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Article

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Novel Pyrazolo[4,3-d]pyrimidine as Potent and Orally Active Inducible Nitric Oxide Synthase (iNOS) Dimerization Inhibitor with Efficacy in Rheumatoid Arthritis Mouse Model

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ABSTRACT: In order to discover novel anti-inflammatory agents for treatment of arthritis and based on preliminary structure-activity relationships, four series (**A**~**D**) of total 90 new pyrazolo[4,3-d]pyrimidine compounds were designed and synthesized. All the compounds have been tested for their anti-inflammatory activities by inhibiting of LPS-induced NO production. A clear structure-activity relationship has been concluded step by step, and finally 3,4,5-trimethoxystyryl-1H-pyrazolo[4,3-d]pyrimidine was found to be the most active scaffold. Among them, compound **D27** was discovered as the most potent anti-inflammatory agent (IC_{50}=3.17 μM) with low toxicity and strong inhibitory of NO release (IR=90.4% at 10 μM). This compound also showed potent inhibition of iNOS with IC_{50} value of 1.12 μM. Preliminary mechanism studies indicated that it could interfere the stability and formation of active dimeric iNOS. The anti-inflammatory effect of this compound was determined by adjuvant-induced arthritis in rat model. We believe these findings would further support study of rational design of more efficient iNOS inhibitors in the future.

INTRODUCTION

Nitric oxide synthases (NOS[a]) are dimeric enzymes that catalyze the formation of nitric oxide (NO) and citrulline from arginine and molecular oxygen.[1-6] Inflammation can be regulated by the recruitment of macrophages and production of proinflammatory cytokines. Macrophages produce proinflammatory cytokines including nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin (IL-6) and interleukin-1β (IL-1β).[7, 8] NO, being an important signaling molecule, is a unique gasotransmitter in the process of vasodilation, non-specific host defense and chronic or acute inflammation, which is biogenetically produced by NOS. The approach of inhibiting NOS is to either disrupt the function of active NOS dimer or prevent NOS dimerization. Given that inducible NOS (iNOS) is controlled at the transcriptional level, NOS dimerization inhibitors may be expected to work best with iNOS.[9, 10] Thus, iNOS should be a potential target for inflammatory diseases.

In the past decades, several molecules capable of disrupting NOS dimer's stability or preventing NOS dimerization have been developed including clotrimazole, miconazole, PPA250, KLYP961, BBS-1 and BBS-4.[1, 9, 11-16] However, most of them are imidazole-based molecules, which would also inhibit P450 resulting in drug-drug interaction. For example, inhibition of endothelial NOS (eNOS) can always cause serious side effects. Thus, selectively inhibiting NOS has always been a challenge as However, except KLYP961, a non-imidazole-based inhibitor which can prevent dimerization of iNOS and neuronal NOS (nNOS)[1,15-18], no other potent non-imidazole-based NOS inhibitor with low toxicity profile has been reported until

now. Therefore, development of potent, low-toxicity non-imidazole iNOS inhibitors is in urgent need.

The pyrazolo[4,3-d]pyrimidine skeleton, an isostere of adenine, is an important drug-like scaffold and its derivatives have been demonstrated with a myriad of therapeutic indications including anti-inflammation, anti-cancer and anti-infectious effects.[19-23] Also, pyrazolo-pyrimidine derivatives have been reported for treatment of a variety of inflammatory diseases. Based on above findings, we hypothesized that pyrazolo-pyrimidine derivatives could be potential NOS inhibitors.

In our previous study, many pyrazole-pyrimidine derivatives have been synthesized[25] and in-house compounds library screening showed that most compounds had no toxicity against six tumor cell lines. Further activity evaluation showed that they were even less toxic to RAW 264.7 cells. We then preliminarily evaluated their anti-inflammatory activities (compounds of Series **A**, **Figure 1**) by evaluating their inhibitory effects against LPS-stimulated NO, IL-6 and TNF-α productions in RAW 264.7 cells.

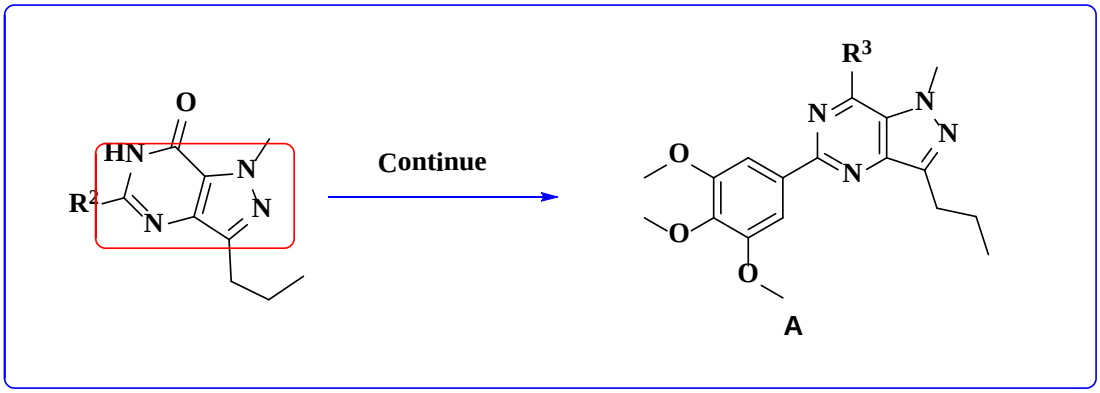


Figure 1. Our previous work

The results revealed that some compounds exhibited moderate anti-inflammatory

effects through decreasing the levels of NO, IL-6 and TNF-α. Interestingly, it was found that most compounds showed stronger inhibitory effects on NO production than IL-6 or TNF-α production (**Table 1**). Thus, in order to find potent anti-inflammatory agents and focusing on improving their inhibitory effect of NO production, compounds of series **B~D** were designed and synthesized based on SARs step by step in this study **(Figure 2**).

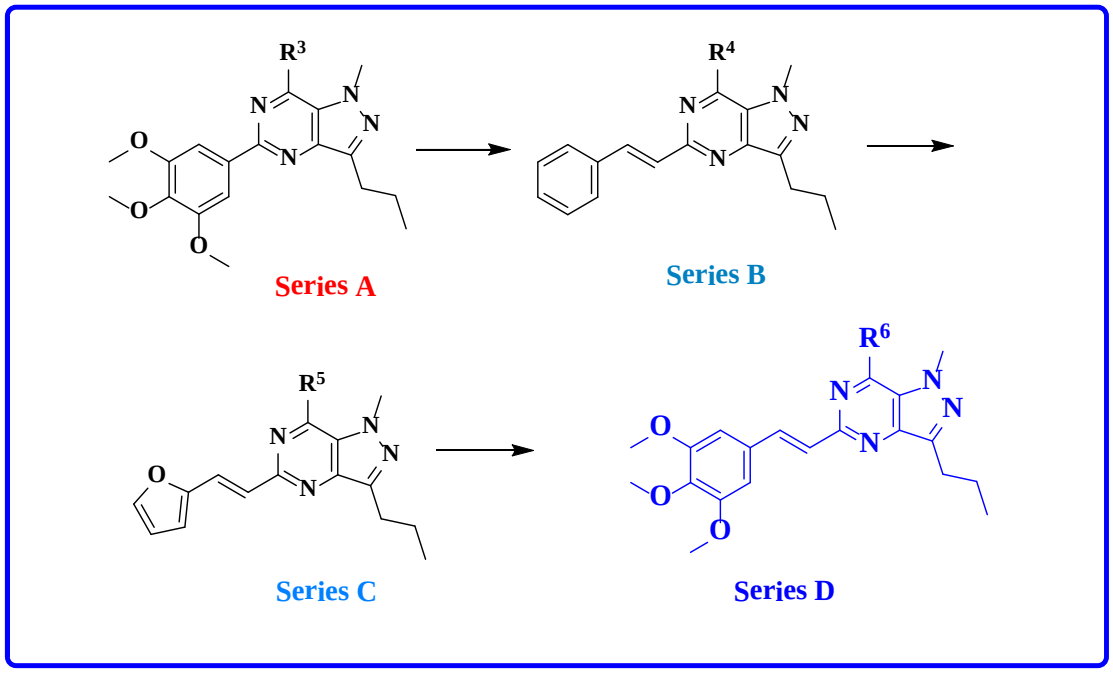


Figure 2. Four series compounds (**A~D**) were designed step by step

RESULTS AND DISCUSSION

Design. 3,4,5-trimethoxyphenyl moiety is crucial to activity in drug design,[25,26] and its derivatives have a wide range of therapeutic indications such as anti-inflammatory[22,27,28] and antinarcotic effects.[29-31] Therefore, some 3,4,5-trimethoxyphenyl pyrazolo[4,3-d]pyrimidines (Series **A**) were designed and synthesized at first.

To further improve the activity of the compounds, stilbene scaffold was introduced into the structure, which has been proved to be an active moiety as a basic element of natural product. Representative chemicals with stilbene moiety including resveratrol (**I**), pterostilbene (**II**) and combretastatin A-4 (**III**) are of significant interest for drug research[32,33] (**Figure 3**). Inspired by this, we then introduced styryl group at C-5 of pyrazolo[4,3-d]pyrimidine scaffold and compounds of Series **B** were designed.

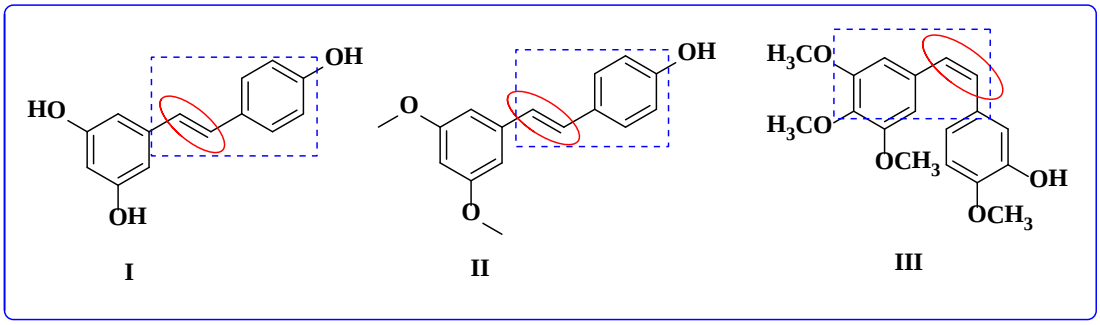
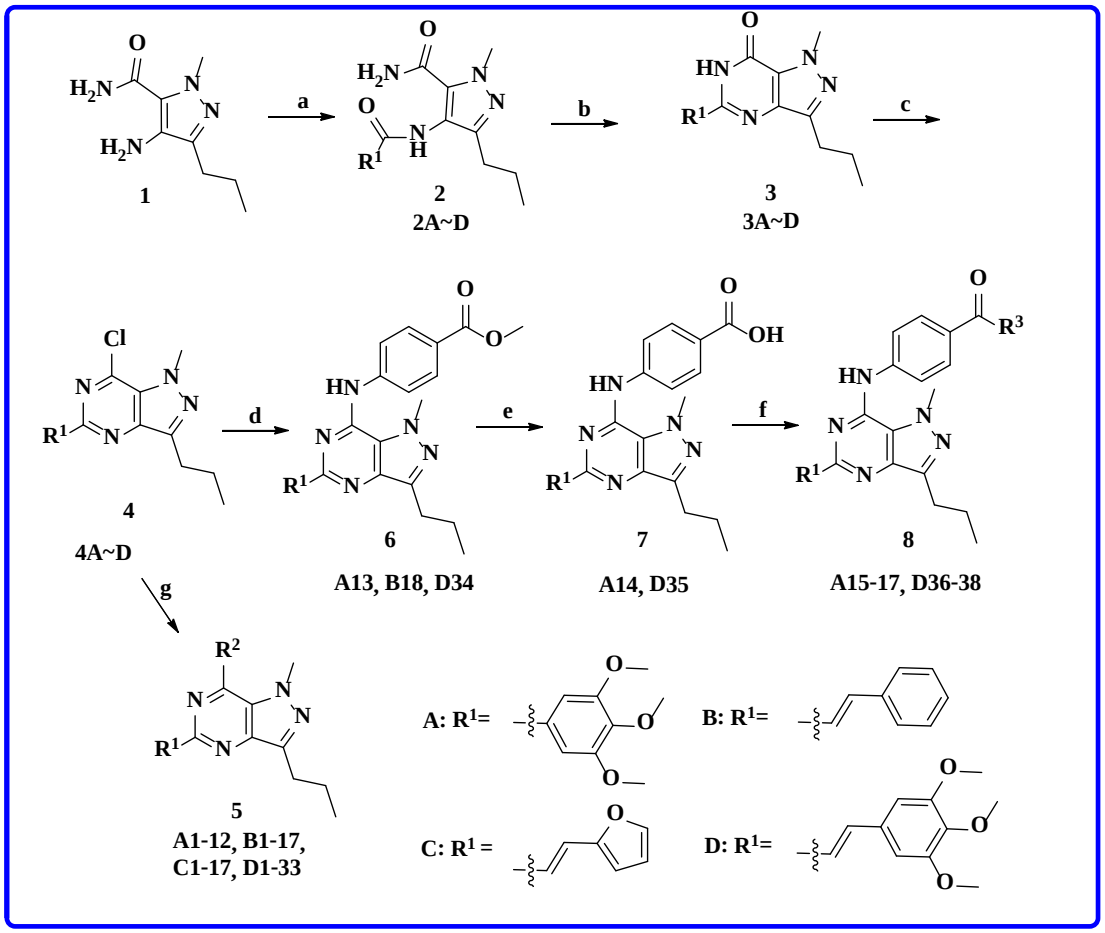


Figure 3. Structures of representative stilbene

In view of the preliminary SARs results, influence of alternative substituents at C-5 of pyrazolo[4,3-d]pyrimidine was found. Based on this finding, the styryl group was changed to 2-(furan-2-yl)vinyl group since it has been successfully used as a good styryl bioisostere (Series **C**).[34,35] Considering the contribution of 3,4,5-trimethoxyphenyl moiety to the activity and the re-summarized SAR, we at last introduced trimethoxystyryl group at C-5 of pyrazolo[4,3-d]pyrimidine scaffold (Series **D**).

Chemistry. According to our previous work, 4-amino-1-methyl-3-propyl-1H-pyrazole-5-carboxamide (compound **1**) was synthesized and used as the lead material. All the following compounds (Series **A~D)**

were synthesized according to the protocol outlined in **Scheme 1**.[36, 37]



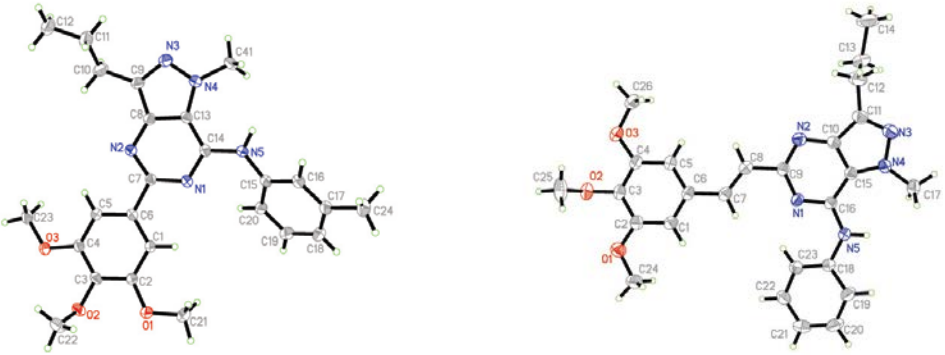
Scheme 1. Synthesis of Compounds Series A, B, C, D [a]

[a] Reaction conditions and reagents: (**a**): (1) substituted carboxylic acid, $(COCl)_2$, CH_2Cl_2, DMF (Cat), 25 ^oC, 3 h; (2) Compound **1**, CH_2Cl_2, TEA, 25 ^oC, 12 h; (**b**): NaOEt, EtOH, reflux, 12 h; (**c**): $POCl_3$, reflux, 3-8 h; (**d**): methyl 4-aminobenzoate, IPA, reflux; (**e**): MeOH, 2N NaOH, rt.; (**f**): amine derivatives, EDCI, HOBt, TEA, rt.; (**g**): amine derivatives, IPA, reflux.

At first, 3,4,5-trimethoxyphenyl-pyrazolo[4,3-d]pyrimidine derivatives (**Series A**) were synthesized. Compound **2A** (**Scheme 1**) was obtained by two steps: the corresponding acyl chloride was prepared with oxalyl chloride at room temperature and formed amide **2A**. Compound **3A** was afforded through intramolecular

cyclization reaction. Synthon **4A** was a key intermediate for title compounds, which was achieved by chlorination reaction in the presence of $POCl_3$.[38] Nucleophilic substitution reactions of compound **4A** with various amines gave title compounds **A1-13** (**Scheme 1**). Based on compound **A13**, compounds **A14-17** were prepared through simple derivation. Compounds of Series B-D were synthesized with similar route.

^{1}H NMR spectrum of alkene (CH=CH, compound **D3**) exhibited chemical shift at 7.15 ppm in $CDCl_3$ with coupling constant of 15.7 Hz. These results demonstrated that the configuration of compound **D3** is *E* geometrical isomer and the result was further confirmed by X-ray. Crystal data of compound **A2** was shown as following: Colorless crystals, yield=55%; mp: 202-203 ^oC; $C_{25}H_{29}N_5O_3$, Monoclinic, space group $P2_1/c$; a=7.2308(3), b=20.7026(8), c=15.7198(6) (Å); α=90, β=96.463(4), γ=90(°), V=2338.24(16) nm^3, T=293(2) K, Z=4, Dc=1.271 g/cm^3, $F(000)$=952.0, Reflections collected/unique =8504/4341, Data / restraints / parameters =4341/0/304, Goodness of fit on F^2 =0.978, Fine, R_1=0.0572, $wR(F^2)$= 0.1281. Crystal data of compound **D3**: Colorless crystals, yield=56%; mp: 109-110 ^oC; $C_{26}H_{29}N_5O_3$, Monoclinic, space group $P2_1/c$; a=10.1561(4), b=19.6033(10), c=14.3506(6) (Å); α=90, β=103.929(5), γ=90(°), V=2773.1(2) nm^3, T=293(2) K, Z=4, Dc=1.211 g/cm^3, $F(000)$=1080.0, Reflections collected/unique=10620/5436, Data/restraints/ parameters =5436/36/367, Goodness of fit on F^2 =1.039, Fine, R_1=0.0725, $wR(F^2)$ = 0.1373. Their structures were shown in **Figure 4**.

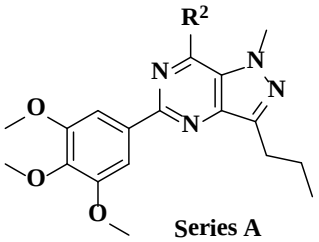


Compound **A2** Compound **D3**

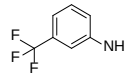
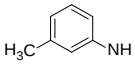
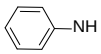
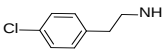
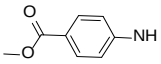
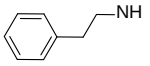
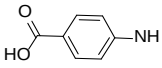
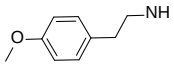
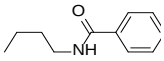
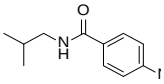
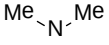
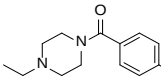
Figure 4. ORTEP drawing of compounds **A2** and **D3**

Inhibition of NO Production in RAW 264.7 Cells. Pre-research results showed that most compounds (Series **A**) had stronger inhibitory effects on NO production than IL-6 or TNF-α production (**Table 1**). Therefore, based on the inhibitory effect of NO production, anti-inflammatory activity of these compounds was evaluated in this study (**Table 1**).

Table 1. **Inhibitory Effects of Compounds A1-17 Against NO Production in RAW 264.7 Cells** [a]



Series A

Compd	R^2	NO [b] inhibition (%) ± SD	Compd	R^2	NO inhibition (%) ± SD
A1		-	**A10**		5.2±11.3
A2		-	**A11**		3.1±9.6
A3		22.2±6.2	**A12**		7.6±10.3
A4		22.2±3.2	**A13**		17.3±3.2
A5		20.2±3.2	**A14**		44.9±3.2
A6		10.2±5.3	**A15**		-
A7		21.6±3.3	**A16**		-
A8		21.2±2.4	**A17**		27.6±4.4
A9		51.9±1.3	**Indomethacin**		59.2±3.7

"-" : No anti-inflammatory activity.

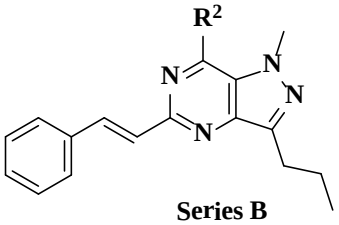
[a] The cells were treated with compounds for 1 h, and then stimulated with LPS (1 μg/mL) for 24 h. The level of NO in the culture medium was measured by nitrite and nitrate assay. All date shown were at least three independent experiments.

[b] The inhibition of NO production in LPS-induced RAW 264.7 was tested at 10 μM.

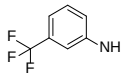
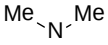
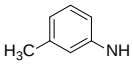
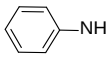
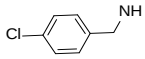
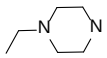
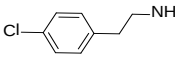
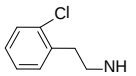
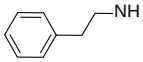
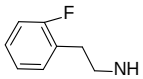
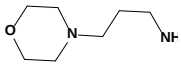
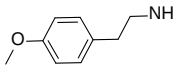
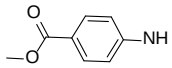
[c] **Indomethacin** as a positive control.

It could be observed that most of 3,4,5-trimethoxyphenyl-pyrazolo[4,3-d] pyrimidine derivatives (Series **A**) showed weak inhibitory activity against LPS-induced NO production at 10 μM (inhibitory rates (IR) were less than 22.2%). Among them, compounds **A1**, **A2**, **A15** and **A16** had no activities. In contrast, compound **A9**, an analog with isopropylamine at C-7 resulted in moderate inhibitory activity (IR=51.9%) compared to that of positive control Indomethacin. Based on above, it is not difficult to find that inhibitory activity of series **A** is closely related to the chemical structure. Because stilbene scaffold is a privileged structure, styryl was then introduced to C-5 of pyrazolo[4,3-d]pyrimidine scaffold (Series **B**). Their activities against NO production were shown in **Table 2**.

Table 2. **Inhibitory Effects of Compounds B1-18 Against NO Production** [a]



Series B

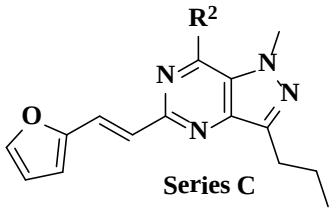
Compd	R^2	NO inhibition (%) ± SD	Compd	R^2	NO inhibition (%) ± SD
B1		14.6±6.2	**B10**		27.1±5.9
B2		15.3±3.3	**B11**		53.9±6.3
B3		4.9±6.3	**B12**		33.3±5.3
B4		24.2±3.5	**B13**		10.2±6.3
B5		10.7±3.3	**B14**		10.2±3.3
B6		14.3±7.6	**B15**		6.3±8.3
B7		48.6±3.3	**B16**		7.7±5.2
B8		43.9±3.7	**B17**		42.4±3.3
B9		48.0±5.3	**B18**		42.4±3.6
Indomethacin		59.2±3.7			

[a] Described as **Table 1**.

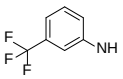
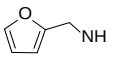
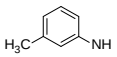
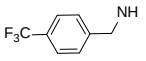
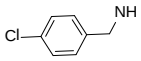
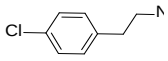
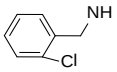
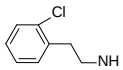
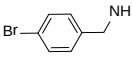
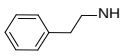
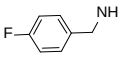
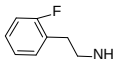
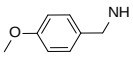
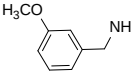
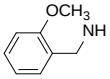
In these styryl-pyrazolo[4,3-d]pyrimidine derivatives (Series **B**), compounds **B1**-**3**, with substituted or unsubstituted *N*-phenyl group at C-7, showed very weak potencies which were very similar to compounds **A1**-**3**. Compound **B4** (IR=24.2%), with *N*-Benzyl group at C-7 negligibly showed any significant effects. After replacement of the *N*-phenyl with *N*-phenethyl moiety, inhibition activities were

found to be greatly enhanced (compounds **B5-9)** with IR increasing from 10.7% to 48.6%. Although only compound **B11** (IR=53.9%) showed moderate inhibitory activity, one-third of the series **B** compounds contained inhibitory activities more than 40%, indicating the merit of derivation strategy. Provided with the results of these preliminary SARs, the influence of alternative substituents at C-5 of pyrazolo[4,3-d]pyrimidine should be further revealed. Thus, compounds **C1-17** were designed and synthesized. Their activities were screened (**Table 3)**.

Table 3. **Inhibitory Effects of Compounds C1-17 Against NO Production** [a]



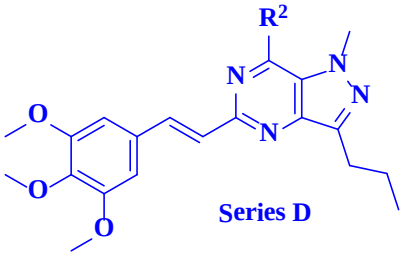
Series C

Compd	R^2	NO inhibition (%) ± SD	Compd	R^2	NO inhibition (%) ± SD
C1		8.9±7.6	**C10**		52.3 ±2.3
C2		8.9±3.5	**C11**		50.2±3.5
C3		56.4±2.5	**C12**		79.2±5.6
C4		54.8±5.2	**C13**		70.8±3.7
C5		40.6±4.1	**C14**		36.4±2.2
C6		50.7±3.3	**C15**		73.0±3.3
C7		43.8±3.7	**C16**		30.6±6.3
C8		35.6±3.3	**C17**		37.7±3.3
C9		38.4±3.3	**Indomethacin**		59.2±3.7

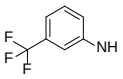
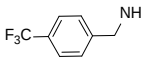
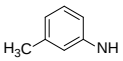
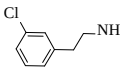
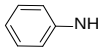
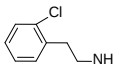
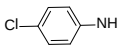
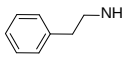
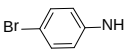
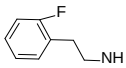
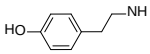
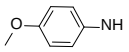
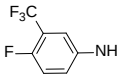
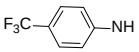
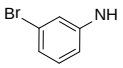
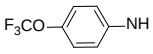
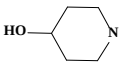
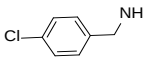
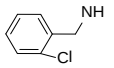
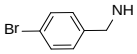
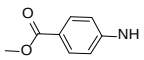
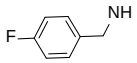
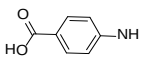
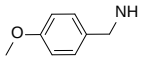
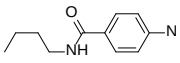
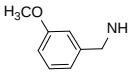
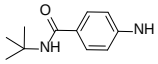
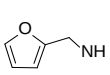
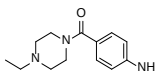
[a] Described as **Table 1**.

As described in **Table 3**, compounds **C1-2**, with substituted *N*-phenyl group at C-7, had very weak potency. Interestingly, compounds **C3** and **B4**, though both having *N*-4-chlorobenzyl group at C-7, showed dramatic difference at inhibitory activity (56.4% and 24.2%). To summarize, nearly half of the compounds (Series **C**) exhibited moderate potencies, with IR ranging from 50.2% to 79.2%. Among the *N*-phenethyl or *N*-alkyl substituted analogues **C12-15**, **C16-17**, most of compounds exhibited moderate inhibitory activities. Compounds **C10**, **C11** and **C13**, substituted with phenethyl amine moiety at the **C**-7, could dramatically elevate inhibitory activities (79.2%, 70.8% and 73.0% respectively). These results indicated that inhibitory activities of compounds **C** series were much higher than that of compounds series **A** and **B**. Focusing on above SARs, in order to improve activity, we will carry out further structural optimization. Compounds **D1-39** were then synthesized. Inhibitory effects against NO production were listed in **Table 4**.

Table 4. **Inhibitory Effects of Compounds D1-39 Against NO Production** [a]



Series D

Compd	R^2	NO inhibition (%) ± SD	Compd	R^2	NO inhibition (%) ± SD
D1		38.2±4.2	**D20**		54.6±3.3
D2		25.8±3.7	**D21**		80.0±3.3
D3		48.1±3.3	**D22**		46.9±2.4
D4		25.3±6.2	**D23**		86.1±4.2
D5		33.0±3.2	**D24**		85.0±3.7
D6		54.4±3.3	**D25**		15.0±6.3
D7		63.2±1.3	**D26**		82.1±3.3
D8		82.6±3.7	**D27**		90.4±1.2
D9		37.8±2.3	**D28**		75.0±3.3
D10		31.0±6.3	**D29**		28.3±3.3
D11		38.5±1.3	**D30**		81.0±3.2
D12		16.4±3.3	**D31**		45.3±3.2
D13		67.2±1.3	**D32**		15.5±3.7
D14		90.0±1.3	**D33**		16.3±4.3
D15		53.9±2.3	**D34**		10.3±6.4
D16		69.7±3.6	**D35**		15.3±6.9
D17		73.1±2.4	**D36**		35.9±4.3
D18		70.9±3.6	**D37**		10.6±6.3
D19		62.1±2.5	**D38**		15.4±5.6
Indomethacin		59.2±3.7			

[a] Described as **Table 1**.

In the series **D**, it could be observed that compounds **D1-3**, with substituted or unsubstituted *N*-phenyl group at C-7, had moderate potency (IR ranging from 25.0% to 48.1%), which were found to be slightly better compared to compounds **A1-3**, **B1-3** and **C1-2**. This means that trimethoxystyryl group may contribute to the activity. Based on this, we continued to synthesize compounds **D4-12**, with substituted or unsubstituted *N*-phenyl group at C-7 and the results showed most of them exhibited potent activities. Among them, compounds **D6-8**, were found to possessing dramatically improved activity with inhibitory rates of 54.4%, 63.2% and 82.6% respectively. Compounds **D13-20**, with substituted *N*-benzyl group at C-7, also exhibited dramatically improved activity compared to compounds **D1-12** and **D21-25**, with IR ranging from 53.9% to 90.0%. These results indicated that introduction of substituted *N*-benzyl group at C-7 was critical to activity. Particularly compounds **D26-28** showed dramatically improved activity with IR ranging from 75.0% to 90.4%, compared to compounds **A8-9**. To summarize, among the compounds of series **D**, eight compounds' inhibition rates are more than 80%, and among them two compounds' IRs are more than 90%, surpassing that of commodity drug Indomethacin. Therefore, after third rounds optimization, lead compound was initially discovered.

Inhibition Activity of iNOS. To explore the relationship between IC_{50} values of NO and iNOS in the interest of further confirmation of the mechanism of anti-inflammatory, selected compounds with high or no NO inhibitory activities were chosen to evaluate their IC_{50s} against iNOS. The results were summarized in **Table 5**.

Table 5. IC_{50} Values of Selected Compounds Against iNOS

Compd	NO IC_{50} (μM)[a]	iNOS IC_{50} (μM)[c]	cytotoxicity IC_{50} (μM) without LPS	with LPS
A9	10.78[b]	7.26[d]	>50	>50
A11	-	-	>50	>50
A14	15.82	10.23	>50	>50
B3	-	-	>50	>50
B11	12.20	8.46	>50	>50
B12	22.11	5.31	>50	>50
C3	9.26	4.25	>50	>50
C12	6.04	2.60	>50	>50
D27	3.17	1.12	>50	>50
D31	13.72	5.23	>50	>50
Indomethacin [e]	45.57	24.89	>50	>50

"-" : No anti-inflammatory activity.

[a]Inhibition(%)=[LPS (OD_{540})-compounds(OD_{570})]/[LPS(OD_{540})-control (OD_{540})]×100

[b] First calculate the inhibition rates for different concentrations. The IC_{50s} of various compounds were calculated by regression analysis through SPSS.

[c] Inhibition (%) = [LPS (AW_{495}, EW_{515})–compounds (AW_{495}, EW_{515})]/[LPS (AW_{495}, EW_{515})- control (AW_{495}, EW_{515})]×100.

[d] First calculate the inhibition rates for different concentrations. The IC_{50s} of various compounds were calculated by regression analysis through SPSS.

[e] Indomethacin as a positive control.

Ten compounds were exposed to investigate the inhibitory effects against NO compared to iNOS. From the **Table 5**, it could be found that compounds **A11** and **B3** had no inhibitory activity against LPS-induced NO production at 10 μM. They also didn't show any activity against iNOS. In contrast, for compounds **B12**,**C12** and **D27**, there was a good correlation between IC_{50} of NO and the iNOS, indicating that inhibition of the NO may result in inhibiting of iNOS. As anticipated, 5-substituted styryl derivative (compound **D27**) exhibited the most potent inhibitory activity against iNOS with the IC_{50} of 1.12 μM (Selectivity=22 times with Indomethacin), this was consistent with the above SARs analysis. Thus, compound **D27** was identified as the title compound for further study.

Inhibition of iNOS Dimerization. From **Tables 1**~**5**, compound D**27** showed very good inhibitory effect against NO inhibition. NO plays an important role in inflammation-related diseases, which is also importantly related to modulation expression of iNOS.[2] Thus, the inhibitory effect of compound D**27** against expression of iNOS in LPS-mediated RAW cells was analyzed by Western blot. From **Figure 5**, we found that this compound could not affect the expression of iNOS. It was reported that iNOS would dimerize to be in an active dimer form in LPS-mediated condition.[1] From previous result, our title compound indicated good NO inhibitory activity but could not affect expression of iNOS. Thus, it would be very possible that inhibition of NO production resulted from inhibition of iNOS dimerization. To confirm this, the dimer of the iNOS in the presence of title compound was investigated using Native Gel Sample Loading Buffer low-temperature SDS-PAGE, followed by

immunoblotting with antibody recognizing the protein of iNOS. Within a certain time, dimeric iNOS was resolved on the basis of having distinct mobility (**Figure 6**). Title compound **D27** was found to inhibit iNOS from forming dimer. This result suggests that compound D**27** may be interfere the formation of stable dimer iNOS so as to reflects anti-inflammatory activity.

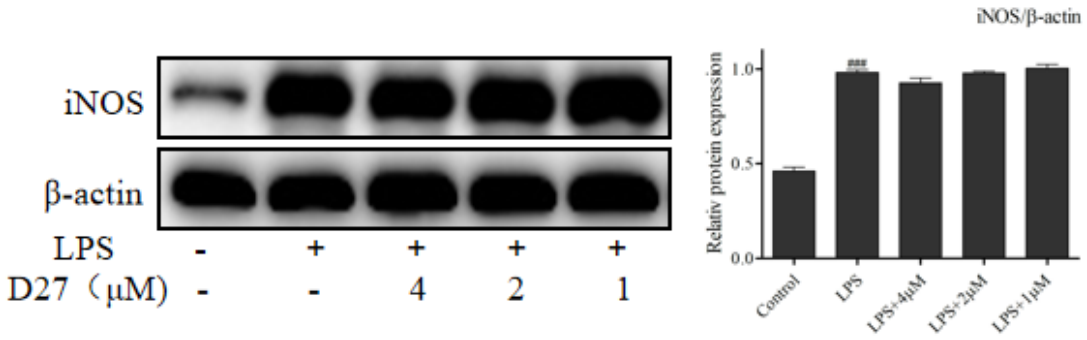


Figure 5. Compound **D27** inhibit expression of iNOS [a]

[a] iNOS expression was detected by Western blot analysis. β-actin was used as loading control; [###]p<0.001 compared with unstimulated cells, Data were obtained by at least three independent experiments, and each was performed in duplicate.

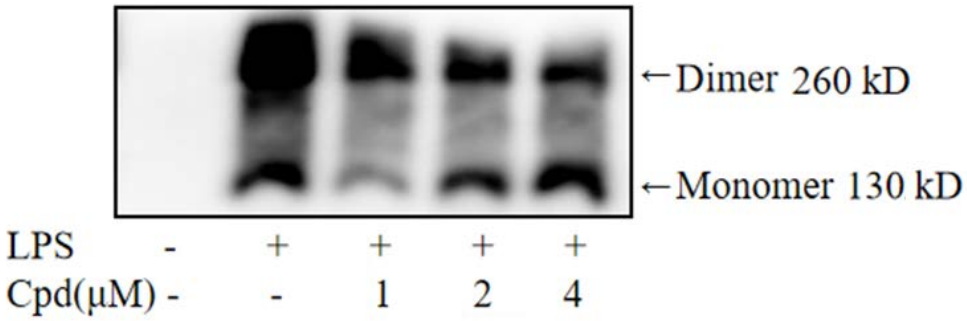
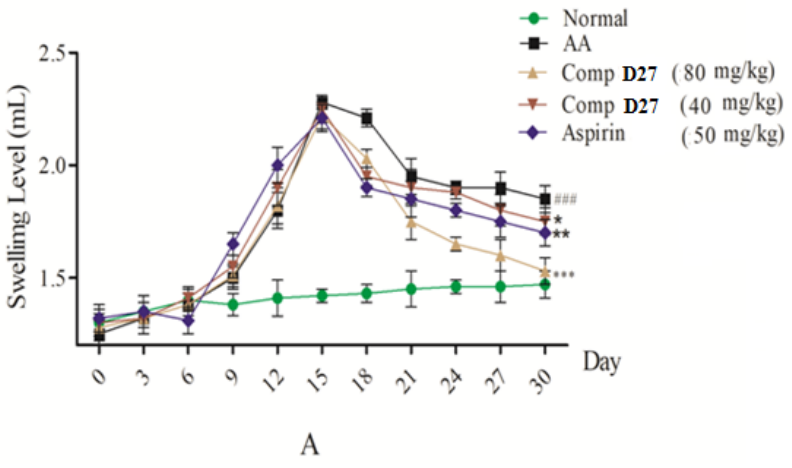


Figure 6. Compound **D27** destabilizes murine iNOS dimer in cells [a]

[a] RAW 264.7 cells were pretreated with the indicated concentrations of compound **D27** for 1 h and treated with LPS (1 μg/mL) for 24 h. Detect proteins were subjected to 8% (iNOS) SDS-PAGE.

Anti-inflammatory Activity *in Vivo*. AIA as a rheumatoid arthritis model was often used for testing anti-inflammatory activity. In this model, 80~90% of rats developed arthritis within 14~21 days after adjuvant injection. Title compound was administered intragastrically from day 14 to 28 after AA immunization. Intragastric frequency was administered once a day. During the whole treatment period, doses (40 or 80 mg/kg) of compound **D27** were administered intragastric administration to the test groups. Aspirin was also administered by intragastric administration. Paw swelling and arthritis index were measured every 3 days. As shown in **Figure 7A**, the swelling peak of AA rats appeared on day 15. Administration of title compound **D27** (40 mg/kg) showed a significant inhibitory effect on hind paw swelling on day 30; which was similar to the aspirin-treated positive control group. As displayed in **Figure 7B**, body weight loss in group of rats treated with compound **D27** was significantly reduced.



A

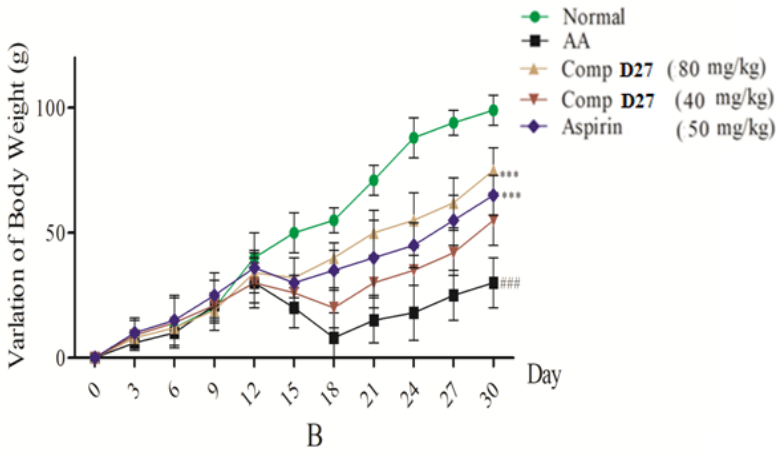


Figure 7. Effects of compound **D27** on hind paw swelling and body weight loss in rats with AA [a]

[a] Data are mean±s.d., n=5 per group. Aspirin was the positive control. ###p<0.01 compared with control; *** p<0.001, ** p<0.01, * p<0.05 *vs* AA group.

Typical photos of non-injected hind paw from sham and AIA rats were taken on day 30 after AIA induction (**Figure 8**). There was a significant increase of secondary hind paw swelling, but title compound could treat secondary hind paw swelling.

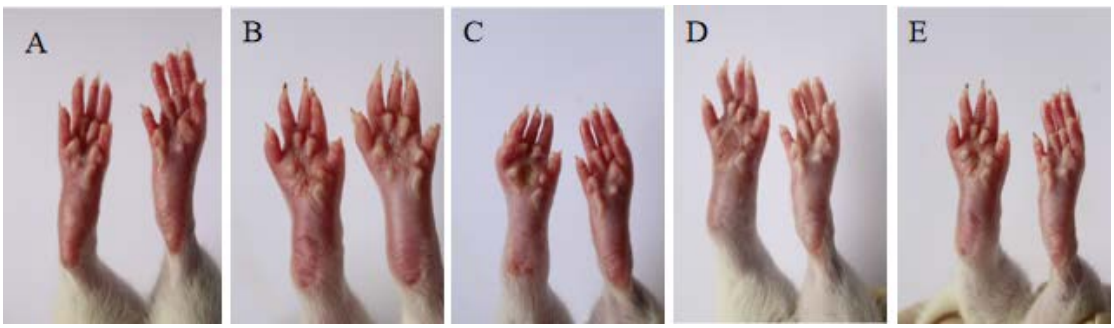


Figure 8. Representative photos of hind paw on day 30 after arthritis induction [a]

[a] Representative photos of hind paw on day 30 after arthritis induction. (**A**) Normal; (**B**) AA; (**C**) Aspirin 50 mg/kg; (**D**) Compound **D27**, 40 mg/kg; (**E**) Compound **D27** 80 mg/kg; Aspirin was the positive control.

To investigate whether compound **D27** relieve the histological changes in knee

joint of AA rats by photomicrographs of knee joints sections stained with H&E. No inflammation was seen in normal rats (**Figure 9A**). AA rats exhibited extensive inflammation, synovial hyperplaisia, bone or cartilage destruction and inflammatory cells infiltration (**Figure 9B**).

AA rats treated with compound **D27** (40 mg/kg) exhibited moderate synovial hyperplasia and inflammatory cells infiltration (**Figure 9D**). Compound **D27** (80 mg/kg) and Aspirin (40 mg/kg) ameliorated inflammatory cells infiltration and cartilage destruction, although slight synovial hyperplasia remained (**Figures 9C** and **9E**).

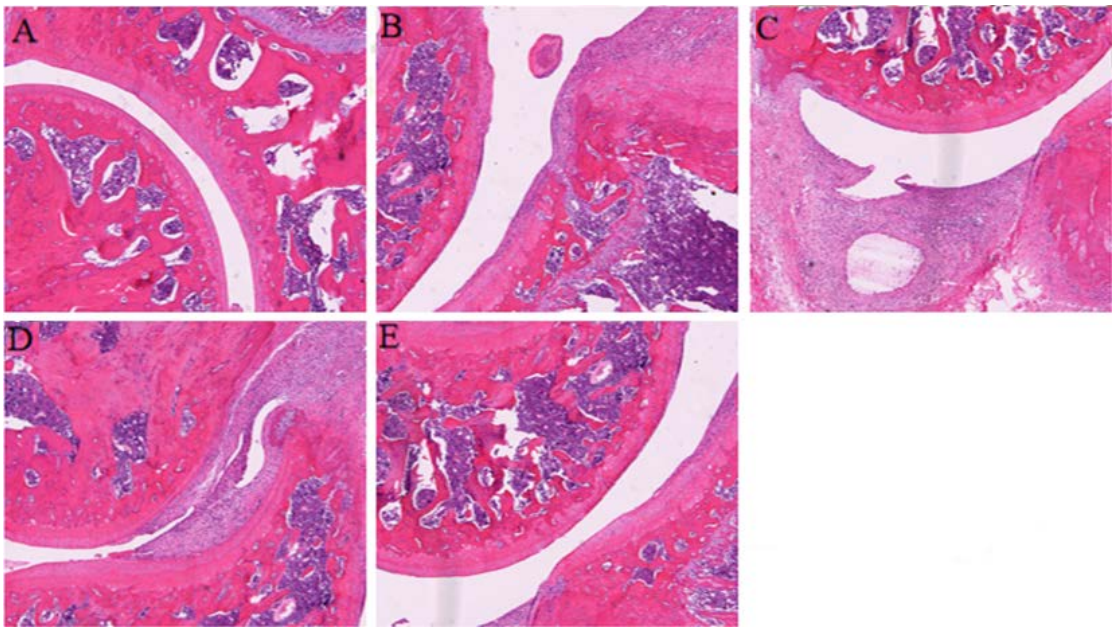


Figure 9. Photomicrographs of sections stained with H&E illustrated the effects of title compound **D27** on joint histology for AA disease

(**A**) Normal; (**B**) AA; (**C**) Aspirin 50 mg/kg; (**D**) Compound **D27** 40 mg/kg; (**E**) Compound **D27** 80 mg/kg, Aspirin was the positive control.

The serum levels of NO, TNF-α, IL-1β and IL-6 significantly elevated in AA model group compared with control group (p<0.01). AA rats treatment with title

compound **D27** (40, 80 mg/kg) showed a dose dependent decrease in serum concentrations of NO, TNF-α, IL-1β and IL-6 compared with AA model group (**Figure 10**). As a positive control, Aspirin showed similar inhibitory effects on IL-1β, TNF-α, NO and IL-6 production in serum.

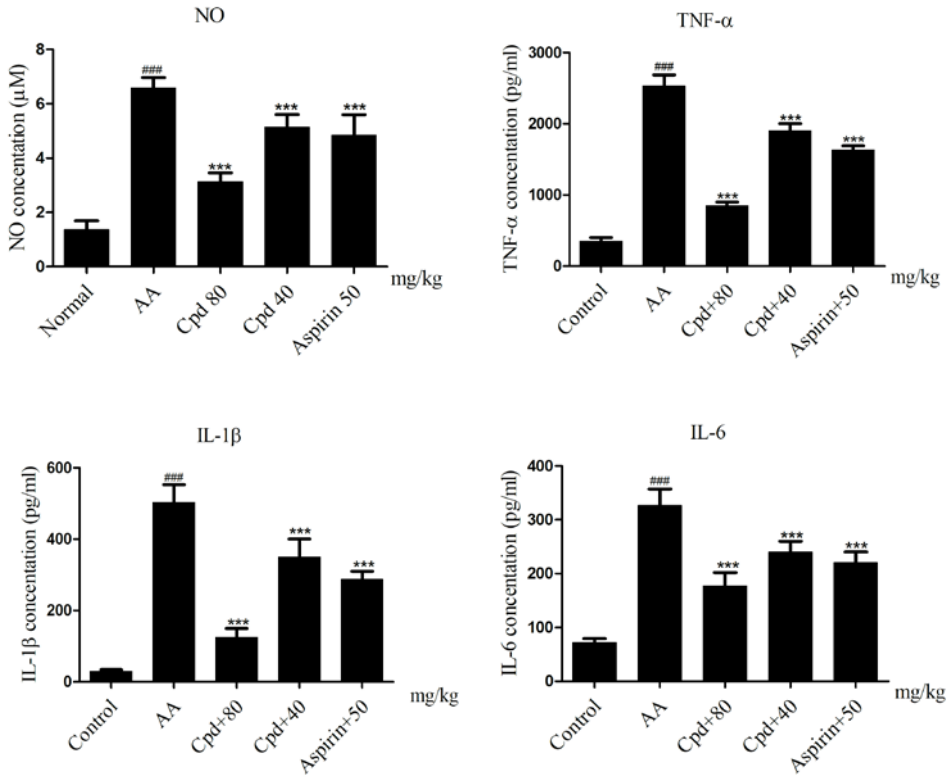


Figure 10. Effects of compound **D27** on productions of NO, TNF-α, IL-1β and IL-6 by serum in rats with adjuvant arthritis (AA) [a]

[a] Data are mean±s.d., n=5 per group. Aspirin used as the positive control. ###p<0.01 compared with control group; *** p<0.001, ** p<0.01, * p<0.05 *vs* AA group.

CONCLUSIONS

In summary, based on the preliminary screening results of inhibitory effects against NO production, guided by structure-activity relationships step by step, four series of total 90 novel pyrazolo[4,3-d]pyrimidine derivatives have been designed、

synthesized and evaluated. After structural optimization through third rounds, target compounds exhibited significant inhibitory activity against NO production in LPS-induced RAW 264.7 cells. A clear structure-activity relationships indicated that substituted 3,4,5-trimethoxystyryl at C-5 and alkylamine at C-7 pyrazolo[4,3-d]pyrimidine scaffold were beneficial to its anti-inflammatory activity. Particularly, compound **D27** with a propan-2-amine side chain displayed potent inhibition towards NO production with IC_{50} value of 3.17 μM. It also showed inhibitory activity against iNOS with IC_{50} of 1.12 μM. Preliminary mechanism studies indicated that title compound could prevent iNOS dimerization. Furthermore, in order to confirm its anti-inflammatory activity *in vivo*, rats models of acute and chronic inflammation were investigated with title compound. For acute inflammatory models in AA rats, treatment of compound **D27** showed significant inhibitory effect on hind paw swelling and body weight loss, which was similar to the effect observed in aspirin-treated group. These observations suggest that title compound **D27** may be an effective agent for treatment of inflammatory diseases through regulation of iNOS dimerization.

EXPERIMENTAL SECTION

General Methods for Chemistry. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica GF254 plates. Column chromatography was performed using 300-400 mesh silica gel purchased from Qingdao Haiyang Chemical Co., Ltd. Melting point was determined on a XT4MP apparatus (Taike

Corp., Beijing, China). ^{1}H and ^{13}C NMR spectra are obtained by Brucker AM-300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or Agilent DD2 600 MHz (^{1}H, 600 MHz; ^{13}C, 151 MHz) spectrometer with $CDCl_3$ or DMSO-d_6 as the solvents and TMS as the internal standard. Chemical shifts are reported in δ (parts per million) values. Coupling constants *J* are reported in Hz. High-resolution electron impact mass spectra (HR-MS) are recorded under electron impact (70 eV) condition using a Micro Mass GCT CA 055 instrument. All chemicals or reagents were purchased from standard commercial suppliers and treated with standard methods before use. Solvents were dried in a routine way and redistilled. The purity of all the tested compounds is at least 95% as determined by HPLC. Crystallographic studies (*Supporting Information).*

General Procedure for Synthesis of Intermediates 4A~D. 3,4,5-trimethoxybenzoic acid (1.06 g, 5 mmol), oxalyl chloride (0.846 mL, 10 mmol) and catalytic DMF were dissolved in dry DCM (10 mL), and the mixture was stirred at 25~30 ^{o}C for 3 h. Then the mixture was concentrated under vacuum and directly used in next without any further purification. The obtained corresponding acyl chloride was dissolved in DCM (1 mL) and the solution was added to the mixture of compound **1**[36, 37] (0.819 g, 4.5 mmol) and TEA (1.34 mL, 10 mmol) in dry DCM (10 mL). The solution was allowed to stir at room temperature for overnight. Reaction mixture was washed with saturated $NaHCO_3$ and water. Organic solvent was dried over anhydrous Na_2SO_4, filtrated, and then evaporated to give a solid. The crude solid product was further recrystallized in ethanol to give compound **2A**.

Compound **2A** (0.376 g, 1 mmol) and sodium ethoxide (0.340 g, 5 mmol) were

dissolved in ethanol (15 mL) and refluxed for 8~12 h. The mixture was concentrated, and the residue was acidified with 1N HCl to pH 7. The precipitate was filtered, washed with water, and dried to give compound **3A.**

Compound **3A** (3.58 g, 10 mmol) was dissolved in phosphorus oxychloride ($POCl_3$) (30 mL) and stirred at 100 ^oC for 8~12 h under N_2 atmosphere. After completion of the reaction, the mixture was concentrated in vacuum to give a dark oil residue. The residue was washed with cold H_2O and extracted with DCM (30 mL×3). The combined organic layer was washed with saturated $NaHCO_3$ solution, saturated brine solution, dried over anhydrous Na_2SO_4, and concentrated in vacuum to give the key intermediate **4A**. Compounds **4B~D** were obtained according to the same procedure as compound **4A**.

1-methyl-3-propyl-*N*-(3-(trifluoromethyl)phenyl)-5-(3,4,5-trimethoxyphenyl)-1 H-pyrazolo[4,3-d]pyrimidin-7-amine (A1). Compound **4A** (200 mg, 0.531 mmol), and 3-trifluoromethylaniline (85.6 mg, 0.531 mmol) were dissolved in *i*-propanol (15 mL) and refluxed for 6~8 h. After completion of the reaction, the solvent was removed, and the residue was purified by column chromatography (gradient elution of PE/EtOAc 85/15 v/v) to obtain compound **A1**. Compounds **A2-13** were obtained according to the same procedure.

Yellow solid (179.2 mg, yield: 67.3%), mp: 164-165 ^oC. ^1H NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H, ArH), 7.92 (d, *J* = 8.0 Hz, 1H, ArH), 7.72 (s, 2H, ArH), 7.52 (t, *J* = 7.9 Hz, 1H, ArH), 7.43 (d, *J* = 7.7 Hz, 1H, ArH), 7.02 (s, 1H, NH), 4.36 (s, 3H, OCH_3), 3.95 (s, 6H, 2×OCH_3), 3.91 (s, 3H, NCH_3), 3.02 (t, *J*=7.6 Hz, 2H, CH_2), 2.03~1.84 (m,

2H,CH_2), 1.06 (t, *J* = 7.4 Hz, 3H,CH_3). HR-MS (ESI): calcd for $C_{25}H_{26}F_3N_5O_3$ $[M+H]^+$, 502.20580; found 502.20583.

1-methyl-3-propyl-*N*-(m-tolyl)-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (A2). Compound was isolated as a yellow solid in 55.4% yieid, mp: 202-203 °C. (recryst. from ethanol). ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.81 (s, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.67 (s, 2H), 7.58 (s, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 6.98 (d, *J* = 7.5 Hz, 1H), 4.31 (s, 3H), 3.84 (s, 6H), 3.71 (s, 3H), 2.91 (t, *J* = 7.4 Hz, 2H), 2.35 (s, 3H), 1.85 (dd, *J* = 14.8, 7.4 Hz, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{25}H_{30}N_5O_3$ $[M+H]^+$, 448.2039; found 448.2039.

1-methyl-*N*-phenyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (A3). Compound was isolated as a yellow solid in 82% yield. mp: 165-166°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, 4H, ArH), 7.41 (t, *J* = 7.9 Hz, 2H, ArH), 7.18 (t, *J* = 7.4 Hz, 1H, ArH), 6.89 (s, 1H, NH), 4.32 (s, 3H, OCH_3), 3.97 (s, 6H, 2 ×OCH_3), 3.90 (s, 3H, NCH_3), 3.02 (t, *J* = 7.6 Hz, 2H, CH_2), 1.93 (dd, *J* = 15.0, 7.5 Hz, 2H, CH_2), 1.06 (t, *J* = 7.4 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{24}H_{28}N_5O_3$ $[M+H]^+$, 434.21496; found 434.21496.

***N*-(4-chlorophenethyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (A4)**. Compound was isolated as a yellow solid in 79% yield, mp: 145-147 °C. (recryst. from ethanol). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.80 (s, 2H, ArH), 7.31 (d, *J* = 8.2 Hz, 2H, ArH), 7.21 (d, *J* = 8.2 Hz, 2H, ArH), 5.12 (s, 1H, NH), 4.09 (s, 3H, OCH_3), 3.99 (m, 8H, 2×OCH_3+CH_2), 3.91 (s, 3H, NCH_3), 3.09 (t, *J* = 6.9 Hz, 2H, NCH_2), 2.98 (t, *J* = 7.6 Hz, 2H, CH_2), 1.97~1.85 (m, 2H, CH_2),

1.04 (t, *J* = 7.4 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{26}H_{31}ClN_5O_3$ $[M+H]^+$, 496.2110; found 496.2112.

1-methyl-*N*-phenethyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (A5). Compound was isolated as a yellow solid in 68% yield, mp: 135-136 ^oC. (recryst. from ethanol). ^1H NMR (600 MHz, $CDCl_3$) δ 7.81 (s, 2H, ArH), 7.35 (t, *J* = 7.5 Hz, 2H, ArH), 7.28 (d, *J* = 7.3 Hz, 3H, ArH), 5.11 (t, *J* = 5.2 Hz, 1H, NH), 4.06~4.01 (m, 5H, OCH_3+NCH_2), 3.99 (s, 6H, 2×OCH_3), 3.91 (s, 3H, NCH_3), 3.10 (t, *J* = 6.8 Hz, 2H, NCH_2), 2.97 (t, *J* = 7.6 Hz, 2H, CH_2), 1.94~1.86 (m, 2H, CH_2), 1.04 (t, *J* = 7.4 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{26}H_{32}N_5O_3$ $[M+H]^+$, 462.2500; found 462.2502.

***N*-(4-methoxyphenethyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (A6)**. Compound was isolated as a yellow solid in 77% yield, mp:146-148 ^oC. (recryst. from ethanol). ^1H NMR (300 MHz, $CDCl_3$): δ 7.81 (s, 2H, ArH), 7.18 (d, *J* = 8.5 Hz, 2H, ArH), 6.87 (d, *J* = 8.6 Hz, 2H, ArH), 5.16 (t, *J* = 5.3 Hz, 1H, NH), 4.04 (s, 3H, OCH_3), 3.99 (s, 6H, 2×OCH_3), 3.95 (d, *J* = 5.9 Hz, 2H, NCH_2), 3.91 (s, 3H, NCH_3), 3.79 (s, 3H, OCH_3), 3.12~2.88 (m, 4H, 2×CH_2), 1.90 (m, 2H, CH_2), 1.04 (t, *J* = 7.4 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{27}H_{34}N_5O_4$ $[M+H]^+$, 492.25700; found 492.25700.

***N*,1-dimethyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d]pyrimidin -7-amine (A7)**. Compound was isolated as a yellow solid in 65% yield, mp:184-185 ^oC. (recryst. from ethanol). ^1H NMR (600 MHz, $CDCl_3$): δ 7.80 (s, 2H, ArH), 5.15 (d, *J* = 4.2 Hz, 1H, NH), 4.22 (s, 3H, OCH_3), 3.99 (s, 6H, 2×OCH_3), 3.90 (s, 3H, NCH_3),

3.27 (d, *J* = 4.8 Hz, 3H, NCH_3), 2.98 (t, *J* = 7.6 Hz, 2H, CH_2), 1.95~1.86 (m, 2H, CH_2), 1.04 (t, *J* = 7.4 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{19}H_{26}N_5O_3$ $[M+H]^+$, 372.20302; found 372.20122.

***N,N*,1-trimethyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (A8)**. Compound was isolated as a yellow solid in 49% yield, mp: 122-123 ^oC. (recryst. from ethanol). ^1H NMR (600 MHz, $CDCl_3$): δ 7.80 (s, 2H, ArH), 4.13 (s, 3H, OCH_3), 3.99 (s, 6H, 2×OCH_3), 3.91 (s, 3H, NCH_3), 3.20 (s, 6H, 2×CH_3), 3.02 (t, *J* = 7.6 Hz, 2H, CH_2), 2.00~1.86 (m, 2H, CH_2), 1.06 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{20}H_{28}N_5O_3$ $[M+H]^+$, 386.2187; found 386.2189.

***N*-isopropyl-1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (A9)**. Compound was isolated as a white solid yield 68%, mp:166-167^oC , ^1H NMR (600 MHz, $CDCl_3$): δ 7.79 (s, 2H, ArH), 4.87 (d, *J* = 6.5 Hz, 1H, NH), 4.62 (dq, *J* = 13.0, 6.5 Hz, 1H, 2×CH_3), 4.23 (s, 3H, OCH_3), 3.99 (s, 6H, 2×OCH_3), 3.90 (s, 3H, NCH_3), 2.98 (t, *J* = 7.6 Hz, 2H, CH_2), 1.95~1.85 (m, 2H, CH_2), 1.42 (d, *J* = 6.5 Hz, 6H, 2×CH_3), 1.04 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{21}H_{30}N_5O_3$ $[M+H]^+$, 400.2343; found 400.2345.

4-(1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d]pyrimidin -7-yl)morpholine (A10). Compound was isolated as a yellow solid, yield 74%, mp: 145-146^oC. (recryst. from ethanol). ^1H NMR (600 MHz, $CDCl_3$): δ 7.78 (s, 2H, ArH), 4.12 (s, 3H, OCH_3), 4.00 (s, 6H, 2×OCH_3), 3.98~3.93 (m, 4H, 2×OCH_2), 3.91 (s, 3H, NCH_3), 3.66~3.57 (m, 4H, 2×NCH_2), 3.03 (t, *J* = 7.6 Hz, 2H, CH_2), 1.93 (m, 2H, CH_2), 1.06 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.41, 153.86,

153.25, 147.75, 145.67, 139.58, 134.18, 124.41, 105.30, 66.52, 61.08, 56.29, 50.11, 38.60, 28.02, 22.18, 14.29. HR-MS (ESI): calcd for $C_{22}H_{30}N_5O_4$ $[M+H]^+$, 428.2292; found 428.2292.

1-methyl-3-propyl-7-(pyrrolidin-1-yl)-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo [4,3-d]pyrimidine (A11). Compound was isolated as a white solid in 59% yield, mp: 142-143^{o}C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78 (s, 2H, ArH), 4.17 (s, 3H, OCH_3), 3.99 (s, 6H, 2×OCH_3), 3.90 (s, 3H, NCH_3), 3.87 (Brs, 4H, 2×NCH_2), 3.01 (t, *J* = 7.6 Hz, 2H, CH_2), 2.04 (Brs, 4H, 2×CH_2), 1.91 (dt, *J* = 14.7, 7.4 Hz, 2H, CH_2), 1.06 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 155.99, 153.07, 151.09, 146.56, 145.09, 139.42, 134.78, 123.30, 105.27, 61.00, 56.21, 50.36, 41.47, 27.94, 25.71, 22.18, 14.28. HR-MS (ESI): calcd for $C_{22}H_{30}N_5O_3$ $[M+H]^+$, 412.2343; found 412.2346.

1-methyl-7-(piperidin-1-yl)-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo [4,3-d]pyrimidine (A12). Compound was isolated as a white solid in 61% yield, mp: 129-130^{o}C. ^{1}H NMR (600 MHz,$CDCl_3$): δ 7.80 (s, 2H, ArH), 4.11 (s, 3H, OCH_3), 4.00 (s, 6H, 2×OCH_3), 3.91 (s, 3H, NCH_3), 3.55 (s, 4H, 2×NCH_2), 3.02 (t, *J* = 7.6 Hz, 2H, CH_2), 1.97~1.88 (m, 2H, CH_2), 1.82 (d, *J* = 4.9 Hz, 4H, 2×CH_2), 1.75 (d, *J* = 4.6 Hz, 2H, CH_2), 1.06 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.34, 154.55, 153.17, 147.38, 145.28, 139.56, 134.54, 124.62, 105.30, 61.03, 56.24, 50.66, 38.58, 28.02, 25.61, 24.59, 22.20, 14.27. HR-MS (ESI): calcd for $C_{23}H_{32}N_5O_3$ $[M+H]^+$, 426.2500; found 426.2503.

Methyl 4-((1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d]

pyrimidin-7-yl)amino)benzoate (A13). Compound was isolated as a yellow solid in 71%, mp: 242-243 °C. (recryst. from ethanol). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.99 (d, *J* = 8.6 Hz, 2H, ArH), 7.80 (d, *J* = 8.6 Hz, 2H, ArH), 7.67 (s, 2H, ArH), 6.97 (s, 1H, NH), 4.25 (s, 3H, OCH_3), 3.90 (s, 6H, 2×OCH_3), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, NCH_3), 2.95 (t, *J* = 7.6 Hz, 2H, CH_2), 1.86 (dd, *J* = 15.0, 7.5 Hz, 2H, CH_2), 0.99 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 166.63, 156.52, 153.25, 146.50, 146.12, 145.24, 142.92, 139.74, 133.95, 130.78, 125.37, 120.96, 119.95, 105.19, 61.08, 56.19, 52.23, 39.28, 27.88, 22.14, 14.26. HR-MS (ESI): calcd for $C_{26}H_{30}N_5O_5$ $[M+H]^+$, 492.2241; found 492.2243.

4-((1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl) amino) benzoic acid (A14). Aqueous NaOH solution (2 N, 10 mL, 20 mmol, 10 equiv) was added to a solution of compound **A13** (200 mg, 0.415 mmol) in MeOH (6 mL) at room temperature. The mixture was stirred at room temperature for 5~6 h. After completion of the reaction, aqueous HCl solution (1 N, ~20 mL) was added dropwise to bring pH to 5~6. The precipitation was then filtered, washed with water, and dried to give the title compound as a yellow solid (164.3mg, 84.5%), mp: 275-277 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.76 (brs, 1H, OH), 9.19 (s, 1H, NH), 7.99 (q, *J* = 8.9 Hz, 4H, ArH), 7.68 (s, 2H, ArH), 4.32 (s, 3H, OCH_3), 3.86 (s, 6H, 2×OCH_3), 3.73 (s, 3H, NCH_3), 2.93 (t, *J* = 7.4 Hz, 2H, CH_2), 1.87 (h, *J* = 7.4 Hz, 2H, CH_2), 1.00 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, DMSO-d_6): δ 167.03, 154.85, 152.76, 146.48, 144.30, 143.93, 143.56, 138.95, 133.50, 129.85, 125.02, 121.21, 120.97, 104.57, 60.11, 55.57, 48.62, 27.18, 21.38, 13.97. HR-MS (ESI): calcd

for $C_{25}H_{28}N_5O_5$ $[M+H]^+$, 478.2085; found 478.2085.

***N*-butyl-4-((1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl)amino)benzamide (A15)**. Compound **A14** (200 mg, 0.428 mmol) was dissolved in anhydrous DCM (10 mL). EDCI (123 mg, 0.642 mmol) and HOBt (86.9 mg, 0.642 mmol) were added and the mixture was allowed to stir for 30 min under N_2 at room temperature. Then a solution of butan-1-amine (35.3 mg, 0.428 mmol) and DIEA (62.3 mg, 0.05 mmol) in DCM (10 mL) were added to the reaction mixture and the reaction mixture was stirred at room temperature for another 12 h under N_2. The solvent was then removed under reduced pressure, and purified by column chromatography (gradient elution of PE/EtOAc 60/40 v/v then 50/50 v/v) to obtain compound **A15** as a white solid (133.1 mg, yield: 51%). mp: 244-246 ^oC. ^1H NMR (600 MHz,$CDCl_3$+DMSO-d_6): δ 8.74 (s, 1H, NH), 7.92 (brs, 1H, NH), 7.86 (s, 4H, ArH), 7.66 (s, 2H, ArH), 4.29 (s, 3H, OCH_3), 3.86 (s, 6H, 2×OCH_3), 3.76 (s, 3H, NCH_3), 3.31 (dd, J = 13.2, 6.7 Hz, 2H, NCH_2), 2.92 (t, J = 7.5 Hz, 2H, CH_2), 1.84 (dd, J = 14.9, 7.4 Hz, 2H, CH_2), 1.61~ 1.46 (m, 2H, CH_2), 1.34 (dd, J = 14.9, 7.5 Hz, 2H, CH_2), 0.98 (t, J = 7.4 Hz, 3H, CH_3), 0.90 (t, J = 7.4 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$+DMSO-d_6): δ 165.37, 154.44, 151.72, 145.75, 144.13, 143.24, 140.93, 137.90, 133.04, 128.38, 126.50, 120.36, 119.90, 103.74, 59.42, 54.80, 38.34, 38.16, 30.56, 26.48, 20.78, 18.99, 12.99, 12.79. HR-MS (ESI): calcd for $C_{29}H_{37}N_6O_4$ $[M+H]^+$, 533.2871; found 533.2869.

General Procedures for Compounds A16-A17. Compounds **A16-A17** were obtained starting from compound **A14** (200 mg, 0.428 mmol) and amine derivatives

according to the same procedure as described for compound **A15**.

***N*-isobutyl-4-((1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl)amino)benzamide (A16)**. Compound was isolated as a white solid (171.4 mg, yield: 77%). mp: 239-241 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.66 (d, *J* = 8.6 Hz, 4H, ArH), 7.53 (s, 2H, ArH), 6.99 (s, 1H, NH), 6.65 (s, 1H, NH), 4.16 (s, 3H, OCH_3), 3.88 (s, 3H, NCH_3), 3.82 (s, 6H, 2× OCH_3), 3.27 (d, *J* = 5.4 Hz, 2H, NCH_2), 2.94 (t, *J* = 6.8 Hz, 2H, CH_2), 1.97~1.84 (m, 3H, CH_2 +CH), 1.05 (t, *J* = 6.6 Hz, 3H, CH_3), 0.98 (d, *J* = 5.7 Hz, 6H, 2×CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 167.39, 155.73, 152.89, 145.94, 145.83, 144.51, 141.43, 139.21, 133.86, 129.76, 127.63, 120.60, 120.04, 104.73, 60.98, 55.90, 47.55, 38.97, 28.75, 27.78, 22.03, 20.30, 14.29. HR-MS (ESI): calcd for $C_{29}H_{37}N_6O_4$ $[M+H]^+$, 533.2871; found 533.2874.

(4-ethylpiperazin-1-yl)(4-((1-methyl-3-propyl-5-(3,4,5-trimethoxyphenyl)-1H-pyrazolo[4,3-d]pyrimidin-7-yl)amino)phenyl)methanone (A17). Compound was isolated as a white solid (112 mg, yield: 46%). mp: 251-252 °C. ^{1}H NMR (600 MHz, $CDCl_3$ + DMSO-d_6): δ 9.00 (s, 1H), 7.92 (d, *J* = 8.4 Hz, 2H), 7.70 (s, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 4.34 (s, 3H), 3.89 (s, 6H), 3.77 (s, 3H), 3.73~3.41 (m, 4H), 2.95 (t, *J* = 7.4 Hz, 2H), 2.41 (dd, *J* = 14.3, 7.1 Hz, 6H), 1.89 (dd, *J* = 14.8, 7.4 Hz, 2H), 1.06 (t, *J* = 7.2 Hz, 3H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$ + DMSO-d_6): δ 168.81, 154.82, 152.45, 146.52, 144.36, 143.77, 140.40, 138.64, 133.57, 130.15, 127.14, 121.16, 121.02, 104.39, 59.92, 56.14, 55.35, 51.47, 38.97, 27.12, 21.38, 13.77, 11.69. HR-MS (ESI): calcd for $C_{31}H_{40}N_7O_4$ $[M+H]^+$, 573.3136; found 574.3138.

(*E*)-1-methyl-3-propyl-5-styryl-*N*-(3-(trifluoromethyl)phenyl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (B1). Intermediate **4B** (200 mg, 0.639 mmol), 3-trifluoromethylaniline (103.1 mg, 0.531 mmol) were dissolved in *i*-propanol (15 mL) and refluxed for 8~10 h. The solvent was removed under vacuum, and the residue was purified by column chromatography (gradient elution of PE/EtOAc 90/10 v/v then 80/20 v/v) to obtain **B1**. Compounds **B2-18** were obtained according to the same procedure. Yellow solid (155.2 mg, yield: 56%). mp:109-110 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.84 (brs, 1H), 8.42 (s, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.81~7.54 (m, 5H), 7.45 (dd, *J* = 15.8, 8.2 Hz, 4H), 4.40 (s, 3H), 2.94 (t, *J* = 7.3 Hz, 2H), 1.77 (dd, *J* = 14.6, 7.2 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{24}H_{23}F_3N_5$ $[M+H]^+$, 438.19; found 438.1904.

(*E*)-1-methyl-3-propyl-5-styryl-*N*-(m-tolyl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (B2). Compound was isolated as a yellow solid in 69% yield. mp: 112-113 ^{o}C ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 10.14 (s, 1H), 7.93~7.31 (m, 10H), 7.19 (d, *J* = 7.1 Hz, 1H), 4.48 (s, 3H), 3.21~3.02 (m, 2H), 2.47 (s, 3H), 1.82 (dd, *J* = 13.4, 6.1 Hz, 2H), 1.03 (t, *J* = 6.3 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 152.72, 148.26, 142.92, 139.87, 137.62, 135.44, 133.61, 129.77, 128.17, 127.69, 127.55, 126.78, 124.76, 121.34, 120.48, 118.07, 117.92, 39.49, 26.88, 21.53, 20.67, 12.90. HR-MS (ESI): calcd for $C_{24}H_{26}N_5$ $[M+H]^+$, 384.2181; found 384.2181.

(*E*)-1-methyl-*N*-phenyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (B3). Compound was isolated as a yellow solid in 81% yield, mp: 102-103 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 15.6 Hz, 1H),

7.51~7.28 (m, 8H), 7.10 (t, *J* = 7.4 Hz, 1H), 4.46 (s, 3H), 2.82 (t, *J* = 7.6 Hz, 2H), 1.84~1.62 (m, 2H), 0.95 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{23}H_{24}N_5O$ $[M+H]^+$, 370.2026; found 370.2030.

(*E*)-*N*-(4-chlorobenzyl)-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (B4). Compound was isolated as a white solid in 93% yield, mp: 144-145 °C. ^{1}H NMR (600 MHz, $CDCl_3$+DMSO-d_6): δ 7.76 (s, 1H), 7.66 (d, *J* = 15.8 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 2H), 7.48 (d, *J* = 8.3 Hz, 3H), 7.37 (t, *J* = 7.6 Hz, 2H), 7.31 (s, 1H), 7.28 (t, *J* = 7.4 Hz, 1H), 7.09 (d, *J* = 15.8 Hz, 1H), 4.87 (d, *J* = 5.6 Hz, 2H), 4.27 (s, 3H), 2.88 (t, *J* = 7.6 Hz, 2H), 1.82 (dd, *J* = 15.1, 7.5 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$ + DMSO-d_6): δ 156.89, 149.02, 144.62, 142.46, 138.69, 136.61, 134.71, 132.02, 129.05, 129.02, 128.63, 128.20, 128.10, 126.96, 121.13, 43.60, 39.16, 27.55, 22.06, 13.99. HR-MS (ESI): calcd for $C_{24}H_{25}ClN_5[M+H]^+$, 418.1793; found 418.1796.

(*E*)-*N*-(4-chlorophenethyl)-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (B5). Compound was isolated as a white solid in 87% yield, mp: 171-173 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.86 (s, 1H, NH), 7.77 (d, *J* = 15.9 Hz, 1H, =CH), 7.67 (d, *J* = 7.4 Hz, 2H), 7.46~7.27 (m, 7H), 7.14 (d, *J* = 15.9 Hz, 1H, =CH), 4.15 (s, 3H, NCH_3), 3.82 (m, 2H, CH_2), 3.08~2.99 (m, 2H, CH_2), 2.80 (t, *J* = 7.5 Hz, 2H, CH_2), 1.76 (dd, *J* = 14.9, 7.4 Hz, 2H, CH_2), 0.95 (t, *J* = 7.4 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{25}H_{27}ClN_5$ $[M+H]^+$, 432.1950; found 432.1952.

(*E*)-*N*-(2-chlorophenethyl)-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (B6). Compound was isolated as a yellow solid in 82% yield, mp:

176-178 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.91 (d, *J* = 15.8 Hz, 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.43~7.36 (m, 3H), 7.33~7.27 (m, 2H), 7.22 (dt, *J* = 14.0, 6.2 Hz, 3H), 5.16 (s, 1H, NH), 4.11 (s, 3H), 4.05 (dd, *J* = 12.7, 6.5 Hz, 2H), 3.25 (t, *J* = 6.8 Hz, 2H), 2.98~2.91 (m, 2H), 1.85 (dd, *J* = 15.1, 7.5 Hz, 2H), 1.02 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.73, 149.23, 145.61, 136.90, 136.75, 135.44, 134.15, 131.15, 129.80, 128.96, 128.65, 128.32, 128.20, 127.31, 127.14, 121.08, 40.65, 38.94, 33.09, 27.73, 22.25, 14.06. HR-MS (ESI): calcd for $C_{25}H_{27}ClN_5[M+H]^+$, 432.195; found 432.1951.

(*E*)-1-methyl-*N*-phenethyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (B7). Compound was isolated as a yellow solid in 73% yield, mp:164-165 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.81 (d, *J* = 15.9 Hz, 1H, =CH), 7.67 (d, *J* = 7.5 Hz, 2H, ArH), 7.48~7.30 (m, 8H, ArH+NH), 7.30~7.21 (m, 1H, ArH), 7.16 (d, *J* = 15.9 Hz, 1H, =CH), 4.16 (s, 3H, NCH_3), 3.86~3.79 (m, 2H, CH_2), 3.12~2.96 (m, 2H, CH_2), 2.80 (t, *J* = 7.5 Hz, 2H, CH_2), 1.76 (dd, *J* = 14.8, 7.4 Hz, 2H, CH_2), 0.95 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{25}H_{28}N_5[M+H]^+$, 398.2339; found 398.2337.

(*E*)-*N*-(2-fluorophenethyl)-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (B8). Compound was isolated as a yellow solid in 85% yield, mp: 169-171 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.91 (d, *J* = 15.8 Hz, 1H), 7.63 (d, *J* = 7.6 Hz, 2H), 7.38 (t, *J* = 7.6 Hz, 2H), 7.29 (dd, *J* = 12.3, 7.4 Hz, 2H), 7.25 (s, 1H), 7.22 (d, *J* = 15.8 Hz, 1H), 7.16~7.07 (m, 2H), 5.22 (s, 1H, NH), 4.12 (s, 3H), 4.00 (dd, *J* = 12.5, 6.5 Hz, 2H), 3.15 (t, *J* = 6.7 Hz, 2H), 2.97~2.91 (m, 2H), 1.85 (dd, *J* = 15.1, 7.5

Hz, 2H), 1.02 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 161.36 (d, *J* = 244.0 Hz), 157.75, 149.22, 145.60, 136.92, 135.48, 131.30 (d, *J*= 4.9 Hz), 128.92, 128.68, 128.64, 128.63, 128.18, 127.32, 125.94 (d, *J* = 16.1 Hz), 124.48 (d, *J* = 3.5 Hz), 121.08, 115.52 (d, *J* = 22.2 Hz), 41.23, 38.82, 28.96, 27.72, 22.27, 14.06. HR-MS (ESI): calcd for $C_{25}H_{27}FN_5[M+H]^+$, 416.2245; found 416.2241.

(*E*)-*N*-(4-methoxyphenethyl)-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (B9). Compound was isolated as a white solid in 88% yield, mp: 179-181 °C. ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 7.89 (d, *J* = 15.9 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.45~7.35 (m, 2H), 7.31 (d, *J* = 7.3 Hz, 1H), 7.23 (dd, *J* = 12.1, 7.2 Hz, 3H), 6.90 (d, *J* = 8.5 Hz, 2H), 5.84 (s, 1H), 4.11 (s, 3H), 3.94 (dd, *J* = 12.6, 6.6 Hz, 2H), 3.81 (s, 3H), 3.04 (t, *J* = 7.0 Hz, 3H), 2.92 (t, *J* = 7.6 Hz, 2H), 1.86 (dt, *J* = 15.2, 7.5 Hz, 2H), 1.02 (t, *J* = 7.3 Hz, 3H), 0.00 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.08, 157.28, 149.11, 144.93, 142.29, 136.60, 134.97, 130.94, 129.56, 128.79, 128.44, 127.97, 127.00, 121.01, 113.89, 55.05, 42.09, 38.74, 34.05, 27.46, 22.00, 13.83. HR-MS (ESI): calcd for $C_{26}H_{30}N_5O$ $[M+H]^+$, 428.2445; found 428.2445.

(*E*)-*N*,*N*,1-trimethyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (B10). Compound was isolated as a white solid in 78% yield, mp: 112-113 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88 (d, *J* = 15.8 Hz, 1H), 7.62 (d, *J* = 7.7 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.28 (t, *J* = 7.4 Hz, 1H), 7.26~7.22 (d, *J* = 15.8 Hz, 1H), 4.11 (s, 3H), 3.17 (s, 6H), 2.98 (t, *J* = 7.7 Hz, 2H), 1.96~1.82 (m, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{19}H_{24}N_5$ $[M+H]^+$, 322.2066; found 322.2028.

(*E*)-*N*-isopropyl-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (B11). Compound was isolated as a white solid in 81% yield, mp: 109-110°C.^{1}H NMR (600 MHz, $CDCl_3$+DMSO-d_6): δ 8.21 (d, *J* = 7.5 Hz, 1H, NH), 8.02 (d, *J* = 15.6 Hz, 1H, ArH), 7.80 (d, *J* = 15.6 Hz, 1H, ArH), 7.67 (d, *J* = 4.2 Hz, 2H, ArH), 7.40 (s, 3H, ArH), 4.89 (dq, *J* = 13.6, 6.7 Hz, 1H, NCH), 4.40 (s, 3H, NCH_3), 3.08 (t, *J* = 7.4 Hz, 2H, CH_2), 1.85~1.74 (m, 2H, CH_2), 1.51 (d, *J* = 6.6 Hz, 6H, 2×CH_3), 1.01 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$+DMSO-d_6): δ 152.94, 149.33, 142.33, 139.74, 133.95, 129.86, 128.67, 128.32, 127.83, 120.41, 118.36, 44.34, 39.59, 27.11, 21.83, 21.08, 13.10. HR-MS (ESI): calcd for $C_{20}H_{26}N_5$ $[M+H]^+$, 336.2183; found 336.2184.

(*E*)-*N*-isobutyl-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (B12). Compound was isolated as a white solid in 86% yield, mp: 131-132 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.72 (brs, 1H, NH), 8.05 (d, *J* = 15.7 Hz, 1H, =CH), 7.71 (d, *J* = 6.3 Hz, 2H, ArH), 7.60~7.42 (m, 4H, ArH), 4.29 (s, 3H, NCH_3), 3.63 (t, *J* = 6.3 Hz, 2H, CH_2), 2.92 (t, *J* = 7.4 Hz, 2H, CH_2), 2.24~2.08 (m, 1H, CH), 1.71 (dd, *J* = 14.8, 7.4 Hz, 2H, CH_2), 1.02 (d, *J* = 6.6 Hz, 6H, 2×CH_3), 0.96 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{21}H_{28}N_5[M+H]^+$, 350.2339; found 350.2342.

(*E*)-7-(4-ethylpiperazin-1-yl)-1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d] pyrimidine (B13). Compound was isolated as a yellow solid in 64% yield, mp: 147-148 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.92 (Brs, 1H), 8.04 (d, *J* = 15.8 Hz, 1H), 7.74 (d, *J* = 6.9 Hz, 2H), 7.54 (d, *J* = 16.5 Hz, 1H), 7.51~7.38 (m, 3H),4.47 (d, *J* = 13.6 Hz, 2H), 4.14 (s, 3H), 3.86 (t, *J* = 12.4 Hz, 2H), 3.60 (d, *J* = 11.9 Hz, 2H),

3.36~3.09 (m, 4H), 2.97 (t, *J* = 7.5 Hz, 2H), 1.75 (dt, *J* = 14.4, 7.2 Hz, 2H), 1.31 (dd, *J* = 12.5, 5.3 Hz, 3H), 0.98 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{23}H_{31}N_6$ $[M+H]^+$, 391.2605; found 391.2604.

(*E*)-4-(1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-yl)morpholine (B14). Compound was isolated as a yellow solid in 57% yield, mp: 138-140 ^oC. (recryst. from ethanol). ^1H NMR (600 MHz, $CDCl_3$): δ 7.86 (d, *J* = 15.9 Hz, 1H, =CH), 7.62 (d, *J* = 7.6 Hz, 2H, ArH), 7.38 (t, *J* = 7.5 Hz, 2H, ArH), 7.30 (t, *J* = 7.3 Hz, 1H, ArH), 7.25 (d, *J* = 15.9 Hz, 1H, =CH), 4.10 (s, 3H, NCH_3), 3.97~3.92 (m, 4H, 2 × OCH_2), 3.62~3.56 (m, 4H, 2×NCH_2), 2.99 (t, *J* = 7.7 Hz, 2H, CH_2), 1.89 (dd, *J* = 15.1, 7.5 Hz, 2H, CH_2), 1.04 (t, *J* = 7.4 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.31, 153.72, 147.57, 145.39, 136.83, 135.88, 128.80, 128.58, 128.48, 127.46, 124.40, 66.55, 50.10, 38.60, 27.98, 22.27, 14.21. HR-MS (ESI): calcd for $C_{21}H_{26}N_5O$ $[M+H]^+$, 364.2132; found 364.2136.

(*E*)-1-methyl-3-propyl-7-(pyrrolidin-1-yl)-5-styryl-1H-pyrazolo[4,3-d]pyrimidine (B15). Compound was isolated as a yellow solid in 73% yield, mp: 142-143 ^oC. ^1H NMR (600 MHz, $CDCl_3$): δ 7.85 (d, *J* = 15.8 Hz, 1H, =CH), 7.61 (d, *J* = 7.6 Hz, 2H, ArH), 7.36 (t, *J* = 7.6 Hz, 2H, ArH), 7.31~7.25 (m, 1H, ArH), 7.22 (d, *J* = 15.8 Hz, 1H,=CH), 4.15 (s, 3H, NCH_3), 3.85 (t, *J* = 6.4 Hz, 4H, 2×NCH_2), 3.01~2.93 (m, 2H, CH_2), 2.02 (t, *J* = 6.5 Hz, 4H, CH_2), 1.91~1.84 (m, 2H, 2×CH_2), 1.04 (t, *J* = 7.3 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.84, 151.08, 146.39, 144.57, 137.04, 135.24, 129.02, 128.71, 128.21, 127.40, 123.46, 50.43, 41.41, 27.90, 25.71, 22.30, 14.23. HR-MS (ESI): calcd for $C_{21}H_{26}N_5$ $[M+H]^+$, 348.2183; found 348.2180.

(*E*)-1-methyl-7-(piperidin-1-yl)-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidine (B16). Compound was isolated as a white solid in 61% yield, mp: 152-154 ^{o}C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.10 (d, *J* = 15.6 Hz, 1H), 8.00 (d, *J* = 15.6 Hz, 1H), 7.74 ~7.70 (m, 2H), 7.43~7.38 (m, 3H), 4.11 (s, 3H), 3.94 (Brs, 4H), 3.25 (t, *J* = 7.5 Hz, 2H), 1.90~1.81 (m, 8H), 1.06 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{22}H_{28}N_5$ $[M+H]^+$, 362.2339; found 362.2335.

(*E*)-1-methyl-*N*-(3-morpholinopropyl)-3-propyl-5-styryl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (B17). Compound was isolated as a white solid in 47% yield, mp: 149-151^{o}C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.84 (d, *J* = 15.8 Hz, 1H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.36 (t, *J* = 7.6 Hz, 2H), 7.28 (d, *J* = 7.3 Hz, 1H), 7.20 (d, *J* = 15.8 Hz, 1H), 6.09 (s, 1H), 4.25 (s, 3H), 3.83 (dd, *J* = 11.5, 6.0 Hz, 2H), 3.72 (s, 4H), 2.97~2.91 (m, 2H), 2.58 (t, *J* = 5.9 Hz, 2H), 2.52 (brs, 4H), 1.95 (dd, *J* = 12.1, 6.0 Hz, 2H), 1.90 – 1.82 (m, 2H), 1.02 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{24}H_{33}N_6O$ $[M+H]^+$, 421.2710; found 421.2714.

Methyl (*E*)-4-((1-methyl-3-propyl-5-styryl-1H-pyrazolo[4,3-d]pyrimidin-7-yl) amino)benzoate (B18). Compound was isolated as a white solid in 90% yield, mp: 178-179 ^{o}C. ^{1}H NMR (600 MHz,DMSO-d_6) δ 9.76~9.64 (m, 1H), 8.10 (d, *J* = 8.6 Hz, 2H), 8.00 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 15.8 Hz, 1H), 7.67 (d, *J* = 7.4 Hz, 2H), 7.45 (t, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.2 Hz, 1H), 7.36 (d, *J* = 15.8 Hz, 1H), 4.36 (s, 3H), 3.88 (s, 3H), 2.92 (t, *J* = 7.5 Hz, 2H), 1.78 (dd, *J* = 14.9, 7.4 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{25}H_{26}N_5O_2$ $[M+H]^+$, 428.2081; found 428.2084.

(*E*)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-*N*-(3-(trifluoromethyl)phenyl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (C1). Intermediate **4C** (200 mg, 0.661 mmol), 3-trifluoromethylaniline (128.3 mg, 0.661 mmol) were dissolved in *i*-propanol (15 mL), and refluxed for 8-10 h. Then, the solvent was removed under reduced pressure and the residues were purified by column chromatography gradient elution of PE/EtOAc 90/10 v/v then 80/20 v/v to obtain compound **C1**. Compounds **C2-17** were obtained according to the same procedure.

White solid (193.2 mg, yield: 68 %). mp:133-134 ^{o}C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.66 (brs, 1H, NH), 8.34 (s, 1H, ArH), 8.04 (d, J = 8.1 Hz, 1H, ArH), 7.88 (s, 1H, ArH), 7.72 (d, J = 7.8 Hz, 1H, ArH), 7.62 (d, J = 7.6 Hz, 1H, ArH), 7.49 (d, J = 15.5 Hz, 1H, =CH), 7.14 (d, J = 15.5 Hz, 1H, =CH), 6.76 (d, J = 3.3 Hz, 1H, ArH), 6.66 (d, J = 1.8 Hz, 1H, ArH), 4.39 (s, 3H, NCH_3), 2.94 (t, J = 7.4 Hz, 2H, CH_2), 1.76 (dd, J = 14.7, 7.4 Hz, 2H, CH_2), 0.97 (t, J = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{22}H_{21}F_3N_5O$ $[M+H]^+$, 428.1693; found 428.1697.

(*E*)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-*N*-(m-tolyl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (C2). Compound was isolated as a white solid in 82% yield, mp: 127-128 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6): δ 10.09 (s, 1H), 7.81 (s, 1H), 7.54 (dd, J = 15.1, 4.4 Hz, 3H), 7.40 (t, J = 7.8 Hz, 1H), 7.19 (dd, J = 22.5, 11.4 Hz, 2H), 6.81 (d, J = 3.2 Hz, 1H), 6.62 (dd, J = 3.3, 1.8 Hz, 1H), 4.42 (s, 3H), 2.97 (t, J = 7.5 Hz, 2H), 2.43 (s, 3H), 1.75 (dt, J = 14.7, 7.4 Hz, 2H), 0.99 (t, J = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{22}H_{24}N_5O$ $[M+H]^+$, 374.1975; found 374.1979.

(*E*)-*N*-(4-chlorobenzyl)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-1H-pyrazolo

[4,3-d]pyrimidin-7-amine (C3). Compound was isolated as a white solid in 67% yield, mp:118-119 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.90 (t, *J* = 5.6 Hz, 1H, NH), 7.73 (s, 1H, ArH), 7.50 (m, 3H, ArH), 7.44~7.34 (m, 2H, ArH), 6.78 (d, *J* = 15.8 Hz, 1H, =CH), 6.72 (d, *J* = 3.3 Hz, 1H, ArH), 6.63~6.52 (m, 1H, ArH), 4.78 (d, *J* = 5.6 Hz, 2H), 4.22 (s, 3H), 4.02 (s, 1H), 2.78 (t, *J* = 7.5 Hz, 2H), 1.73 (dt, *J* = 14.9, 7.4 Hz, 2H), 0.93 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{22}H_{23}ClN_5O$ $[M+H]^+$, 408.1586; found 408.1580.

(*E*)-*N*-(2-chlorobenzyl)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C4). Compound was isolated as a white solid in 78% yield, mp: 122-123 °C.^{1}H NMR (600 MHz, $CDCl_3$): δ 7.65 (dd, *J* = 15.6, 1.5 Hz, 1H), 7.57 (t, *J* = 5.6 Hz, 0H), 7.44 (s, 1H), 7.40 (t, *J* = 5.6 Hz, 0H), 7.26~7.21 (m, 2H), 7.11 (d, *J* = 15.7 Hz, 1H), 6.50 (d, *J* = 2.0 Hz, 1H), 6.44 (dd, *J* = 3.3, 1.6 Hz, 1H), 5.70 (t, *J* = 6.0 Hz, 1H), 4.99 (d, *J* = 6.0 Hz, 1H), 4.21 (s, 2H), 2.91 (t, *J* = 7.1 Hz, 2H), 1.83 (q, *J* = 7.5 Hz, 2H), 1.00 (t, *J* = 7.8 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.56, 153.30, 148.98, 145.75, 143.34, 143.00, 135.95, 133.85, 131.03, 129.79, 129.27, 127.44, 127.27, 122.98, 121.14, 111.88, 110.31, 42.78, 39.12, 27.84, 22.36, 14.17. HR-MS (ESI): calcd for $C_{22}H_{23}ClN_5O$ $[M+H]^+$, 408.11586; found 408.1580.

(*E*)-*N*-(4-bromobenzyl)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C5). Compound was isolated as a yellow solid in 64% yield, mp:139-140 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.57 (d, *J* = 15.7 Hz, 1H), 7.50~7.47 (m, 2H), 7.43 (d, *J* = 1.7 Hz, 1H), 7.31 (d, *J* = 8.3 Hz, 2H), 7.10 (d, *J* = 15.7 Hz, 1H), 6.47 (d, *J* = 3.2 Hz, 1H), 6.43 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.37 (t, *J* = 5.6

Hz, 1H), 4.87 (d, *J* = 5.5 Hz, 2H), 4.19 (s, 3H), 2.94~2.90 (m, 2H), 1.84 (q, *J* = 7.5 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.60, 153.20, 149.05, 145.86, 143.46, 143.06, 137.80, 132.03, 129.75, 127.23, 123.13, 121.66, 120.97, 111.90, 110.44, 44.32, 39.20, 27.86, 22.37, 14.19. HR-MS (ESI): calcd for $C_{22}H_{23}BrN_5O$ $[M+H]^+$, 452.108; found 452.1079.

(*E*)-*N*-(4-fluorobenzyl)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C6). Compound was isolated as a yellow solid in 87% yield, mp: 128-129 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.60 (d, *J* = 15.7 Hz, 1H), 7.43 (d, *J* = 1.7 Hz, 1H), 7.41 (dd, *J* = 8.4, 5.4 Hz, 2H), 7.11 (d, *J* = 15.7 Hz, 1H), 7.04 (t, *J* = 8.6 Hz, 2H), 6.47 (d, *J* = 3.3 Hz, 1H), 6.43 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.40 (t, *J* = 5.3 Hz, 1H), 4.87 (d, *J* = 5.4 Hz, 2H), 4.17 (s, 3H), 2.92 (t, *J* = 7.7 Hz, 2H), 1.84 (q, *J* = 7.6 Hz, 2H), 1.01 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 162.27 (d, *J* = 245.8 Hz), 157.47, 153.07, 148.96, 145.64, 143.14, 142.94, 134.37 (d, *J* = 3.4 Hz), 129.65 (d, *J* = 8.2 Hz), 127.04, 123.03, 120.86, 115.68 (d, *J* = 21.4 Hz), 111.77, 110.33, 77.24, 77.03, 76.82, 44.11, 39.05, 27.72, 22.25, 14.05. HR-MS (ESI): calcd for $C_{22}H_{23}FN_5O$ $[M+H]^+$,392.1881; found 392.1880.

(*E*)-5-(2-(furan-2-yl)vinyl)-*N*-(4-methoxybenzyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C7). Compound was isolated as a white solid in 69% yield, mp: 124-125 °C.^{1}H NMR (600 MHz, $CDCl_3$): δ 7.66 (d, *J* = 15.7 Hz, 1H), 7.43 (d, *J* = 1.7 Hz, 1H), 7.39~7.36 (m, 2H), 7.13 (d, *J* = 15.7 Hz, 1H), 6.92 – 6.89 (m, 2H), 6.49 (d, *J* = 3.3 Hz, 1H), 6.43 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.25 (t, *J* = 5.3 Hz, 1H), 4.85 (d, *J* = 5.3 Hz, 2H), 4.16 (s, 3H), 3.81 (s, 3H), 2.93 (t, *J* = 7.7 Hz, 2H), 1.85 (q, *J* = 7.6

Hz, 2H), 1.01 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 159.21, 157.56, 153.18, 149.04, 145.64, 143.13, 142.87, 130.55, 129.38, 127.28, 122.95, 120.90, 114.24, 111.74, 110.21, 55.32, 44.38, 39.03, 27.72, 22.25, 14.05. HR-MS (ESI): calcd for $C_{23}H_{26}N_5O_2$ $[M+H]^+$,404.2081; found 404.2078.

(*E*)-5-(2-(furan-2-yl)vinyl)-*N*-(3-methoxybenzyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C8). Compound was isolated as a white solid in 89% yield, mp: 132-133 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.64 (d, *J* = 15.7 Hz, 1H), 7.43 (d, *J* = 1.7 Hz, 1H), 7.30 (t, *J* = 8.1 Hz, 1H), 7.12 (d, *J* = 15.7 Hz, 1H), 7.04~7.00 (m, 2H), 6.88~6.84 (m, 1H), 6.48 (d, *J* = 3.4 Hz, 1H), 6.43 (dd, *J* = 3.3, 1.8 Hz, 1H), 5.33 (t, *J* = 5.5 Hz, 1H), 4.90 (d, *J* = 5.4 Hz, 2H), 4.19 (s, 3H), 3.80 (s, 3H), 2.95 – 2.91 (m, 2H), 1.89 ~1.81 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 160.11, 157.68, 153.29, 149.18, 145.83, 143.37, 143.02, 140.33, 130.07, 127.37, 123.12, 121.05, 120.27, 113.87, 113.17, 111.88, 110.37, 55.40, 45.00, 39.20, 27.86, 22.39, 14.20. HR-MS (ESI): calcd for $C_{23}H_{26}N_5O_2$ $[M+H]^+$,404.2081; found 404.2090.

(*E*)-5-(2-(furan-2-yl)vinyl)-*N*-(2-methoxybenzyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C9). Compound was isolated as a white solid in 83% yield, mp: 136-137 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.70 (d, *J* = 15.7 Hz, 1H), 7.45 (s, 2H), 7.32~7.27 (m, 1H), 7.12 (d, *J* = 15.7 Hz, 1H), 6.99~ 6.92 (m, 2H), 6.52 (d, *J* = 3.0 Hz, 1H), 6.45 (dd, *J* = 3.1, 1.7 Hz, 1H), 5.85 (s, 1H), 4.92 (d, *J* = 5.7 Hz, 2H), 4.18 (s, 3H), 3.94 (s, 3H), 2.94~2.87 (m, 2H), 1.83 (q, *J* = 7.5 Hz, 2H), 1.00 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.88, 157.71, 153.44, 149.42, 145.67,

142.94, 130.46, 129.22, 127.69, 126.44, 122.92, 121.38, 121.07, 111.86, 110.68, 110.15, 55.60, 41.10, 38.96, 27.85, 22.40, 14.18. HR-MS (ESI): calcd for $C_{23}H_{26}N_5O_2$ $[M+H]^+$,404.2081; found 404.2081.

(*E*)-5-(2-(furan-2-yl)vinyl)-*N*-(furan-2-ylmethyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C10). Compound was isolated as a yellow solid in 74% yield, mp: 104-105 ^oC. ^1H NMR (600 MHz, $CDCl_3$): δ 7.67 (d, *J* = 15.7 Hz, 1H), 7.44 (s, 1H), 7.42~7.38 (m, 1H), 7.12 (d, *J* = 15.7 Hz, 1H), 6.50 (d, *J* = 3.1 Hz, 1H), 6.44 (dd, *J* = 3.1, 1.7 Hz, 1H), 6.37 (dt, *J* = 4.8, 3.0 Hz, 2H), 5.35 (d, *J* = 4.6 Hz, 1H), 4.91 (d, *J* = 5.2 Hz, 2H), 4.20 (s, 3H), 2.95~2.90 (m, 2H), 1.84 (q, *J* = 7.5 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.53, 153.32, 151.54, 148.81, 145.83, 143.39, 143.03, 142.42, 127.36, 123.12, 121.14, 111.89, 110.77, 110.34, 108.06, 39.13, 37.92, 27.86, 22.35, 14.17. HR-MS (ESI): calcd for $C_{20}H_{22}N_5O_2$ $[M+H]^+$, 364.1768; found 364.1772.

(*E*)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-*N*-(4-(trifluoromethyl)benzyl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (C11). Compound was isolated as a yellow solid in 53% yield, mp: 156-157 ^oC. ^1H NMR (600 MHz, $CDCl_3$): δ 7.62 (d, *J* = 8.1 Hz, 2H), 7.57 ~7.52 (m, 3H), 7.42 (s, 1H), 7.09 (d, *J* = 15.7 Hz, 1H), 6.46 (d, *J* = 3.1 Hz, 1H), 6.44 ~6.42 (m, 1H), 5.44 (s, 1H), 4.99 (d, *J* = 5.5 Hz, 2H), 4.21 (s, 3H), 2.95~2.91 (m, 2H), 1.85 (q, *J* = 7.5 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.42, 153.00, 148.89, 145.75, 143.37, 142.92, 142.78, δ 129.87 (q, *J* = 32.5 Hz), 128.03, 126.96, 125.69 (q, *J* = 3.8 Hz),124.03 (d, *J* = 271.9 Hz).,123.02, 120.82, 111.75, 110.32, 44.28, 39.04, 27.70, 22.19, 14.01. HR-MS (ESI): calcd for

$C_{23}H_{23}F_3N_5O$ $[M+H]^+$, 442.1849; found 442.1848.

(*E*)-*N*-(4-chlorophenethyl)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (C12). Compound was isolated as a yellow solid in 67% yield, mp: 121-122 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.76 (s, 1H, ArH), 7.56 (d, *J* = 15.8 Hz, 1H, =CH), 7.32-7.40 (m, 5H, ArH+NH), 6.87 (d, *J* = 15.8 Hz, 1H, =CH), 6.76 (d, *J* = 3.2 Hz, 1H, ArH), 6.60 (s, 1H, ArH), 4.14 (s, 3H, NCH_3), 3.87 – 3.71 (m, 2H, CH_2), 3.01 (t, *J* = 7.3 Hz, 2H, CH_2), 2.78 (t, *J* = 7.5 Hz, 2H, CH_2), 1.75 (dd, *J* = 14.8, 7.4 Hz, 2H, CH_2), 0.94 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{23}H_{25}ClN_5O$ $[M+H]^+$, 422.1742; found 422.1748.

(*E*)-*N*-(2-chlorophenethyl)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-7-amine (C13). Compound was isolated as a yellow solid in 51% yield, mp: 130-132 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.10 (brs, 1H), 7.95 (s, 1H, ArH), 7.84 (d, *J* = 15.3 Hz, 1H, =CH), 7.40 (m, 2H, ArH), 7.25~7.20 (m, 2H, ArH), 7.16 (d, *J* = 15.3 Hz, 1H, =CH), 6.97 (d, *J* = 3.2 Hz, 1H, ArH), 6.73 (s, 1H, ArH), 4.27 (s, 3H, NCH_3), 4.04 (d, *J* = 6.6 Hz, 2H, CH_2), 3.17 (t, *J* = 6.8 Hz, 2H, CH_2), 2.91 (t, *J* = 7.5 Hz, 2H, CH_2), 1.69 (dd, *J*=14.8, 7.5 Hz, 2H, CH_2), 0.94 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{23}H_{25}ClN_5O$ $[M+H]^+$, 422.1742; found 422.1747.

(*E*)-5-(2-(furan-2-yl)vinyl)-1-methyl-*N*-phenethyl-3-propyl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (C14). Compound was isolated as a yellow solid in 60% yield, mp: 117-118 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.95 (Brs, 1H, NH), 7.95 (s, 1H, ArH), 7.84 (d, *J* = 15.4 Hz, 1H, =CH), 7.41~7.13 (m, 6H, ArH), 7.01 (d, *J* = 3.2 Hz,

1H, ArH), 6.78~6.66 (m, 1H, ArH), 4.25 (s, 3H, NCH_3), 4.08~3.91 (m, 2H, CH_2), 3.04 (t, *J* = 7.2 Hz, 2H, CH_2), 2.91 (t, *J* = 7.5 Hz, 2H, CH_2), 1.80~1.59 (m, 2H, CH_2), 0.95 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{23}H_{26}N_5O$ $[M+H]^+$, 388.2132; found 388.2131.

(*E*)-*N*-(2-fluorophenethyl)-5-(2-(furan-2-yl)vinyl)-1-methyl-3-propyl-1H-pyrazolo [4,3-d]pyrimidin-7-amine (C15). Compound was isolated as a yellow solid in 52% yield, mp: 103-104 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.67 (d, *J* = 15.7 Hz, 1H), 7.44 (s, 1H), 7.29 ~ 7.21 (m, 2H), 7.13 ~ 7.05 (m, 3H), 6.50 (d, *J* = 3.2 Hz, 1H), 6.45 ~ 6.43 (m, 1H), 5.17 (t, *J* = 4.8 Hz, 1H), 4.09 (s, 3H), 3.96 (d, *J* = 6.6 Hz, 2H), 3.12 (t, *J* = 6.7 Hz, 2H), 2.91 (t, 2H), 1.83 (q, *J* = 7.5 Hz, 2H), 1.00 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{23}H_{25}FN_5O[M+H]^+$, 406.2038; found 406.2039.

(*E*)-5-(2-(furan-2-yl)vinyl)-*N*,*N*,1-trimethyl-3-propyl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (C16). Compound was isolated as a white solid in 44% yield, mp: 97-98 °C.^{1}H NMR (600 MHz, $CDCl_3$): δ 7.66 (d, *J* = 15.7 Hz, 1H), 7.44 (s, 1H), 7.14 (d, *J* = 15.7 Hz, 1H), 6.50 (d, *J* = 3.0 Hz, 1H), 6.45 – 6.40 (m, 1H), 4.10 (s, 3H), 3.15 (s, 6H), 2.96 (t, *J* = 7.7 Hz, 2H), 1.86 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.97, 154.42, 153.31, 147.25, 144.90, 143.00, 127.20, 124.31, 123.03, 111.88, 110.33, 41.11, 39.29, 27.99, 22.29, 14.21. HR-MS (ESI): calcd for $C_{17}H_{22}N_5O[M+H]^+$, 312.1819; found 312.1818.

(*E*)-5-(2-(furan-2-yl)vinyl)-*N*-isopropyl-1-methyl-3-propyl-1H-pyrazolo[4,3-d] pyrimidin-7-amine (C17). Compound was isolated as a white solid in 61% yield, mp: 109-110 °C.^{1}H NMR (600 MHz, $CDCl_3$): δ 7.61 (d, *J* = 15.7 Hz, 1H), 7.43 (s, 1H),

7.10 (d, *J* = 15.7 Hz, 1H), 6.49 (d, *J* = 3.1 Hz, 1H), 6.43 (dd, *J* = 3.1, 1.7 Hz, 1H), 4.81 (d, *J* = 6.7 Hz, 1H), 4.67~4.60 (m, 1H), 4.21 (s, 3H), 2.94~2.90 (m, 2H), 1.84 (q, *J* = 7.5 Hz, 2H), 1.38 (d, *J* = 6.5 Hz, 6H), 1.01 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.74, 153.38, 148.85, 145.68, 143.06, 142.91, 127.67, 122.87, 121.09, 111.84, 110.12, 42.71, 39.10, 27.83, 22.92, 22.39, 14.18. HR-MS (ESI): calcd for $C_{18}H_{24}N_5O[M+H]^+$, 326.1975; found 326.1976.

(*E*)-1-methyl-3-propyl-*N*-(3-(trifluoromethyl)phenyl)-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D1). Intermediate **4D** (200 mg, 0.496 mmol), 3-trifluoromethylaniline (80.0 mg, 0.531 mmol) were dissolved in *i*-propanol (15 mL), and refluxed for 8~10 h. The solvent was removed under reduced pressure, and the residues were purified by column chromatography gradient elution of PE/EtOAc 80/20 v/v then 70/30 v/v to obtain compound **D1**. Compound **D2**~**D34** were obtained according to the same procedure.

Yellow solid (139 mg, yield: 54%), mp: 244-246 °C. ^{1}H NMR (600 MHz,$CDCl_3$): δ 10.45 (s, 1H), 8.71 (s, 1H), 8.22 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 15.4 Hz, 1H), 7.46 (d, *J* = 15.4 Hz, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.22 (d, *J* = 7.5 Hz, 1H), 6.65 (s, 2H), 4.65 (s, 3H), 3.95 (s, 3H), 3.93 (s, 6H), 2.70 (t, *J* = 7.6 Hz, 2H), 1.66 (m, 2H), 0.91 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{29}F_3N_5O_3[M+H]^+$, 528.2217; found 528.2220.

(*E*)-1-methyl-3-propyl-*N*-(m-tolyl)-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo [4,3-d]pyrimidin-7-amine (D2). Compound was isolated as a yellow solid in 61% yield, mp: 223-224 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78 (d, *J* = 15.7 Hz, 1H), 7.73

(s, 1H, NH), 7.45 (d, *J* = 7.5 Hz, 1H), 7.32 (t, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 15.7 Hz, 1H), 7.01 (d, *J* = 7.3 Hz, 1H), 6.86 (s, 1H), 6.83 (s, 2H), 4.29 (s, 3H), 3.90 (s, 6H), 3.87 (s, 3H), 2.97 (t, *J* = 7.7 Hz, 2H), 2.45 (s, 3H), 1.88 (dd, *J* = 15.0, 7.5 Hz, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{32}N_5O_3$ $[M+H]^+$, 474.25; found 474.2503.

(*E*)-1-methyl-*N*-phenyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (D3). Compound was isolated as a yellow solid in 82% yield, mp:201-202 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76~7.71 (m, 3H, ArH+C=CH), 7.44 (t, *J* = 7.5 Hz, 2H, ArH), 7.20 (t, *J* = 7.4 Hz, 1H, ArH), 7.15 (d, *J* = 15.7 Hz, 1H, =CH), 6.91 (s, 1H, NH), 6.82 (s, 2H, ArH), 4.28 (s, 3H, OCH_3), 3.91 (s, 6H, 2×OCH_3), 3.88 (s, 3H, NCH_3), 2.98 (t, *J* = 7.6 Hz, 2H, CH_2), 1.89 (m, 2H, CH_2), 1.04 (t, *J* = 7.3 Hz, 3H, CH_3). HR-MS (ESI): calcd for $C_{26}H_{30}N_5O_3$ $[M+H]^+$, 460.2343; found 460.2346.

(*E*)-*N*-(4-chlorophenyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D4). Compound was isolated as a yellow solid in 71% yield, mp: 195-196 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.71~7.66 (m, 3H), 7.40 (d, *J* = 8.8 Hz, 2H), 7.13 (d, *J* = 15.7 Hz, 1H), 6.84 (s, 1H), 6.81 (s, 2H), 4.29 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 3.00~2.94 (m, 2H), 1.89 (q, *J* = 7.5 Hz, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 202.56, 157.45, 153.37, 146.46, 146.09, 144.49, 138.82, 137.01, 135.86, 132.25, 129.21, 129.04, 127.92, 122.33, 120.88, 104.64, 60.90, 56.20, 39.13, 27.66, 22.08, 13.98. HR-MS (ESI): calcd for $C_{26}H_{29}ClN_5O_3$ $[M+H]^+$, 494.1953; found 494.1956.

(*E*)-*N*-(4-bromophenyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D5). Compound was isolated as a white solid in 59% yield, mp: 206-207 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.68 (d, *J* = 15.7 Hz, 1H), 7.66~7.63 (m, 2H), 7.56~7.52 (m, 2H), 7.13 (d, *J* = 15.7 Hz, 1H), 6.85 (s, 1H), 6.81 (s, 2H), 4.29 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 2.97 (t, *J* = 7.7 Hz, 2H), 1.88 (q, *J* = 7.5 Hz, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.61, 153.54, 146.55, 146.25, 144.66, 138.95, 137.71, 136.04, 132.42, 132.16, 128.10, 122.78, 121.05, 116.86, 104.77, 61.09, 56.38, 39.31, 27.84, 22.27, 14.17. HR-MS (ESI): calcd for $C_{26}H_{29}BrN_5O_3$ $[M+H]^+$, 538.1448; found 538.1450.

(*E*)-1-methyl-3-propyl-*N*-(o-tolyl)-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (D6). Compound was isolated as a yellow solid in 66% yield, mp: 160-161 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.06 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 15.8 Hz, 1H), 7.35~7.29 (m, 2H), 7.17 (d, *J* = 7.2 Hz, 1H), 7.13 (d, *J* = 15.9 Hz, 1H), 6.80 (s, 2H), 6.77 (s, 1H), 4.22 (s, 3H), 3.90 (s, 6H), 3.87 (s, 3H), 2.98 (t, *J* = 7.7 Hz, 2H), 2.40 (s, 3H), 1.89 (d, *J* = 7.5 Hz, 2H), 1.05 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.56, 153.27, 147.13, 145.95, 138.44, 136.88, 135.82, 132.35, 130.78, 129.14, 127.96, 126.78, 124.86, 122.72, 121.29, 104.36, 60.94, 56.11, 39.19, 27.71, 22.21, 18.19, 14.07. HR-MS (ESI): calcd for $C_{27}H_{32}N_5O_3$ $[M+H]^+$, 474.25; found 474.2501.

(*E*)-*N*-(4-methoxyphenyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D7). Compound was isolated as a yellow solid in 57% yield, mp: 185-186 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 10.02 (s, 1H), 7.68

(d, *J* = 9.0 Hz, 2H), 7.65 (d, *J* = 15.6 Hz, 1H), 7.40 (d, *J* = 15.6 Hz, 1H), 7.12 (d, *J* = 9.0 Hz, 2H), 6.90 (s, 2H), 4.38 (s, 3H), 3.84 (s, 6H), 3.81 (s, 3H), 3.70 (s, 3H), 2.93 (t, *J* = 7.5 Hz, 2H), 1.72 (q, *J* = 7.4 Hz, 2H), 0.96 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{32}N_5O_4$ $[M+H]^+$, 490.2449; found 490.2449.

(*E*)-*N*-(4-fluoro-3-(trifluoromethyl)phenyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D8). Compound was isolated as a yellow solid in 68% yield, mp: 178-179 ^oC. ^1H NMR (600 MHz, DMSO-d_6): δ 9.12 (s, 1H), 8.57 (m, 1H), 8.02 (m, 1H), 7.62 (t, *J* = 9.8 Hz, 1H), 7.54 (d, *J* = 15.8 Hz, 1H), 7.22 (d, *J* = 15.8 Hz, 1H), 6.91 (s, 2H), 4.32 (s, 3H), 3.84 (s, 6H), 3.69 (s, 3H), 2.85 (t, *J* = 7.6 Hz, 2H), 1.78 (q, *J* = 7.5 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{28}F_4N_5O_3$ $[M+H]^+$, 546.2123; found 546.2123.

(*E*)-1-methyl-3-propyl-*N*-(4-(trifluoromethyl)phenyl)-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D9). Compound was isolated as a white solid in 59% yield, mp: 194-195 ^oC. ^1H NMR (600 MHz, $CDCl_3$): δ 7.87 (d, *J* = 8.5 Hz, 2H), 7.74~7.66 (m, 3H), 7.15 (d, *J* = 15.7 Hz, 1H), 7.00 (s, 1H), 6.81 (s, 2H), 4.32 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 2.98 (t, 2H), 1.89 (q, *J* = 7.5 Hz, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz $CDCl_3$): δ 157.40, 153.39, 146.21, 146.07, 144.80, 141.64, 138.88, 136.04, 132.15, 127.74, δ 126.28 (q, *J* = 3.7 Hz), 125.76 (d, *J* = 32.7 Hz), 125.02, 120.87, 120.35, 104.64, 60.90, 56.17, 39.11, 27.65, 22.06, 13.97. HR-MS (ESI): calcd for $C_{27}H_{29}F_3N_5O_3$ $[M+H]^+$, 528.2217; found 528.2218.

(*E*)-*N*-(3-bromophenyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D10). Compound was isolated as a yellow solid

in 55% yield, mp:167-168 °C. ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ 9.40 (s, 1H), 8.39 (s, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 15.7 Hz, 1H), 7.46 (d, *J* = 7.9 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.27 (d, *J* = 15.7 Hz, 1H), 6.97 (s, 2H), 4.35 (s, 3H), 3.85 (s, 6H), 3.70 (s, 3H), 2.88 (t, *J* = 7.5 Hz, 2H), 1.77 (d, *J* = 7.4 Hz, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{26}H_{29}BrN_5O_3$ $[M+H]^+$, 538.1448; found 538.1446.

(*E*)-*N*-(2-bromophenyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D11). Compound was isolated as a white solid in 71% yield, mp: 147-148 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.92 (d, *J* = 8.2 Hz, 1H), 7.76 (d, *J* = 15.7 Hz, 1H), 7.68 (s, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 15.7 Hz, 1H), 7.04 (t, *J* = 7.6 Hz, 1H), 6.84 (s, 2H), 4.44 (s, 3H), 3.92 (s, 6H), 3.89 (s, 3H), 2.99 (t, *J* = 7.7 Hz, 2H), 1.90 (q, *J* = 7.5 Hz, 2H), 1.05 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.48, 153.46, 146.17, 146.04, 144.45, 138.70, 136.48, 135.98, 132.55, 132.40, 128.32, 128.15, 124.56, 122.06, 121.11, 113.94, 104.53, 61.11, 56.29, 39.72, 27.81, 22.36, 14.21. HR-MS (ESI): calcd for $C_{26}H_{29}BrN_5O_3$ $[M+H]^+$, 538.1448; found 538.1446.

(*E*)-1-methyl-3-propyl-*N*-(4-(trifluoromethoxy)phenyl)-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D12). Compound was isolated as a yellow solid in 55% yield, mp: 173-174 °C. ^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ 9.99 (s, 1H), 7.91 (d, *J* = 9.0 Hz, 2H), 7.62 (d, *J* = 15.6 Hz, 1H), 7.56 (d, *J* = 8.5 Hz, 2H), 7.34 (d, *J* = 15.5 Hz, 1H), 6.90 (s, 2H), 4.39 (s, 3H), 3.84 (s, 6H), 3.71 (s, 3H), 2.93 (t, *J* = 7.5 Hz, 2H), 1.75 (q, *J* = 7.4 Hz, 2H), 0.97 (t, *J* = 7.3 Hz, 3H). HR-MS: calcd for $C_{27}H_{28}F_3N_5O_4$ $[M+H]^+$,544.2166 found 544.2166.

(*E*)-*N*-(4-chlorobenzyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D13). Compound was isolated as a white solid in 64% yield, mp: 179-180 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.70 (d, *J* = 15.7 Hz, 1H), 7.41 (d, *J* = 8.5 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.13 (d, *J* = 15.7 Hz, 1H), 6.82 (s, 2H), 5.37 (t, *J* = 5.5 Hz, 1H), 4.95 (d, *J* = 5.4 Hz, 2H), 4.22 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 2.97 ~2.93 (m, 2H), 1.86 (m, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{31}ClN_5O_3$ $[M+H]^+$, 508.211; found 508.2110.

(*E*)-*N*-(2-chlorobenzyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D14). Compound was isolated as a yellow solid in 55% yield, mp:167-169 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, *J* = 15.7 Hz, 1H), 7.60~7.58 (m, 1H), 7.43~7.41 (m, 1H), 7.27~7.26 (m, 1H), 7.25~7.23 (m, 1H), 7.12 (d, *J* = 15.7 Hz, 1H), 6.85 (s, 2H), 5.68 (t, *J* = 5.9 Hz, 1H), 5.05 (d, *J* = 5.9 Hz, 2H), 4.23 (s, 3H), 3.92 (s, 6H), 3.88 (s, 3H), 2.95~2.90 (m, 2H), 1.84 (q, *J* = 7.5 Hz, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.55, 153.32, 148.88, 145.58, 143.16, 138.34, 135.83, 135.35, 133.73, 132.53, 130.77, 129.72, 129.21, 128.39, 127.18, 121.08, 104.26,60.97, 56.13, 42.73, 39.05, 27.69, 22.28, 14.04. HR-MS (ESI): calcd for $C_{27}H_{30}ClN_5O_3$ $[M+H]^+$, 508.211; found 508.2110.

(*E*)-*N*-(4-bromobenzyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D15). Compound was isolated as a white solid in 67% yield, mp: 183-185 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.68 (d, *J* = 15.7 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 7.12 (d, *J* = 15.7 Hz, 1H), 6.81 (s, 2H), 5.35 (d, *J* = 4.0 Hz, 1H), 4.92 (d, *J* = 5.2 Hz, 2H), 4.20 (s, 3H), 3.90 (s, 6H),

3.87 (s, 3H), 2.94 (t, *J* = 7.7 Hz, 2H), 1.90 – 1.81 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{31}BrN_5O_3$ $[M+H]^+$, 552.1605; found 552.1605.

(*E*)-*N*-(4-fluorobenzyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D16). Compound was isolated as a white solid in 62% yield, mp: 160-162 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.73 (d, *J* = 15.7 Hz, 1H), 7.49~7.41 (m, 2H), 7.14 (d, *J* = 15.7 Hz, 1H), 7.10~7.05 (m, 2H), 6.83 (s, 2H), 5.32 (t, *J* = 5.5 Hz, 1H), 4.95 (d, *J* = 5.4 Hz, 2H), 4.21 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 2.98~2.92 (m, 2H), 1.90~1.81 (m, 2H), 1.03 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{31}FN_5O_3$ $[M+H]^+$, 492.2405; found 492.2406.

(*E*)-*N*-(4-methoxybenzyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D17). Compound was isolated as a white solid in 79% yield, mp: 159-160 °C. ^{1}H NMR (600 MHz, $CDCl_3$): 7.79 (d, *J* = 15.7 Hz, 1H), 7.40 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 15.7 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 6.85 (s, 2H), 5.28 (t, *J* = 5.3 Hz, 1H), 4.90 (d, *J* = 5.2 Hz, 2H), 4.18 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 3.82 (s, 3H), 2.95 (t, *J* = 7.7 Hz, 2H), 1.86 (h, *J* = 7.5 Hz, 2H), 1.03 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{28}H_{34}N_5O_4$ $[M+H]^+$, 504.2605; found 504.2605.

(*E*)-*N*-(3-methoxybenzyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D18). Compound was isolated as a white solid in 81% yield, mp: 127-128 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, *J* = 15.7 Hz, 1H), 7.32 (t, *J* = 8.1 Hz, 1H), 7.14 (d, *J* = 15.7 Hz, 1H), 7.07~7.03 (m, 2H), 6.89~6.86 (m, 1H), 6.85 (s, 2H), 5.35 (t, *J* = 5.4 Hz, 1H), 4.95 (d, *J* = 5.3 Hz, 2H), 4.21 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 3.81 (s, 3H), 2.98~2.92 (m, 2H), 1.86 (q, *J* = 7.5 Hz, 2H),

1.03 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{28}H_{34}N_5O_4$ $[M+H]^+$, 504.2605; found 504.2604.

(*E*)-*N*-(furan-2-ylmethyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D19). Compound was isolated as a yellow solid in 63% yield, mp: 175-176 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81 (d, *J* = 15.7 Hz, 1H), 7.43 (d, *J* = 0.8 Hz, 1H), 7.15 (d, *J* = 15.7 Hz, 1H), 6.87 (s, 2H), 6.41 (d, *J* = 3.1 Hz, 1H), 6.39 (d, *J* = 1.7 Hz, 1H), 5.37 (s, 1H), 4.97 (d, *J* = 5.2 Hz, 2H), 4.23 (s, 3H), 3.92 (s, 6H), 3.88 (s, 3H), 2.97~2.92 (m, 2H), 1.86 (m, 2H), 1.02 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{25}H_{30}N_5O_4$ $[M+H]^+$, 464.2292; found 464.2293.

(*E*)-1-methyl-3-propyl-*N*-(4-(trifluoromethyl)benzyl)-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D20). Compound was isolated as a white solid in 49% yield, mp: 182-183 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.66 (d, *J* = 10.9 Hz, 1H), 7.66 ~7.57 (m, 4H), 7.12 (d, *J* = 15.7 Hz, 1H), 6.80 (s, 2H), 5.44 (t, *J* = 5.3 Hz, 1H), 5.03 (d, *J* = 5.5 Hz, 2H), 4.23 (s, 3H), 3.89 (s, 6H), 3.87 (s, 3H), 2.95 (t, *J* = 7.7 Hz, 2H), 1.86 (q, *J* = 7.5 Hz, 2H), 1.03 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{28}H_{31}F_3N_5O_3$ $[M+H]^+$, 542.2374; found 542.2374.

(*E*)-*N*-(3-chlorophenethyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D21). Compound was isolated as a white solid in 75% yield, mp: 78-79 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, *J* = 15.8 Hz, 1H), 7.32~7.19 (m, 4H), 7.15 (d, *J* = 15.6 Hz, 1H), 6.87 (s, 2H), 5.10 (s, 1H), 4.09 (s, 3H), 4.06~3.97 (m, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 3.09 (t, *J* = 6.8 Hz, 2H), 2.94 (t, *J* = 7.7 Hz, 2H), 1.86 (q, *J* = 7.6 Hz, 2H), 1.02 (t, *J* = 7.3 Hz, 3H). ^{13}CNMR (151 MHz,

$CDCl_3$): δ 157.83, 153.49, 149.26, 145.79, 143.25, 141.20, 138.56, 135.41, 134.81, 132.65, 130.25, 129.15, 128.66, 127.22, 127.17, 121.22, 104.48, 61.12, 56.29, 41.94, 39.02, 35.17, 27.86, 22.41, 14.20. HR-MS (ESI): calcd for $C_{28}H_{33}ClN_5O_3$ $[M+H]^+$, 522.2266; found 522.2268.

(*E*)-*N*-(2-chlorophenethyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D22). Compound was isolated as a white solid in 54% yield, mp: 160-161 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81 (d, *J* = 15.7 Hz, 1H), 7.41 (m, 1H), 7.33~7.29 (m, 1H), 7.23~7.20 (m, 2H), 7.13 (d, *J* = 15.7 Hz, 1H), 6.87 (s, 2H), 5.15 (t, *J* = 5.7 Hz, 1H), 4.12 (s, 3H), 4.06 (dd, *J* = 12.6, 6.8 Hz, 2H), 3.92 (s, 6H), 3.88 (s, 3H), 3.25 (t, *J* = 6.8 Hz, 2H), 2.95~2.90 (m, 2H), 1.88~1.80 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{28}H_{33}ClN_5O_3$ $[M+H]^+$, 522.2266; found 522.2265.

(*E*)-1-methyl-*N*-phenethyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo [4,3-d]pyrimidin-7-amine (D23). Compound was isolated as a white solid in 51% yield, mp: 147-148 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81 (d, *J* = 15.7 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 2H), 7.31 (d, *J* = 7.2 Hz, 2H), 7.29 (s, 1H), 7.14 (d, *J* = 15.7 Hz, 1H), 6.86 (s, 2H), 5.06 (t, *J* = 5.6 Hz, 1H), 4.06 – 4.01 (m, 2H), 4.01 (s, 3H), 3.92 (s, 6H), 3.88 (s, 3H), 3.10 (t, *J* = 6.8 Hz, 2H), 2.93 (t, *J* = 7.7 Hz, 2H), 1.85 (q, *J* = 7.5 Hz, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.84, 153.44, 149.36, 145.65, 143.04, 138.98, 138.43, 135.39, 132.69, 129.03, 129.01, 128.64, 127.03, 121.23, 104.38, 61.10, 56.24, 41.96, 38.93, 35.31, 27.83, 22.42, 14.19. HR-MS (ESI): calcd for $C_{28}H_{34}N_5O_3$ $[M+H]^+$, 488.2656; found 488.2656.

(*E*)-*N*-(2-fluorophenethyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-amine (D24). Compound was isolated as a white solid in 64% yield, mp: 144-146 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.83 (d, *J* = 15.7 Hz, 1H), 7.32~7.23 (m, 2H), 7.16~7.07 (m, 3H), 6.88 (s, 2H), 5.23 (t, *J* = 5.5 Hz, 1H), 4.13 (s, 3H), 4.01 (td, *J* = 6.8, 5.5 Hz, 2H), 3.93 (s, 6H), 3.89 (s, 3H), 3.17 (t, *J* = 6.8 Hz, 2H), 2.95~2.91 (m, 2H), 1.89~1.82 (m, 2H), 1.02 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{28}H_{33}FN_5O_3$ $[M+H]^+$, 506.2562; found 506.2561.

(*E*)-4-(2-((1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl)amino)ethyl)phenol (D25). Compound was isolated as a white solid in 38% yield, mp: 122-123 °C.^{1}H NMR (600 MHz, DMSO-*d*$_6$): δ 9.22 (s, 1H, OH), 7.73 (d, *J* = 15.7 Hz, 1H, =CH), 7.30 (t, *J* = 5.4 Hz, 1H, NH), 7.15 (m, 3H, ArH + =CH), 6.95 (s, 2H, ArH), 6.73 (d, *J* = 8.2 Hz, 2H, ArH), 4.15 (s, 3H), 3.87 (s, 6H), 3.75 (dd, *J* = 14.4, 6.3 Hz, 2H), 3.69 (s, 3H), 2.94~2.88 (m, 2H), 2.78 (t, *J* = 7.5 Hz, 2H), 1.75 (dd, *J* = 14.9, 7.4 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-*d*$_6$): δ 154.94, 153.97, 151.35, 147.17, 142.03, 140.26, 135.97, 132.66, 130.34, 127.94, 127.76, 126.97, 119.11, 113.42, 102.47, 58.31, 54.12, 40.81, 37.20, 32.12, 25.57, 20.02, 12.17. HR-MS (ESI): calcd for $C_{28}H_{34}N_5O_4$$[M+H]^+$, 504.2605; found 504.2605.

(*E*)-*N*,*N*,1-trimethyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-amine (D26). Compound was isolated as a yellow solid in 62% yield, mp: 173-174 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.80 (d, *J* = 15.7 Hz, 1H), 7.16 (d, *J* = 15.7 Hz, 1H), 6.86 (s, 2H), 4.12 (s, 3H), 3.92 (s, 6H), 3.89~3.87 (m, 3H), 3.19 (s,

6H), 2.98 (t, *J* = 7.7 Hz, 2H), 1.89 (q, *J* = 7.5 Hz, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.94, 154.34, 153.33, 147.06, 144.79, 138.56, 135.29, 132.50, 128.20, 124.25, 104.49, 60.88, 56.14, 40.98, 39.14, 27.82, 22.09, 14.01. HR-MS (ESI): calcd for $C_{22}H_{30}N_5O_3[M+H]^+$, 412.2343; found 412.2342.

(*E*)-*N*-isopropyl-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo [4,3-d]pyrimidin-7-amine (D27). Compound was isolated as a white solid in 69% yield, mp: 133-134 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (d, *J* = 15.7 Hz, 1H), 7.13 (d, *J* = 15.7 Hz, 1H), 6.85 (s, 2H), 4.85 (d, *J* = 6.9 Hz, 1H), 4.70 (dd, *J* = 12.9, 6.4 Hz, 1H), 4.23 (s, 3H), 3.92 (s, 6H), 3.88 (s, 3H), 2.94 (t, *J* = 7.5 Hz, 2H), 1.86 (dd, *J* = 14.8, 7.3 Hz, 2H), 1.41 (d, *J* = 6.1 Hz, 6H), 1.02 (t, *J* = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.71, 153.27, 148.79, 145.47, 142.83, 138.25, 135.08, 132.60, 128.67, 121.01, 104.25, 60.94, 56.11, 42.57, 38.99, 27.68, 22.85, 22.26, 14.03. HR-MS (ESI): calcd for $C_{23}H_{32}N_5O_3$ $[M+H]^+$, 426.2500; found 426.2503.

(*E*)-*N*-(sec-butyl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo [4,3-d]pyrimidin-7-amine (D28). Compound was isolated as a white solid in 72% yield, mp:154-155 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.73 (d, *J* = 15.6 Hz, 1H), 7.12 (d, *J* = 15.6 Hz, 1H), 6.84 (s, 2H), 4.84 (d, *J* = 7.6 Hz, 1H), 4.58 – 4.50 (m, 1H), 4.23 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 2.93 (t, *J* = 7.7 Hz, 2H), 1.85 (q, *J* = 7.5 Hz, 2H), 1.78 – 1.69 (m, 2H), 1.37 (d, *J* = 6.6 Hz, 3H), 1.05 (t, *J* = 7.5 Hz, 3H), 1.02 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.90, 153.45, 149.25, 145.69, 143.09, 138.49, 135.21, 132.78, 128.90, 121.23, 104.48, 61.10, 56.29, 47.84, 39.15, 29.75, 27.85, 22.41, 20.44, 14.19, 10.56. HR-MS (ESI): calcd for $C_{24}H_{33}N_5O_3$ $[M+H]^+$,

440.2656 found 440.2657

(*E*)-7-(4-ethylpiperazin-1-yl)-1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidine (D29). Compound was isolated as a yellow solid in 41% yield, mp:123-124 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78 (d, *J* = 15.7 Hz, 1H), 7.17 (d, *J* = 15.7 Hz, 1H), 6.86 (s, 2H), 4.10 (s, 3H), 3.92 (s, 6H), 3.88 (s, 3H), 3.64 (s, 4H), 3.01~2.95 (m, 2H), 2.72 (s, 4H), 2.55 (d, *J* = 7.2 Hz, 2H), 1.88 (q, *J* = 7.5 Hz, 2H), 1.17 (t, *J* = 7.2 Hz, 3H), 1.04 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.11, 153.62, 153.34, 147.23, 145.05, 138.59, 135.50, 132.41, 128.06, 124.36, 104.47, 60.93, 56.17, 52.37, 52.23, 49.31, 38.55, 27.81, 22.11, 14.01, 11.86. HR-MS (ESI): calcd for $C_{26}H_{37}N_6O_3$ $[M+H]^+$,481.2922 found 481.2922.

(*E*)-4-(1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl)morpholine (D30). Compound was isolated as a white solid in 69% yield, mp: 142-143 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, *J* = 15.7 Hz, 1H), 7.17 (d, *J* = 15.7 Hz, 1H), 6.85 (s, 2H), 4.11 (s, 3H), 3.97~3.94 (m, 4H), 3.92 (s, 6H), 3.88 (s, 3H), 3.59 (m, 4H), 2.99 (t, *J* = 7.7 Hz, 2H), 1.88 (dd, *J* = 15.0, 7.5 Hz, 2H), 1.04 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.20, 153.74, 153.44, 147.41, 145.25, 138.57, 135.75, 132.42, 127.99, 124.35, 104.40, 66.52, 61.07, 56.23, 50.09, 38.60, 27.93, 22.27, 14.18. HR-MS (ESI): calcd for $C_{24}H_{32}N_5O_4$ $[M+H]^+$, 454.2449; found 454.2452.

(*E*)-1-(1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl)piperidin-4-ol (D31). Compound was isolated as a white solid in 57% yield, mp: 162-163 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.78 (d, *J* = 15.7 Hz, 1H), 7.17

(d, *J* = 15.6 Hz, 1H), 6.86 (s, 2H), 4.11 (s, 3H), 4.05 (m, 1H), 3.92 (s, 6H), 3.90(Brs,1H), 3.89 (s, 3H), 3.32 (t, *J* = 11.1 Hz, 2H), 2.99 (t, *J* = 7.3 Hz, 2H), 2.20~2.10 (m, 2H), 1.88 (m, 2H), 1.86~1.74 (m, 4H), 1.04 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.19, 154.00, 153.45, 147.22, 144.99, 138.52, 135.62, 132.55, 128.18, 124.53, 104.41, 67.61, 61.10, 56.26, 47.12, 38.56, 34.03, 27.96, 22.35, 14.21.HR-MS (ESI): calcd for $C_{25}H_{34}N_5O_4$ $[M+H]^+$, 468.2605; found 468.2609.

(*E*)-1-methyl-3-propyl-7-(pyrrolidin-1-yl)-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidine (D32). Compound was isolated as a white solid in 49% yield, mp: 201-202 ^{o}C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.75 (d, *J* = 15.7 Hz, 1H), 7.13 (d, *J* = 15.7 Hz, 1H), 6.85 (s, 2H), 4.16 (s, 3H), 3.91 (s, 6H), 3.87 (s, 3H), 3.86 (m, 4H), 2.99 ~2.94 (m, 2H), 2.08~2.00 (m, 4H), 1.87 (q, *J* = 7.6 Hz, 2H), 1.03 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 156.81, 153.41, 151.15, 146.39, 144.63, 138.35, 135.08, 132.77, 128.62, 123.52, 104.36,61.09, 56.24, 50.48, 41.43, 27.92, 25.74, 22.32, 14.23. HR-MS (ESI): calcd for $C_{24}H_{31}N_5O_3$ [M+H]+, 438.25; found 438.2498.

(*E*)-1-methyl-7-(piperidin-1-yl)-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidine (D33). Compound was isolated as a white solid in 68% yield, mp: 165-167 ^{o}C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, *J* = 15.6 Hz, 1H), 7.16 (d, *J* = 15.6 Hz, 1H), 6.85 (s, 2H), 4.10 (s, 3H), 3.91 (s, 6H), 3.87 (s, 3H), 3.51 (s, 4H), 2.97 (t, *J* = 7.6 Hz, 2H), 1.87 (dd, *J* = 14.9, 7.4 Hz, 2H), 1.82 (m, 4H), 1.74 (m, 2H), 1.03 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 157.24, 154.59, 153.46, 147.13, 144.85, 138.59, 135.48, 132.67, 128.39, 124.68, 104.47, 61.10, 56.27, 50.75,

38.63, 27.99, 25.71, 24.63, 22.37, 14.22. HR-MS (ESI): calcd for $C_{25}H_{34}N_5O_3$ $[M+H]^+$, 452.2656; found 452.2656.

(*E*)-methyl 4-((1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl)amino)benzoate (D34). Compound was isolated as a yellow solid in 81% yield, mp: 192-194 ^oC. ^1H NMR (600 MHz, $CDCl_3$): δ 10.47 (s, 1H), 8.09 (d, *J* = 8.4 Hz, 2H), 7.92 (d, *J* = 8.3 Hz, 2H), 7.61 (d, *J* = 15.3 Hz, 1H), 7.49 (d, *J* = 15.4 Hz, 1H), 6.68 (s, 2H), 4.58 (s, 3H), 3.94 (s, 6H), 3.93 (s, 6H), 2.72 (t, *J* = 7.5 Hz, 2H), 1.65 (dd, *J* = 15.0, 7.5 Hz, 2H), 0.89 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{28}H_{32}N_5O_5$ $[M+H]^+$, 518.2398; found 518.2398.

(*E*)-4-((1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d] pyrimidin-7-yl)amino)benzoic acid (D35). Aqueous NaOH solution (2 N, 10 mL, 20 mmol, 10 equiv) was added to a solution of compound **D34** (200 mg, 0.386 mmol) in MeOH (6 mL each) at room temperature. The mixture was stirred at room temperature for 5-6 h. After completion of the reaction, aqueous HCl solution (1 N, ~20 mL) was added to bring the pH to 5~6. The compound **D35** was collected by filtration, washed with water, and dried to give as yellow solid (176.3 mg, 85%), mp: 231-233 ^oC. ^1H NMR (600 MHz, DMSO-d_6): 9.29 (s, 1H), 8.04 (d, *J* = 8.3 Hz, 2H), 7.97 (d, *J* = 8.6 Hz, 2H), 7.66 (d, *J* = 15.7 Hz, 1H), 7.22 (dd, *J* = 15.9, 1H), 6.99 (s, 2H), 4.31 (s, 3H), 3.86 (s, 6H), 3.70 (s, 3H), 3.17 (s, 1H), 2.88 (t, *J* = 7.5 Hz, 2H), 1.84~1.76 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI): calcd for $C_{27}H_{30}N_5O_5$ $[M+H]^+$, 504.2241; found 504.2242.

(*E*)-*N*-butyl-4-(1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]

pyrimidin-7-ylamino)benzamide (D36). Compound **D35** (200 mg, 0.397 mmol) was dissolved in anhydrous DCM (10 mL), EDCI (114.1 mg, 0.596 mmol) and HOBt (83.2 mg, 0.596 mmol) were then added and allowed to stir until completely dissolved under N_2. After 30 min, butylamine (28.9 mg, 0.397 mmol) and DIEA (62.3 mg, 0.05 mmol) were mixed in DCM (10 mL) and added to the reaction mixture. The mixture was allowed to stir at room temperature overnight. The solvent was removed under reduced pressure, and the residues were purified by column chromatography gradient elution of PE/EtOAc 60/40 v/v then 50/50 v/v to obtain compound **D36**, and compounds **D37-38** were obtained according to the same procedure.

White solid (129 mg, 58%), mp: 250-252 ^{o}C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.86~7.82 (m, 4H), 7.69 (d, *J* = 15.7 Hz, 1H), 7.14 (d, *J* = 15.7 Hz, 1H), 7.09 (s, 1H), 6.82 (d, *J* = 17.4 Hz, 2H), 6.24 (t, *J* = 5.3 Hz, 1H), 4.32 (s, 3H), 3.90 (s, 6H), 3.89 (s, 3H), 3.48 (dd, *J* = 13.2, 6.9 Hz, 2H), 2.98 (t, *J* = 7.7 Hz, 2H), 1.88 (dt, *J*= 14.0, 7.0 Hz, 2H), 1.66~1.59 (m, 2H), 1.48~1.38 (m, 2H), 1.05 (t, *J* = 7.3 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$): δ 166.80, 157.36, 153.32, 146.16, 146.00, 144.49, 141.37, 138.58, 135.78, 132.24, 129.82, 127.96, 120.91, 119.99, 109.98, 104.41, 60.97, 56.20, 39.86, 39.20, 31.78, 27.66, 22.16, 20.18, 14.05, 13.80. HR-MS (ESI): calcd for $C_{31}H_{39}N_6O_4$ $[M+H]^+$, 559.3027; found 559.3025.

(*E*)-*N*-tert-butyl-4-(1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo [4,3-d]pyrimidin-7-ylamino)benzamide (D37). Compound was isolated as a white solid in 52% yield, mp: 263-265 ^{o}C. ^{1}H NMR (600 MHz, chloroform-*d*) δ = 7.80 (s, 4H), 7.71 (d, *J*=15.7, 1H), 7.16 (d, *J*=15.7, 1H), 7.08 (s, 1H), 6.83 (s, 2H), 5.97 (s,

1H), 4.33 (s, 3H), 3.92 (s, 6H), 3.88 (s, 3H), 2.97 (t, *J*=7.7, 2H), 1.88 (q, *J*=7.5, 2H), 1.49 (s, 9H), 1.03 (t, *J*=7.4, 3H). HR-MS (ESI): calcd for $C_{31}H_{39}N_6O_4$ $[M+H]^+$, 559.3027; found 559.3027.

(*E*)-(4-ethylpiperazin-1-yl)(4-((1-methyl-3-propyl-5-(3,4,5-trimethoxystyryl)-1H-pyrazolo[4,3-d]pyrimidin-7-yl)amino)phenyl)methanone (D38). Compound was isolated as a white solid in 61% yield, mp: 247-249 ^oC. ^1H NMR (300 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 15.8 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 15.8 Hz, 1H), 7.08 (s, 1H), 6.82 (s, 2H), 4.34 (s, 3H), 3.91 (s, 6H), 3.88 (s, 3H), 3.71 (d, *J* = 27.3 Hz, 4H), 2.97 (t, *J* = 7.7 Hz, 2H), 2.45 (q, *J* = 7.2 Hz, 6H), 1.88 (q, *J* = 7.5 Hz, 2H), 1.10 (t, *J* = 7.2 Hz, 3H), 1.03 (t, *J* = 7.3 Hz, 3H). HR-MS (ESI): calcd for $C_{33}H_{41}N_7O_4$ $[M+H]^+$, 600.3293 found 600.3294.

Biological Tests *in Vitro* And *in Vivo.* The procedures of cell culture, Assay of NO Production, Assay of iNOS Activity, Western Blotting, AIA in Rats and Evaluation were summarized in Supporting Information.

Ethics Approval and Consent to Participate. The research was approved by the Ethics Committee of Anhui Medical University on the care, and all animals received humane care according to the National Institutes of Health (USA) guidelines.

Statistical Analysis. Results are expressed as the mean values standard deviation (SD) and were analyzed by SPSS software, and differences between groups were assessed with the Tukey's method. A value of $p<0.05$ was considered to be statistically significant.

ASSOCIATED CONTENT

Supporting Information

The following files are available free.

Crystallographic data (excluding structure factors) for the structures had been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-1479900, CCDC-1479901. Biological tests *in vitro* and *in vivo* containing **Stable 1** and **SFigure 1**; assay procedures; HR-MS, HPLC, ^{1}H NMR and ^{13}C NMR spectra of synthesized compounds; HPLC of compound **D27**; and the molecular formula strings (CSV).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AIA, adjuvant-induced arthritis; ALI, acute lung injury; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; NSAID, nonsteroidal anti-inflammatory drug; ^{1}H NMR, proton nuclear magnetic resonance; ^{13}C NMR, carbon nuclear magnetic resonance; MS, mass spectroscopy; LPS, lipopolysaccharide; ELISA, enzyme linked immunosorbent assay; SARs, structure-activity relationships; IC_{50}, half maximal inhibitory concentration; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; MeOH, methanol; EtOAc, ethyl acetate; HCl, hydrochloric acid; HPLC, high-performance liquid chromatography; DMSO, dimethyl sulfoxide; Pd/C, palladium/carbon; DCM, dichloromethane; DMA, dimethyl adipate; TBTU, tributylthiourea; DIPEA, *N*,*N*-diisopropylethylamine.

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Graphic Abstract