacrificed and the spleens were removed aseptically. Mononuclear cell suspensions were prepared after cell debris, and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Cells were washed and resuspended in RPMI 1640 media containing 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were counted by trypan blue exclusion.

2.4. Cell Viability Assay. Cell viability was assessed with CCK-8 reagent. Briefly, fresh splenocytes (1×10^6 cells) were cultured in 96-well plates in triplicate with 200 μL RPMI 1640 media containing 10% FBS, penicillin (100 U/mL), and streptomycin (100 μg/mL) in a humidified, 37 °C, 5% CO_2-containing incubator for 48 h in the presence or absence of indicated concentrations of compounds. Subsequently, a total of 20 μL CCK-8 was added to each well. After 6-8 h incubation, the absorbance value at 450 nm (570 nm calibration) was collected by a microplate reader (Molecular Devices, Sunnyvale, CA, USA) and the cell viability was calculated. The values of CC_{50} were estimated using the log(inhibitor) vs. normalized response non-linear fit (GraphPad Prism 6.0).

2.5. Con A and LPS-induced Proliferation Assay. Splenocytes (5×10^5 cells) were cultured in triplicate for 48 h, in the presence or absence of indicated concentrations of compounds, were stimulated with 5 μg/ml of Con A or 10 μg/ml of LPS to induce T cells or B cells proliferation, respectively. The cell culture was incubated in a humidified, 37 °C, 5% CO_2-containing

incubator for 48 h. Cells were pulsed with 0.5 μCi/well of [^{3}H] thymidine for 8 h and harvested onto glass fiber filters. The incorporated radioactivity was counted using a Beta Scintillation Counter (MicroBeta Trilux, PerkinElmer Life Sciences, Boston, MA). The values of IC_{50} were estimated using the log(inhibitor) vs. normalized response non-linear fit (GraphPad Prism 6.0).

2.6. Purification of CD4$^+$ T cells and CD19$^+$ B cells. After mononuclear cell suspensions preparation, mouse splenic polyclonal CD4$^+$ T cells and CD19$^+$ B cells were isolated using EasySep™ mouse CD4$^+$ T Cell isolation kit (Stemcell, Vancouver, BC, Canada) and EasySep™ mouse CD19$^+$ B Cell isolation kit (Stemcell) according to the manufacturer's instructions, respectively. To acquire CD4$^+$ T cells, immunomagnetic negative selection were performed for removal with biotinylated antibodies recognizing specific cell surface markers. Unwanted cells (CD8$^+$ cells, B220$^+$ cells, CD11b$^+$ cells, and I-A$^+$ antigen presenting cells from splenocytes) were separated with an EasySep™ magnet. The purity of the CD4$^+$ T cells was consistently >98% determined by flow cytometry. Similarly, to acquire CD19$^+$ B cells, mAb cocktails were added to deplete CD4$^+$ cells, CD8$^+$ cells and CD11b$^+$ cells from splenocytes by negative selection. The purity of the CD19$^+$ B cells was consistently >98%.

2.7. *In vitro* Stimulation of Splenic CD4$^+$ T cells and CD19$^+$ B cells. For *in vitro* study, purified CD4$^+$ T cells were cultured with medium alone or anti-CD3 antibodies (5 μg/ml) and anti-CD28 antibodies (2 μg/ml). Purified CD19$^+$ B cells were stimulated with medium alone or F(ab')2 anti-mouse IgM (10 μg/ml) plus anti-CD40 (1 μg/ml). After incubation, for 96-well plates, the cultures were pulsed with 0.5 μCi/well [^{3}H] thymidine to determine CD4$^+$ T and CD19$^+$ B cells proliferation activity. For 24-well plates, the supernatants were collected to determine the cytokine levels, meanwhile, the activation of T cells and B cells were detected by flow cytometry.

2.8. ELISA. Cytokines in culture supernatants were determined by using mouse IL-2, IL-6, IL-10 and IFN-γ ELISA kits (BD Pharmingen) according to the manufacturer's instructions.

Statistical analyses were conducted using GraphPad Prism 6.0 software and all data are presented as the mean ± sem.

2.9. Flow Cytometry. Cells were washed with phosphate buffered saline (PBS) and then stained with fixable viability dye eFluor™ 780 (eBioscience, San Diego, CA, USA) for 30 min at 4 ℃ to identify viable cells from the dead cells. Following, cells were blocked with anti-CD16/CD32 mAb (eBioscience) and stained with fluorescein isothiocyanate (FITC)-conjugated CD19, phycoerythrin (PE)-conjugated CD25, and allophycocyanin (APC)-conjugated IgD. All immunofluorescent mAbs used in this research were obtained from BD Biosciences (Franklin Lakes, NJ, USA). The data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA).

■ ASSOCIATED CONTENT

Supporting Information

Molecular Formula Strings (MFS), figures of NMR spectra, MS spectra, and HPLC purity for the biologically evaluated compounds. The Supporting Information is available free of charge on the ACS Publications website.

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Author Contributions

[1] These two authors contributed equally.

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Con A, concanavalin A; LPS, lipopolysaccharide; TCR, T cell receptor; BCR, B cell receptor; CsA, cyclosporin A; MMF, mycophenolate mofetil; MNP, marine natural product; DAT, dopamine transporter; PTP1B, protein tyrosine phosphatase-1B; SAR, structure-activity relationship; TFA, trifluoroacetic acid; HATU, 2-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; DIPEA, diisopropylethylamine; PE, petroleum ether; FBS, fetal bovine serum.

■ REFERENCES

(1) Kahan, B. D. Individuality: the barrier to optimal immunosuppression. *Nat. Rev. Immunol*. **2003**, *3*, 831−838.

(2) Meyer, K. C.; Decker, C.; Baughman, R. Toxicity and monitoring of immunosuppressive therapy used in systemic autoimmune diseases. *Clin. Chest Med.* **2010**, *31*, 565−588.

(3) Ader, J. L.; Rostaing, L. Cyclosporin nephrotoxicity: pathophysiology and comparison with FK-506. *Curr. Opin. Nephrol. Hypertens*. **1998**, 7, 539−545.

(4) Wang, X. L.; Tang, F.; Li, H. M. Bad responses of common immunosuppressants in organ transplantation. *J. Chin. Phys*. **2002**, 4, 1291−1293 (Chinese).

(5) Mignat, C. Clinically significant drug interactions with new immunosuppressive agents. *Drug. Saf.* **1997**, 16, 267-278.

(6) Wang, Y. R. Recent advances in immunosuppressive drugs. *Chin. J. New Drugs.* **2002**, 11, 512−515.

(7) Smith, J. M.; Nemeth, T. L.; McDonald, R. A. Current immunosuppressive agents: efficacy, side effects, and utilization. *Pediatr. Clin. North Am*. **2003**, 50, 1283−1300.

(8) Ye, F.; Zhu, Z. D.; Chen, J. S.; Li, J.; Gu, Y. C.; Zhu, W. L.; Li, X. W.; Guo, Y. W. Xishacorenes A-C, diterpenes with bicyclo[3.3.1]nonane nucleus from the Xisha soft coral *Sinularia polydactyla*. *Org. Lett*. **2017**, 19, 4183−4186.

(9) Ye, F.; Li, J.; Wu, Y.; Zhu, Z. D.; Mollo, E.; Gavagnin, M.; Zhu, W. L.; Li, X. W.; Guo, Y. W. Sarinfacetamides A and B, nitrogenous diterpenoids with tricyclo[6.3.1.$0^{1,5}$]dodecane scaffold from the south China Sea soft coral *Sarcophyton infundibuliforme*. *Org. Lett*. **2018**, 20, 2637−2640.

(10) Carbone, M.; Li, Y.; Irace, C.; Mollo, E.; Castelluccio, F.; Pascale, A. D.; Cimino, G.; Santamaria, R.; Guo, Y. W.; Gavagnin, M. Structure and cytotoxicity of phidianidines A and B: First finding of 1,2,4-oxadiazole system in a marine natural product. *Org. Lett*. **2011**, 13, 2516–2519.

(11) Brogan, J. T.; Stoops, S. L.; Lindsley, C. W. T. Total synthesis and biological evaluation of phidianidines A and B uncovers unique pharmacological profiles at CNS targets. *ACS Chem. Neurosci.* **2012**, 3, 658–664.

(12) Jiang, C. S.; Fu, Y.; Zhang, L.; Gong, J. X.; Wang, Z. Z.; Xiao, W.; Zhang, H. Y.; Guo, Y. W. Synthesis and biological evaluation of novel marine-derived indole-based 1,2,4-oxadiazoles derivatives as multifunctional neuroprotective agents. *Bioorg. Med. Chem. Lett*. **2015**, 25, 216–220.

(13) Zhang, L.; Jiang, C. S.; Gao, L. X.; Gong, J. X.; Wang, Z. Z.; Li, J. Y.; Li, J.; Li, X. W.; Guo, Y. W. Design, synthesis and in vitro activity of phidianidine B derivatives as novel PTP1B inhibitors with specific selectivity. *Bioorg. Med. Chem. Lett*. **2016**, 26, 778–781.

(14) Li, Z.; Chen, W.; Hale, J. J.; Lynch, C.; Mills, S. G.; Hajdu, R.; Keohane, C. A.; Rosenbach,

M. J.; Milligan, J.A.; Shei, G. J.; Chrebet, G.; Parent, S. A.; Bergstrom, J.; Card, D.; Forrest, M.; Quackenbush, E. J.; Wickham, L. A.; Vargas, H.; Evans, R. M.; Rosen, H.; Mandala, S. Discovery of potent 3,5-diphenyl-1,2,4-oxadiazole sphingosine-1-phosphate-1 (S1P1) receptor agonists with exceptional selectivity against S1P2 and S1P3. *J. Med. Chem.* **2005**, 48, 6169-6173.

(15) Vu, C. B.; Corpuz, E. G.; Merry, T. J.; Pradeepan, S. G.; Bartlett, C.; Bohacek, R. S.; Botfield, M. C.; Eyermann, C. J.; Lynch, B. A.; MacNeil, I. A.; Ram, M. K.; van Schravendijk, M. R.; Violette, S.; Sawyer, T. K. Discovery of potent and selective SH2 inhibitors of the tyrosine kinase ZAP-70. *J. Med. Chem.* **1999**, 42, 4088-4098.

(16) Liu, J.; Chen, Y.; Li, J. Y.; Luo, C.; Li, J.; Chen, K. X.; Li, X. W.; Guo, Y. W. Function-oriented synthesis of marine phidianidine derivatives as potential PTP1B inhibitors with specific selectivity. *Mar. Drugs.* **2018**, 16, 97.

(17) Yang, S. H.; Chang, S. Highly efficient and catalytic conversion of aldoximes to nitriles. *Org. Lett*. **2001**, 3, 4209–4211.

(18) Hamze, A.; Hernandez, J. F.; Fulcrand, P.; Martinez, J. Synthesis of various 3-substituted 1,2,4-oxadiazole-containing chiral β3- and α-amino acids from Fmoc-protected aspartic acid. *J. Org. Chem*. **2003**, 68, 7316–7321.

(19) Loeffler, L. J.; Britcher, S. F.; Baumgarten, W. Bridged bicyclic and polycyclic amino acids. Potent new inhibitors of the fibrinolytic process. *J. Med. Chem.* **1970**, 13, 926−935.

(20) Al Hussainy, R.; Verbeek, J.; van der Born, D.; Braker, A. H.; Leysen, J. E.; Knol, R. J.; Booij, J.; Herscheid, J. K. Design, synthesis, radiolabeling, and in vitro and in vivo evaluation of bridgehead iodinated analogues of N-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl}-N-(pyridin-2-yl) cyclohexanecarboxamide (WAY-100635) as potential SPECT ligands for the 5-HT1A receptor. *J. Med. Chem*. **2011**, 54, 3480–3491.

(21) Song, B.; Guan, S.; Lu, J.; Chen, Z.; Huang, G.; Li, G.; Xiong, Y.; Zhang S.; Yue, Z.; Deng, X. Suppressive effects of fisetin on mice T lymphocytes *in vitro* and *in vivo*. *J. Surg. Res.* **2013**, *185*, 399–409.

(22) Li, T.; Chen, H.; Yang, Z.; Wang, W.; Wang, Y.; Zhang, L.; Zhao, J.; Zhou, X.; Li, Y. Effect of etanercept and lithium chloride on preventing secondary tissue damage in rats with experimental diffuse severe brain injury. *Eur. J. Pharmacol.* **2014**, *745*, 10–18.

(23) Kumar, D.; Patel, G.; Chavers, A. K.; Chang, K. H.; Shah, K. Synthesis of novel 1,2,4-oxadiazoles and analogues as potential anticancer agents. *Eur. J. Med. Chem.*, **2011**, *46*, 3085–3092.

(24) Dohi, T.; Suda, M.; Morikawa, S.; Kitamura, T.; Ishkawa, T. STAT6 activation inhibitors and pharmaceutical compositions containing diphenyloxadiazoles and their uses. JP 2007297307 A 20071115. Nov. 15, 2007. [P].

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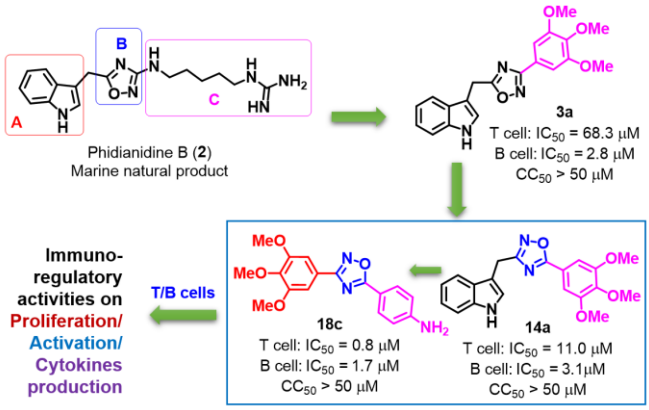
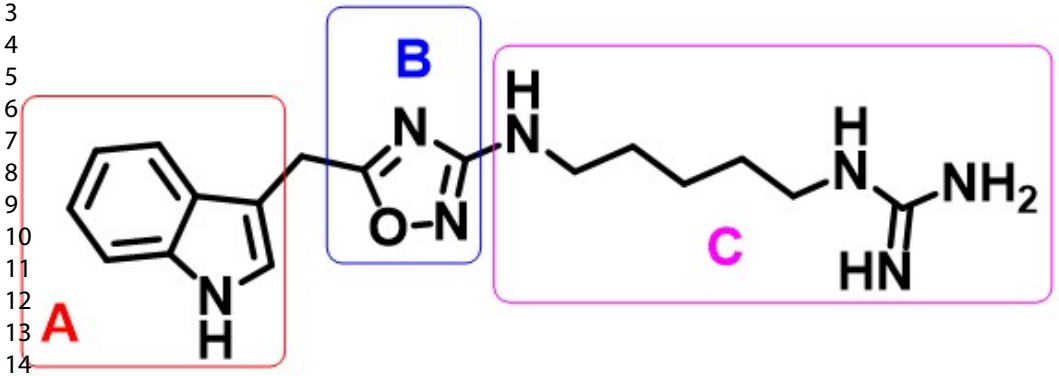
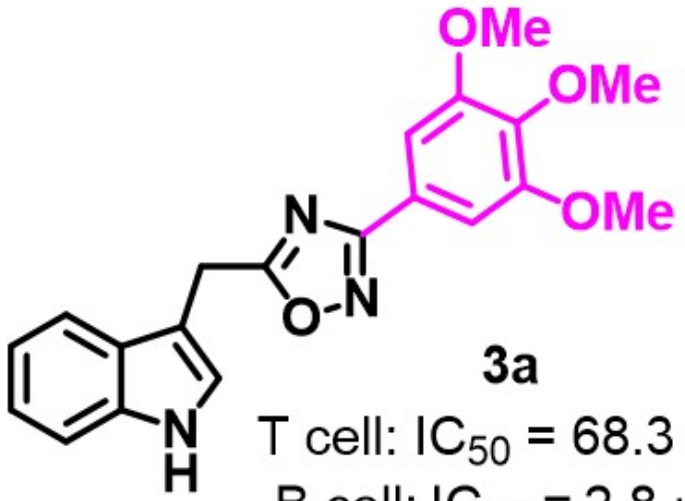




Phidianidine B (**2**)
Marine natural product



3a
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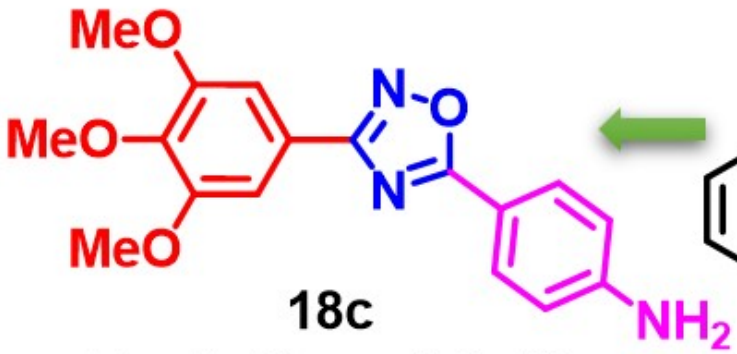
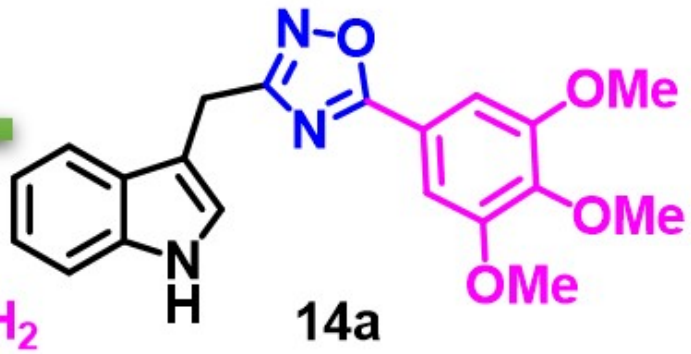
B cell: IC_{50} = 2.8 μM
CC_{50} > 50 μM



18c
T cell: IC_{50} = 0.8 μM
B cell: IC_{50} = 1.7 μM
CC_{50} > 50 μM



14a
T cell: IC_{50} = 11.0 μM
B cell: IC_{50} = 3.1μM
CC_{50} > 50 μM

T/B cells

Immuno-regulatory activities on Proliferation/ Activation/ Cytokines production