Med Chem Res
DOI 10.1007/s00044-015-1323-y

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of the anti-inflammatory activity of quinoline derivatives

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Received: 15 September 2014 / Accepted: 4 January 2015

Abstract Three types of quinoline derivatives, quinolin-2-one, 1-oxa-3,5-diaza-anthracen-6-one, and cyclopenta [*a*]anthracene, were designed and synthesized as potential anti-inflammatory agents. Their anti-inflammatory activities were evaluated using the xylene-induced ear-edema test in mice. Pharmacological analyses showed that compounds 3-(4-methyl-benzyl)-3,4,7,8-tetrahydro-2*H*,5*H*-1-oxa-3,5-diaza-anthracen-6-one (**3g**) and 9-(4-fluoro-phenyl)-5,8,9,10-tetrahydro-4*H*-7-oxa-2,3,9,11*b*-tetraaza-cyclopenta[*a*] anthracene (**6d**) exhibited the greatest anti-inflammatory activity (63.19 and 68.28 % inhibition, respectively, 30 min after intraperitoneal administration) and were more potent than the reference drug ibuprofen. The peak activity of **3g** and **6d** was observed 3 h after oral administration, and the compounds exhibited stronger anti-inflammatory activity than ibuprofen at 50 mg/kg at this time point. The most effective compound, **6d** (hydrochloride salt), was evaluated in lipopolysaccharide (LPS)-stimulated RAW264.7 mouse macrophages, in which it significantly inhibited LPS-induced production of tumor necrosis factor-α and interleukin-6 in a dose-dependent manner.

Keywords Anti-inflammatory · IL-6 inhibition · Mannich reaction · Quinoline · Synthesis · TNF-α

Introduction

Inflammation remains a common and life-threatening problem in cases of allergy, autoimmune diseases, and rejection of transplanted organs (Huerre and Gounon, 1996). Treatment options for inflammatory diseases produce adverse effects and show a lack of efficacy. Non-steroidal anti-inflammatory drugs (NSAIDs) are very useful in the treatment of acute and chronic inflammation (Sng and Schug, 2009), pain (Zahradnik *et al*., 2010), and fever (Eccles, 2006), but long-term use of NSAIDs is associated with gastrointestinal lesions, bleeding, and nephrotoxicity (Mukherjee *et al*., 2001). The discovery of novel and safe anti-inflammatory drugs remains a challenging goal (Vane and Botting, 1995). There is an increasing need for novel structures that may be used in the design of new, potent, and less toxic anti-inflammatory agents.

Quinoline derivatives have a wide spectrum of biological activities, including anti-microbial (Rajanarendar *et al*., 2012), anti-depressant (Sun *et al*., 2011), anti-convulsant (Guan *et al*., 2008), anti-malarial (N'Da *et al*., 2010), and anti-inflammatory effects (El-Gazzar *et al*., 2009). Accordingly, much effort has been spent developing new and more effective quinoline-based therapeutics. In our studies, 6-alkyl-3,4-dihydro-1*H*-quinolin-2-one quinoline derivatives (**2a**–**2i**) were obtained by alkylation of 6-hydroxy-3,4-dihydro-1*H*-quinolin-2-one and alkyl

Electronic supplementary material The online version of this article (doi:10.1007/s00044-015-1323-y) contains supplementary material, which is available to authorized users.

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Published online: 24 January 2015

halides, and some of these derivatives were found to possess modest anti-inflammatory activity. It has been reported that small heterocycles such as oxazine (e.g., oxazine (Barco *et al*., 2007; Kourounakis *et al*., 2010; Liu and Ying, 2010) and triazole (Abdel-Aziz *et al*., 2013; Abuo-Rahma Gel *et al*., 2014) can result in increased biological activity. Therefore, to further investigate the biological activity of quinoline derivatives, we introduced an oxazine ring at the sixth and seventh positions of the quinoline ring and designed a novel series of 1-oxa-3,5-diaza-anthracen-6-one (**3a**–**3l**) derivatives. Furthermore, we also introduced a triazole ring at the fifth and sixth positions of the 1-oxa-3,5-diaza-anthracene ring and designed another novel series of cyclopenta[*a*]anthracene (**6a**–**6l**) derivatives (Fig. 1).

We report the synthesis of three types of quinoline derivatives and the evaluation of their anti-inflammatory activity in a xylene-induced ear-edema test in vivo. The inflammatory response after lipopolysaccharide (LPS) challenge was associated with the release of pro-inflammatory cytokines and other inflammatory mediators, including tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) (Soromou *et al*., 2012). Macrophages are an essential interface between innate and adaptive immunity during the inflammatory response. Herein, we studied active compounds to investigate their protective effects against inflammation in LPS-stimulated RAW264.7 mouse macrophages in vitro.

Results and discussion

Chemistry

Based on previous studies carried out by our research team, we designed three series of 6-alkyl-3,4-dihydro-1*H*-quinolin-2-one (**2a**–**2j**), 1-oxa-3,5-diaza-anthracen-6-one (**3a**–**3l**), and cyclopenta[*a*]anthracene (**6a**–**6l**) quinoline derivatives and synthesized them according to Scheme 1. Compounds **2a**–**2j** were synthesized according to a method described previously (Sun *et al*., 2006; Wei *et al*., 2010; Sun *et al*., 2013). The starting material, compound **1**, was treated with benzyl chloride in a solution of sodium hydroxide in ethanol to yield 6-alkoxy-3,4-dihydroquinolin-2(1*H*)-one (**2a**–**2i**). Next, compounds **3a**–**3l** were synthesized by the Mannich reaction. Subsequently, the starting material, compound **1**, was reacted with phosphorous pentasulfide in the presence of triethylamine in acetonitrile to obtain compound **4**. A key substrate for the

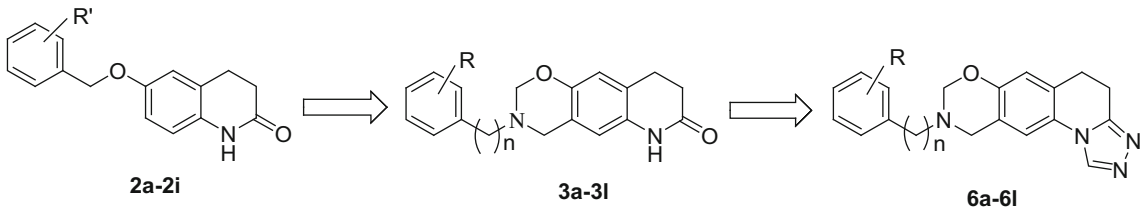
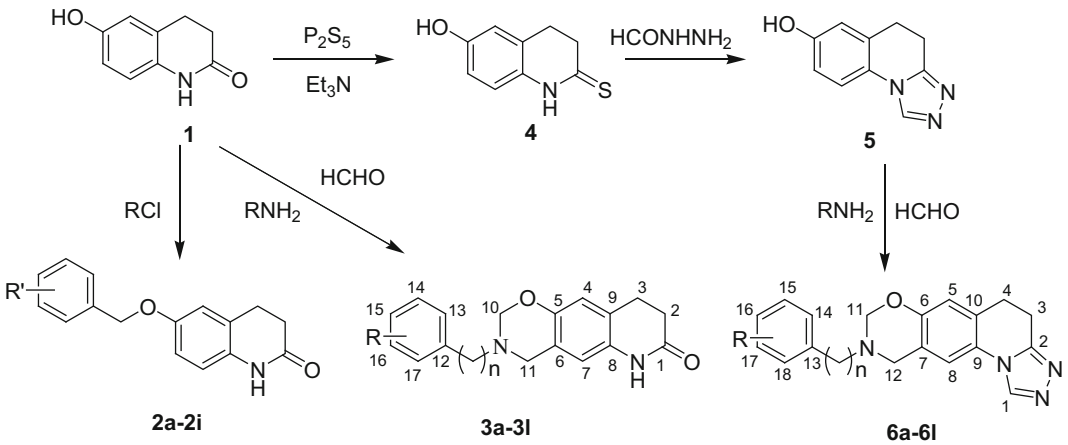


Fig. 1 Target compounds **2a**–**2i**, **3a**–**3l**, and **6a**–**6l**



Scheme 1 Synthesis of compounds **2a–i**, **3a–l**, and **6a–l**. **R′**: **2a** H, **2b** *p*-CH_3, **2c** *p*-OCH_3, **2d** *o*-F, **2e** *m*-F, **2f** *p*-F, **2g** *o*-Cl, **2h** *m*-Cl, **2i** *p*-Cl; **R:** $n = 0$, **3a–6a** H, **3b–6b** *p*-CH_3, **3c–6c** *p*-OCH_3, **3e–6e** *p*-F; $n = 1$, **3f–6f** H, **3g–6g** *p*-CH_3, **3h–6h** *p*-OCH_3, **3i–6i** *p*-F, **3j–6j** *p*-Cl, **3k–6k** *p*-CN; **3e–6e** *p*-F; $n = 2$, **3l–6l** H

production of **6a–6l**, compound **5**, was prepared by an established procedure involving the reaction of compound **4** with carbohydrazide in boiling butanol (Xie *et al*., 2005). Finally, test derivatives **6a–6l** were prepared by the Mannich reaction of compound **5** with diverse amines and formaldehyde in a solution of ethanol. All synthesized compounds were confirmed by ^{1}H NMR, ^{13}C NMR, IR spectroscopy, and MS.

Pharmacology

In vivo inhibition of xylene-induced ear edema

In the primary screening, we used dimethyl sulfoxide (DMSO) as the vehicle and ibuprofen as the reference drug. In this test, all synthesized compounds were screened in a xylene-induced ear-edema test in mice, in which anti-

Table 1 Anti-inflammatory activity of compounds **2a–i**, **3a–l**, and **6a–l** after i.p. administration

Comp.	R	Dose (mg/kg)	Number of mice	Edema mean ± SEM (mg)	Inhibition rate (%)
2a	H	100	10	5.25 ± 1.10**	56.18
2b	*p*-CH_3	100	10	5.00 ± 1.04*	58.26
2c	*p*-OCH_3	100	10	8.07 ± 0.72	32.64
2d	*o*-F	100	10	7.10 ± 1.47	40.73
2e	*m*-F	100	10	5.05 ± 1.36*	57.85
2f	*p*-F	100	10	4.70 ± 0.26**	60.77
2g	*o*-Cl	100	10	6.38 ± 1.17*	46.74
2h	*m*-Cl	100	10	4.85 ± 1.02**	59.52
2i	*p*-Cl	100	10	8.52 ± 1.81	28.88
3a	H	100	10	5.33 ± 1.42**	55.51
3b	*p*-CH_3	100	10	7.20 ± 1.14*	39.90
3c	*p*-OCH_3	100	10	7.60 ± 0.92*	36.56
3d	*p*-F	100	10	8.00 ± 0.98*	33.22
3e	*p*-Cl	100	10	6.56 ± 1.48*	45.24
3f	H	100	10	6.73 ± 0.52**	43.82
3g	*p*-CH_3	100	10	4.41 ± 1.16**	63.19
3h	*p*-OCH_3	100	10	10.42 ± 0.91	–
3i	*p*-F	100	10	6.37 ± 1.09**	46.83
3j	*p*-Cl	100	10	8.35 ± 1.01	30.30
3k	*p*-CN	100	10	9.54 ± 1.38	20.37
3l	H	100	10	8.76 ± 1.88	26.88
6a	H	100	10	9.38 ± 1.07	21.70
6b	*p*-CH_3	100	10	6.90 ± 0.99**	42.40
6c	*p*-OCH_3	100	10	10.75 ± 1.97	–
6d	*p*-F	100	10	3.80 ± 1.11**	68.28
6e	*p*-Cl	100	10	6.30 ± 1.46**	47.41
6f	H	100	10	5.43 ± 0.89**	54.67
6g	*p*-CH_3	100	10	4.93 ± 0.82**	58.85
6h	*p*-OCH_3	100	10	4.48 ± 1.22**	62.60
6i	*p*-F	100	10	7.98 ± 1.21*	33.39
6j	*p*-Cl	100	10	9.30 ± 1.06	22.37
6k	*p*-CN	100	10	6.05 ± 1.06**	49.50
6l	H	100	10	7.31 ± 1.54*	38.98
DMSO	–	100	10	11.98 ± 1.16	–
Ibuprofen	–	100	10	4.42 ± 0.81**	63.11

– no anti-inflammatory activity

* $0.01 < p < 0.05$; ** $p < 0.01$ compared to the vehicle group

Table 2 Anti-inflammatory activity of compounds **3g** and **6d** administered orally at different times before xylene application

Time (h)	Dose (mg/kg)	Inhibition (%)		
		3g	**6d**	Ibuprofen
1	100	24.88	28.18	20.53
2	100	34.19	40.76*	26.65
3	100	58.13*	64.73*	41.58
4	100	30.33*	33.53**	21.17
5	100	23.27	31.59*	20.81
24	100	–	–	–

– No anti-inflammatory activity

* $0.01 < p < 0.05$; ** $p < 0.01$ compared to ibuprofen at the corresponding time

inflammatory activity was assessed by the ability of each compound to prevent edema. Table 1 showed that most of the newly synthesized compounds had modest anti-inflammatory activity when administered at a dose of 100 mg/kg via the intraperitoneal route. Compounds 3-(4-methyl-benzyl)-3,4,7,8-tetrahydro-2*H*,5*H*-1-oxa-3,5-diaza-anthracen-6-one (**3g**) and 9-(4-fluoro-phenyl)-5,8,9,10-tetrahydro-4*H*-7-oxa-2,3,9,11*b*-tetraaza-cyclopenta[*a*]anthracene (**6d**) inhibited inflammation by 63.19 and 68.28 %, respectively, and were thus more effective than ibuprofen (63.11 %). Among the novel compounds, **2a**, **2f**, **2h**, **3a**, **3f**, **3i**, **6b**, **6e–h**, and **6k** possessed anti-inflammatory activity similar to that of ibuprofen. In addition, **2b**, **2e**, **2g**, **3b–d**, **3e**, **6i**, and **6l** revealed modest anti-inflammatory activity in comparison with the vehicle group. The other compounds did not exhibit anti-inflammatory activity.

On the basis of the considerable anti-inflammatory activity demonstrated by the results listed in Tables 1, two outstanding quinoline derivatives, **3g** and **6d**, were chosen for further screening, whereby 100 mg/kg of each compound was administered via the oral route at multiple time points (1, 2, 3, 4, and 5 h) prior to xylene application. Similarities in anti-inflammatory activity were obvious among **3g**, **6d**, and ibuprofen (Table 2; Fig. 2). As the interval lengthened, the anti-inflammatory activities of **3g**, **6d**, and ibuprofen first increased and then declined. Peak activity was observed at 3 h, and they did not exhibit anti-inflammatory activity at 5 h. Compounds **3g** and **6d** showed stronger anti-inflammatory activity than ibuprofen at all intervals (1–5 h), and these differences were especially significant at the 3 h interval.

In a separate experiment, the inhibitory effects of compounds **3g**, **6d**, and ibuprofen against ear inflammation were evaluated and compared at lower doses (50 mg/kg) administered via the oral route 3 h before xylene application (Table 3; Fig. 3). Compounds **3g** and **6d** possessed more activity than ibuprofen at a dose of 50 mg/kg when administered 3 h prior to xylene administration.

Finally, at a dose of 100 mg/kg administered via the intraperitoneal route, **6d** (hydrochloride salt) continued to exhibit potent anti-inflammatory activity (Fig. 4). However, in this test, **3g** (hydrochloride salt) showed strong toxicity: All 10 mice in the group treated with **3g** died after the compound was administered.

In vitro inhibition of cytokine (TNF-α and IL-6) production

To ascertain the toxicity of **6d** (hydrochloride salt) and **3g** (hydrochloride salt) to RAW264.7 cells, cell viability was evaluated by the MTT assay after incubating RAW264.7 cells for 18 h in the absence or presence of LPS. We found that **6d** had no effect on the viability of RAW264.7 cells at 10, 20, or 40 μg/mL. However, concentrations of **6d** up to 50 μg/mL reduced cell viability. The hydrochloride salt **3g** was found to reduce cell viability at 2, 10, 20, 40, and 50 μg/mL. These observations showed that the effects of **6d** were not due to cytotoxicity.

LPS is a major pro-inflammatory constituent of the cell wall of gram-negative bacteria and has a key role in many inflammatory diseases (Asti *et al*., 2000). LPS enters the bloodstream and elicits inflammatory responses that may lead to shock and death (Hudson *et al*., 1995). Production of pro-inflammatory cytokines by macrophages in response to inflammatory stimuli and microbial products is well established (Kim *et al*., 2002). TNF-α and IL-6 are the primary cytokines involved in inflammatory processes in LPS-stimulated RAW264.7 cells. TNF-α is the earliest and primary endogenous mediator involved in inflammation in RAW264.7 cells (Yang *et al*., 2004), and it promotes the aggregation and activation of leukocytes at sites of inflammation and stimulates the release of various inflammatory mediators (Jiang *et al*., 2004). IL-6 is an important inflammatory mediator released from monocytes and vascular endothelial cells. In the present study, cells were treated with LPS alone or LPS with different concentrations of the test compounds for 24 h, and levels of

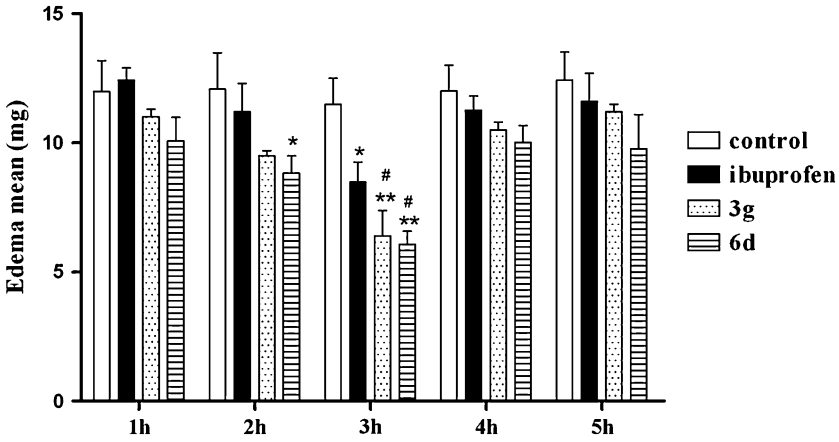


Fig. 2 Anti-inflammatory activities of the compounds were tested in the xylene-induced ear-edema test in mice. **3g**, **6d**, and ibuprofen were administrated p.o. at a dose of 100 mg/kg, and edema was quantified at different intervals (1, 2, 3, 4, and 5 h). $*p < 0.05$; $**p < 0.01$ versus the control group, and $^{\#}p < 0.05$ versus the ibuprofen group at the same time point

Table 3 Anti-inflammatory activity of compounds **3g** and **6d** administered orally at different doses

Time (h)	Dose (mg/kg)	Inhibition (%)		
		3g	**6d**	Ibuprofen
3	100	56.41*	60.55*	39.84
3	50	23.92	28.72*	18.62

– No anti-inflammatory activity

* $0.01 < p < 0.05$; * $p < 0.01$ compared to ibuprofen at the corresponding dose

TNF-α and IL-6 in culture supernatants were detected using enzyme-linked immunosorbent assays (ELISA) (Fig. 5a, b). In comparison with the control group, treatment of RAW264.7 cells with LPS alone led to a significant increase in cytokine production. However, upon treatment with LPS and different concentrations of **6d**, levels of TNF-α and IL-6 fell significantly in a dose-dependent manner, which suggested that the anti-inflammatory activity of **6d** was due to inhibition of production of TNF-α and IL-6.

Structure–activity relationships

By evaluating the activities of the synthesized test compounds, we obtained structure–activity relationships (SARs). Compounds **2a–2i**, **2f**, and **2h** showed significant anti-inflammatory activity with inhibitory rates of 56.18, 60.77, and 59.52 %, respectively. These results showed that quinolin-2-one possessed potential anti-inflammatory activity. As mentioned above, compounds **3a–3l** and **6a–6l** were prepared to evaluate the influence of the oxazine ring and the triazole ring, respectively, on anti-inflammatory activity. Among compounds **3a–3l**, **3a**, **3f**, **3g**, and **3i** showed significant anti-inflammatory activity, and the anti-inflammatory effect of **3g** was 63.19 %. Among **6a–6l**, compounds **6b**, **6d**, **6e**, **6f**, **6g**, **6h**, and **6k** possessed significant anti-inflammatory activity, and the anti-inflammatory effect of **6d** was 68.28 %. Compounds **6a–6l** possessed more potential anti-inflammatory activity than **3a–3l**. These findings suggest that the introduction of an oxazine ring to the derivatives conferred anti-inflammatory activity, and that the triazole ring dramatically improved this activity.

Next, we assessed the SARs of all compounds sequentially for each series. First, comparing derivatives **2a–2i** with different halogen substituents on the benzene ring, the order of activity was *m*-Cl > *p*-F > *m*-F > *o*-Cl > *o*-F > *p*-Cl. Comparing compounds with different electron-donating substituents, the order of activity was *p*-CH_3 > *p*-OCH_3. Electron-donating groups seemed to be a more beneficial structural feature than electron-withdrawing groups for anti-inflammatory activity.

Second, for compounds **3a–3l** (with an oxazine ring), different substitutions at the third position appeared to greatly influence the activity. Comparing derivatives without substitutions on the benzene ring, the order of activity was $-C_6H_5 > -CH_2C_6H_5 > -CH_2CH_2C_6H_5$. For derivatives with different electron-donating substituents (methyl and methoxy), the order of activity was $-CH_3 > -OCH_3$. Moreover, most of the derivatives with electron-withdrawing substituents on the benzene ring showed modest activity, and the order of activity was F > Cl > CN.

Third, the effects of different substitutions at the ninth position on the activities of compounds **6a–6l** (with oxazine and triazole rings) were observed. Comparing derivatives without substitutions on the benzene ring, the order of activity was $-CH_2C_6H_5 > -CH_2CH_2C_6H_5 > -C_6H_5$. Comparison of halogen-substituted derivatives indicated that

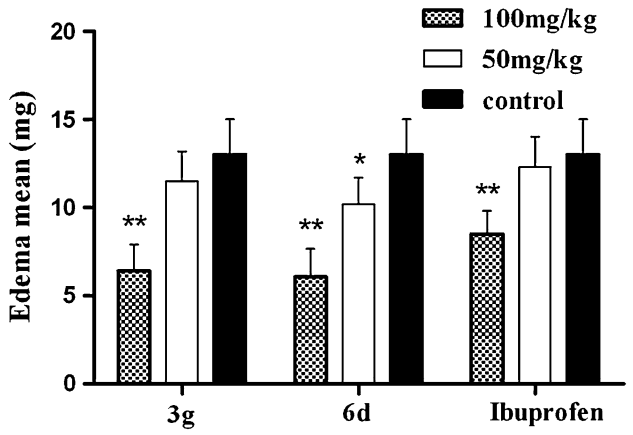


Fig. 3 **3g**, **6d**, and ibuprofen were evaluated and compared at lower doses (50 mg/kg) that were orally administered 3 h before xylene application. $*p < 0.05$; $**p < 0.01$ versus the control group

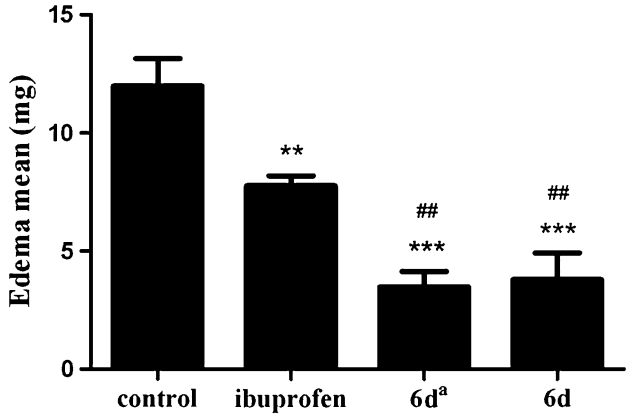


Fig. 4 **6d** and **6d**[a] (hydrochloride salt) were homogenized with physiological saline at a dose of 100 mg/kg and administered i.p. to mice. $**p < 0.01$, $***p < 0.001$ versus the control group, and $^{\#\#}p < 0.01$ versus the ibuprofen group

different halogen atoms contributed to the anti-inflammatory activity in the order F > Cl. Comparing derivatives with different electron-donating substituents (methyl and methoxy), the order of activity was $–CH_3 > –OCH_3$ for the phenyl groups and $–CH_3 = –OCH_3$ for the benzyl groups. Finally, the electron-donating and electron-withdrawing groups made similar contributions to the anti-inflammatory activity.

Conclusion

Three categories of derivatives, quinolin-2-one, 1-oxa-3,5-diaza-anthracen-6-one, and cyclopenta[*a*]anthracene, were synthesized, and their anti-inflammatory activities were evaluated. In the xylene-induced ear-edema test in mice, only two of the newly synthesized compounds (**3h, 6c**) showed weak anti-inflammatory activity; most compounds showed modest anti-inflammatory activity. In particular, 3-(4-methyl-benzyl)-3,4,7,8-tetrahydro-2*H*,5*H*-1-oxa-3,5-diaza-anthracen-6-one (**3g**) and 9-(4-fluorophenyl)-5,8,9,10-tetrahydro-4*H*-7-oxa-2,3,9,11*b*-tetraaza-cyclopenta[*a*]anthracene (**6d**) showed potent anti-inflammatory activity at 100 mg/kg (intraperitoneally), with inflammatory inhibition values of 63.19 and 68.28 %, respectively, which were greater than that of ibuprofen (63.11 %). Compounds **3g** and **6d** showed anti-inflammatory activity upon oral administration at doses of 50 and 100 mg/kg.

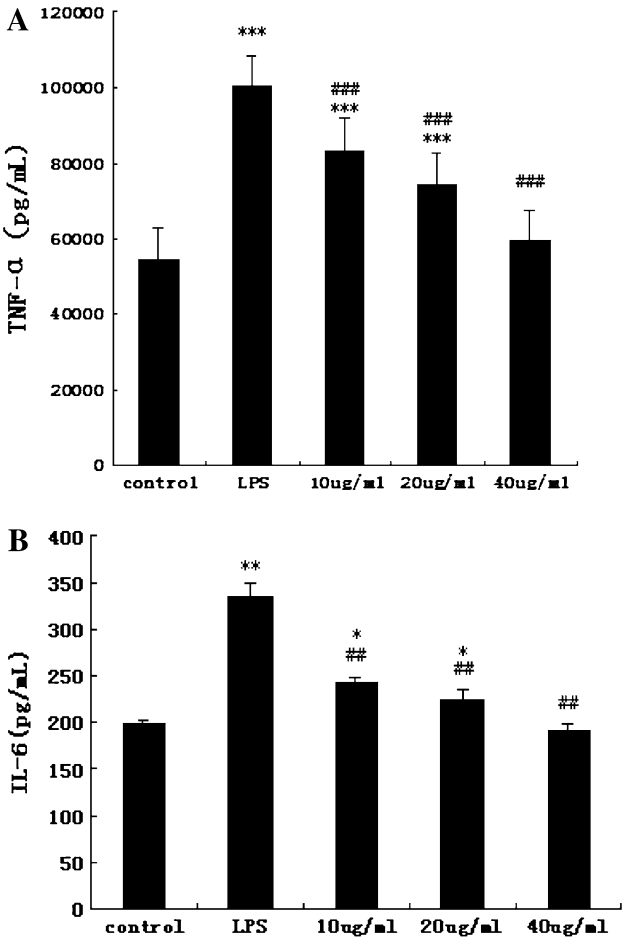


Fig. 5 Cells were treated with LPS alone or LPS plus different concentrations of the test compounds for 24 h. The values presented are the mean ± SEM of three independent experiments. $^{\#\#}p < 0.01$ versus the control group; $**p < 0.01$ versus the LPS group

SAR analyses suggested that oxazine and triazole rings could improve the anti-inflammatory therapeutic efficacy of quinoline derivatives. Active compounds were selected for further anti-inflammatory investigation in vitro, and **6d** (hydrochloride salt) showed dose-dependent inhibition of LPS-induced TNF-α and IL-6 expression.

Further studies are necessary to gain insight into the mechanisms of actions of these quinoline analogs, but these

results suggest that further development of such compounds may be of therapeutic interest.

Experimental protocols

Chemistry

Reactions were monitored by thin-layer chromatography on silica gel plates precoated with F_{254} gel. Developed plates were examined under an ultraviolet lamp (254 nm). Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) using an FTIR1730 Spectrometer (Perkin-Elmer, Waltham, MA, USA).^{1}H NMR and ^{13}C NMR spectra were recorded using an AV-300 Spectrometer (Bruker Daltonik, Bremen, Germany), and all chemical shifts were described in parts per million relative to that of tetramethylsilane. Mass spectra were measured using an HP1100 LC Spectrometer (Agilent Technologies, Santa Clara, CA, USA). High-resolution mass spectra were measured on an MALDI–TOF/TOF mass spectrometer (Bruker Daltonik). Chemicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

*General method for the synthesis of compounds **2a–2i***

Compounds **2a**–**2i** were synthesized according to previously described methods (Sun *et al*., 2006; Wei *et al*., 2010; Sun *et al*., 2013).

*General method for the synthesis of compounds **3a–3l***

Compound **1** (10 mmol) was added to a stirred solution of amine (15 mmol) and methanal (60 mmol) in ethanol (30 mL), and the reaction mixture was refluxed for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 (30 mL), washed with NaOH (1 mol/L, 2 × 30 mL) and H_2O (30 mL), and saturated with NaCl (30 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, after which the obtained crude product was recrystallized in dioxane to afford white needle-shaped crystal compounds (**3a**–**3l**).

*3-Phenyl-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one (**3a**)* Yield: 29.41 %; m.p.: 197 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.60 (t, 2H, J = 7.5 Hz, H-8), 2.90 (t, 2H, J = 7.5 Hz, H-7), 4.60 (s, 2H, –CH_2N), 5.35 (s, 2H, –CH_2O–), 6.50 (s, 1H, H-10), 6.64 (s, 1H, H-9), 6.93–7.31 (m, 5H, H–Ar), 8.84 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.28 (C-3), 30.64 (C-2), 50.22 (C-11), 79.62 (C-10), 113.18 (C-4), 116.26 (C-6), 118.37 (C-14, C-16), 119.69 (C-7), 121.63 (C-15), 123.82 (C-8), 129.30 (C-13, C-17), 130.83 (C-9), 148.26 (C-12), 150.09 (C-5), 171.83 (C-1). IR (KBr, cm^{-1}): 3,202, 1,514 (N–H), 1,678 (C=O), 1,230, 1,024 (C–O–C), 1,094 (C–N). ESI-HRMS calcd. for $C_{17}H_{16}N_2O_2^+$ (M^+): 280.1212; found: 280.1206.

*3-p-Tolyl-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one (**3b**)* Yield: 24.44 %; m.p.: 230 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.27 (s, 3H, –CH_3), 2.60 (t, 2H, J = 7.5 Hz, H-8), 2.89 (t, 2H, J = 7.5 Hz, H-7), 3.52 (s, 2H, –CH_2N), 4.55 (s, 2H, –CH_2O–), 6.51 (s, 1H, H-10), 6.62 (s, 1H, H-9), 6.99–7.09 (m, 4H, H–Ar), 9.17 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.54 (CH_3), 25.28 (C-3), 30.65 (C-2), 50.40 (C-11), 80.11 (C-10), 113.27 (C-4), 116.15 (C-6), 118.70 (C-14, C-16), 119.72 (C-7), 123.73 (C-8), 129.82 (C-13, C-17), 130.79 (C-15), 131.24 (C-9), 145.94 (C-12), 150.07 (C-5), 172.02 (C-1). IR (KBr, cm^{-1}): 3,203, 1,514 (N–H), 1,672 (C=O), 1,230, 1,028 (C–O–C), 1,095 (C–N). ESI-HRMS calcd. for $C_{18}H_{18}N_2O_2^+$ (M^+): 294.1368; found: 294.1360.

*3-(4-Methoxy-phenyl)-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one (**3c**)* Yield: 21.05 %; m.p.: 199 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.60 (t, 2H, J = 7.5 Hz, H-8), 2.90 (t, 2H, J = 7.5 Hz, H-7), 3.76 (s, 3H, –OCH_3), 4.51 (s, 2H, –CH_2N), 5.27 (s, 2H, –CH_2O–), 6.45 (s, 1H, H-10), 6.64 (s, 1H, H-9), 6.81–7.08 (m, 4H, H–Ar), 8.39 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.29 (C-3), 30.64 (C-2), 50.91 (C-11), 55.51 (OCH_3), 80.82 (C-10), 113.19 (C-4), 114.53 (C-14, C-16), 116.17 (C-6), 119.69 (C-7), 120.78 (C-13, C-17), 122.30 (C-8), 123.77 (C-9), 130.73 (C-12), 142.18 (C-15), 150.09 (C-5), 171.84 (C-1). IR (KBr, cm^{-1}): 3,206, 1,512 (N–H), 1,672 (C=O), 1,247, 1,035 (C–O–C), 1,093 (C–N). ESI-HRMS calcd. for $C_{18}H_{18}N_2O_3^+$ (M^+): 310.1317; found: 310.1312.

*3-(4-Fluoro-phenyl)-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one (**3d**)* Yield: 27.78 %; m.p.: 228 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.60 (t, 2H, J = 7.5 Hz, H-8), 2.90 (t, 2H, J = 7.5 Hz, H-7), 4.54 (s, 2H, –CH_2N), 5.28 (s, 2H, –CH_2O–), 6.46 (s, 1H, H-10), 6.65 (s, 1H, H-9), 6.93–7.10 (m, 4H, H–Ar), 8.39 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.29 (C-3), 30.62 (C-2), 50.88 (C-11), 80.31 (C-10), 113.11 (C-4), 115.65 (C-13, C-17), 115.95 (C-14, C-16), 116.26 (C-6), 119.38 (C-7), 120.60 (C-8), 123.94 (C-9), 130.92 (C-12), 144.80 (C-15), 149.95 (C-5), 171.77 (C-1). IR (KBr, cm^{-1}): 3,199, 1,510 (N–H), 1,672 (C=O), 1,228, 1,030 (C–O–C), 1,093 (C–N). ESI-HRMS calcd. for $C_{17}H_{16}FN_2O_2^+$ (M^+): 298.1118; found: 298.1117.

*3-(4-Chloro-phenyl)-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one (**3e**)* Yield: 16.79 %; m.p.: 223 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.60 (t, 2H, J = 7.5 Hz, H-8), 2.90 (t, 2H, J = 7.5 Hz, H-7), 4.56 (s, 2H,

–CH_2N), 5.30 (s, 2H, –CH_2O–), 6.44 (s, 1H, H-10), 6.65 (s, 1H, H-9), 7.02–7.24 (m, 4H, H–Ar), 8.09 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.27 (C-3), 30.60 (C-2), 50.45 (C-11), 79.51 (C-10), 113.16 (C-4), 116.30 (C-6), 119.30 (C-7), 119.74 (C-13, C-17), 124.00 (C-15), 126.70 (C-8), 129.22 (C-14, C-16), 130.98 (C-9), 146.92 (C-12), 149.88 (C-5), 171.89 (C-1). IR (KBr, cm^{-1}): 3,200, 1,518 (N–H), 1,670 (C=O), 1,228, 1,030 (C–O–C), 1,099 (C–N). ESI-HRMS calcd. for $C_{17}H_{16}ClN_2O_2^+$ (M^+): 314.0822; found: 314.0812.

3-Benzyl-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one ***(3f)*** Yield: 20.67 %; m.p.: 211 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.61 (t, 2H, J = 7.5 Hz, H-8), 2.92 (t, 2H, J = 7.5 Hz, H-7), 3.90 (s, 4H, –CH_2NCH_2), 4.84 (s, 2H, –CH_2O–), 6.41 (s, 1H, H-10), 6.65 (s, 1H, H-9), 7.29–7.36 (m, 5H, H–Ar), 9.06 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.33 (C-3), 30.70 (C-2), 49.31 (CH_2), 55.51 (C-11), 82.27 (C-10), 114.11 (C-4), 115.74 (C-6), 118.85 (C-7), 123.58 (C-15), 127.44 (C-8), 128.48 (C-14, C-16), 128.93 (C-13, C-17), 130.71 (C-9), 138.00 (C-12), 149.85 (C-5), 171.88 (C-1). IR (KBr, cm^{-1}): 3,205, 1,514 (N–H), 1,674 (C=O), 1,236, 1,022 (C–O–C), 1,095 (C–N). ESI-HRMS calcd. for $C_{18}H_{18}N_2O_2^+$ (M^+): 294.1368; found: 294.1363.

3-(4-Methyl-benzyl)-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one ***(3g)*** Yield: 22.88 %; m.p.: 202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H, –CH_3), 2.62 (t, 2H, J = 7.5 Hz, H-8), 2.93 (t, 2H, J = 7.5 Hz, H-7), 3.88 (s, 2H, –CH_2–Ar), 3.92 (s, 2H, H-4), 4.85 (s, 2H, –CH_2O–), 6.38 (s, 1H, H-10), 6.66 (s, 1H, H-9), 7.18–7.26 (m, 4H, H–Ar), 8.39 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 21.14 (CH_3), 25.34 (C-3), 30.71 (C-2), 49.26 (CH_2), 55.22 (C-11), 82.19 (C-10), 114.13 (C-4), 115.71 (C-6), 118.91 (C-7), 123.54 (C-8), 128.93 (C-14, C-16), 129.16 (C-13, C-17), 130.70 (C-9), 134.91 (C-12), 137.09 (C-15), 149.88 (C-5), 171.91 (C-1). IR (KBr, cm^{-1}): 3,200, 1,514 (N–H), 1,675 (C=O), 1,222, 1,020 (C–O–C), 1,095 (C–N). ESI-HRMS calcd. for $C_{19}H_{21}N_2O_2^+$ ([M + H]$^+$): 309.1598; found: 309.1598.

3-(4-Methoxy-benzyl)-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one ***(3h)*** Yield: 35.35 %; m.p.: 212 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H, –CH_3), 2.62 (t, 2H, J = 7.5 Hz, H-8), 2.93 (t, 2H, J = 7.5 Hz, H-7), 3.52 (s, 3H, –OCH_3), 3.83 (s, 2H, –CH_2–Ar), 3.85 (s, 2H, H-4), 4.84 (s, 2H, –CH_2O–), 6.36 (s, 1H, H-10), 6.67 (s, 1H, H-9), 6.67–6.91 (m, 4H, H–Ar), 7.99 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.34 (C-3), 30.71 (C-2), 49.19 (CH_2), 54.85 (OCH_3), 55.30 (C-11), 82.02 (C-10), 113.86 (C-14, C-16), 114.05 (C-4), 115.71 (C-6), 118.89 (C-7), 123.58 (C-8), 129.98 (C-12), 130.19 (C-13, C-17), 130.65 (C-9), 149.89 (C-5), 159.01 (C-15), 171.73 (C-1). IR (KBr, cm^{-1}): 3,200, 1,512 (N–H), 1,672 (C=O), 1,242, 1,029 (C–O–C), 1,097 (C–N). ESI-HRMS calcd. for $C_{19}H_{21}N_2O_3^+$ ([M + H]$^+$): 325.1547; found: 325.1550.

3-(4-Fluoro-benzyl)-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one ***(3i)*** Yield: 21.55 %; m.p.: 227 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.62 (t, 2H, J = 7.5 Hz, H-8), 2.93 (t, 2H, J = 7.5 Hz, H-7), 3.88 (s, 2H, –CH_2–Ar), 3.91 (s, 2H, H-4), 4.84 (s, 2H, –CH_2O–), 6.36 (s, 1H, H-10), 6.67 (s, 1H, H-9), 7.02–7.35 (m, 4H, H–Ar), 8.02 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.32 (C-3), 30.69 (C-2), 49.30 (CH_2), 54.79 (C-11), 82.05 (C-10), 114.06 (C-4), 115.16 (C-14, C-16), 115.44 (C-6), 115.78 (C-13, C-17), 118.71 (C-7), 123.67 (C-8), 130.41 (C-9), 130.52 (C-12), 133.66 (C-5), 149.80 (C-15), 171.80 (C-1). IR (KBr, cm^{-1}): 3,199, 1,514 (N–H), 1,685 (C=O), 1,222, 1,018 (C–O–C), 1,095 (C–N). ESI-HRMS calcd. for $C_{18}H_{18}FN_2O_2^+$ ([M + H]$^+$): 313.1347; found: 313.1347.

3-(4-Chloro-benzyl)-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one ***(3j)*** Yield: 20.39 %; m.p.: 214 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.62 (t, 2H, J = 7.5 Hz, H-8), 2.93 (t, 2H, J = 7.5 Hz, H-7), 3.88 (s, 2H, –CH_2–Ar), 3.91 (s, 2H, H-4), 4.84 (s, 2H, –CH_2O–), 6.37 (s, 1H, H-10), 6.67 (s, 1H, H-9), 7.31–7.32 (m, 4H, H–Ar), 8.23 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.33 (C-3), 30.68 (C-2), 49.36 (CH_2), 54.80 (C-11), 82.14 (C-10), 114.03 (C-4), 115.80 (C-6), 118.65 (C-7), 123.70 (C-8), 128.62 (C-14, C-16), 130.20 (C-13, C-17), 130.76 (C-9), 133.19 (C-15), 136.52 (C-12), 149.76 (C-5), 171.76 (C-1). IR (KBr, cm^{-1}): 3,188, 1,510 (N–H), 1,670 (C=O), 1,240, 1,020 (C–O–C), 1,091 (C–N). ESI-HRMS calcd. for $C_{18}H_{18}ClN_2O_2^+$ ([M + H]$^+$): 329.1051; found: 329.1048.

4-(6-Oxo-5,6,7,8-tetrahydro-4H-1-oxa-3,5-diaza-anthracen-3-ylmethyl)-benzonitrile ***(3k)*** Yield: 20.45 %; m.p.: 242 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.39 (t, 2H, J = 7.5 Hz, H-8), 2.79 (t, 2H, J = 7.5 Hz, H-7), 3.82 (s, 2H, –CH_2–Ar), 3.94 (s, 2H, H-4), 4.80 (s, 2H, –CH_2O–), 6.44 (s, 1H, H-10), 6.62 (s, 1H, H-9), 7.54–7.83 (m, 4H, H–Ar), 9.87 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.33 (C-3), 30.70 (C-2), 49.31 (CH_2), 55.51 (C-11), 82.27 (C-10), 109.77 (CN), 114.11 (C-4), 115.74 (C-6), 118.85 (C-7), 123.58 (C-15), 127.44 (C-8), 128.48 (C-14, C-16), 128.93 (C-13, C-17), 130.71 (C-9), 138.00 (C-12), 149.85 (C-5), 171.88 (C-1). IR (KBr, cm^{-1}): 3,208, 1,519 (N–H), 1,685 (C=O), 1,230, 1,071 (C–O–C), 1,116 (C–N). ESI-HRMS calcd. for $C_{19}H_{18}N_3O_2^+$ ([M + H]$^+$): 320.1394; found: 320.1397.

3-Phenethyl-3,4,7,8-tetrahydro-2H,5H-1-oxa-3,5-diaza-anthracen-6-one ***(3l)*** Yield: 49.12 %; m.p.: 180 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.61 (t, 2H, J = 7.5 Hz, H-8), 2.88 (t, 2H, J = 7.5 Hz, –CH_2CH_2N), 3.03 (t, 2H, J = 7.5 Hz,

H-7), 3.52 (t, 2H, $J = 7.5$ Hz, $–CH_2CH_2N$), 3.99 (s, 2H, H-4), 4.87 (s, 2H, $–CH_2O–$), 6.38 (s, 1H, H-10), 6.62 (s, 1H, H-9), 7.21–7.29 (m, 5H, H–Ar), 7.90 (s, 1H, –HNCO–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 25.32 (C-3), 30.71 (C-2), 34.90 (CH_2), 50.12 (CH_2), 53.13 (C-11), 82.51 (C-10), 113.94 (C-4), 115.75 (C-6), 119.06 (C-7), 123.58 (C-15), 126.21 (C-8), 128.40 (C-13, C-17), 128.67 (C-14, C-16), 130.58 (C-9), 139.68 (C-12), 149.97 (C-5), 171.75 (C-1). IR (KBr, cm^{-1}): 3,209, 1,514 (N–H), 1,676 (C=O), 1,232, 1,021 (C–O–C), 1,091 (C–N). ESI-HRMS calcd. for $C_{19}H_{21}N_2O_2^+$ ($[M + H]^+$): 309.1598; found: 309.1591.

Synthesis of 6-hydroxy-3,4-dihydro-1H-quinoline-2-thione ***(4)*** To a stirring mixture of acetonitrile (120 mL) and triethylamine (80 mL) in a round-bottomed flask in an ice-bath, P_2S_5 (17.70 g, 80 mmol) was added one portion at a time after the previous portion had dissolved completely. Next, compound **1** (10.00 g, 60 mmol) was added, and the solution was refluxed for 3 h. The reaction mixture was concentrated to dryness under reduced pressure. The resulting mixture was diluted with methanol and poured into cool water. The mixture was left to stand for 10 h and filtered under reduced pressure to obtain a yellow residue (10.00 g). Yield: 91.07 %; m.p.: 225 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.79 (t, 2H, $J = 6.6$ Hz, H-4), 2.95 (t, 2H, $J = 6.6$ Hz, H-3,), 6.69–7.04 (m, 3H, H–Ar), 8.32 (s, 1H, –NHCO–), 10.93 (s, 1H, HO–). MS-EI *m/z* 179 ($M + H^+$).

Synthesis of 4,5-dihydro-[1,2,4]triazolo[4,3-a]quinolin-7-ol ***(5)*** A solution of compound **4** (8.00 g, 45 mmol) and formohydrazide (6.86 g, 58 mmol) in *n*-butyl alcohol (80 mL) was heated at reflux for 24 h. Eighty percent of the *n*-butyl alcohol was removed under reduced pressure and cooled to 0 °C. The resulting mixture was filtered under reduced pressure to yield a yellow powder (7.20 g). Yield: 86.15 %; m.p.: 235 °C. ^1H NMR (DMSO-d_6, 300 MHz): δ 2.93 (t, 2H, $J = 7.7$ Hz, H-5), 3.01 (t, 2H, $J = 7.7$ Hz, H-4), 6.74–7.56 (m, 3H, H–Ar), 9.11 (s, 1H, –N=CH–), 9.2 (s, 1H, H–O). MS-EI *m/z* 187 ($M + H^+$).

General method for the synthesis of compounds ***(6a–6l)***

Compound **5** (10 mmol) was added to a stirred solution of amine (15 mmol) and methanal (60 mmol) in ethanol (30 mL), and the reaction mixture was refluxed for 24 h. The reaction mixture was concentrated under reduced pressure, diluted with CH_2Cl_2 (30 mL), washed with NaOH (1 mol/L, 2 × 30 mL) and H_2O (30 mL), and saturated with NaCl (30 mL). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, after which the obtained crude product was purified by column chromatography over SiO_2 (CH_2Cl_2/MeOH = 80/1, v/v) to obtain white solid compounds (**6a–6l**).

9-Phenyl-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene ***(6a)*** Yield: 20.77 %; m.p.: 171 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.88 (t, 2H, $J = 7.5$ Hz, H-5), 3.19 (t, 2H, $J = 7.5$ Hz, H-4), 4.65 (s, 2H, H-10), 5.43 (s, 2H, $–CH_2O–$), 7.02–7.35 (m, 7H, H–Ar), 8.66 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.60 (C-3), 25.80 (C-4), 50.45 (C-12), 79.91 (C-11), 114.49 (C-5), 117.74 (C-16), 118.57 (C-14, C-18), 120.34 (C-7), 122.09 (C-9), 125.62 (C-8), 127.60 (C-10), 129.40 (C-15, C-17), 137.27 (C-1), 147.96 (C-13), 150.32 (C-2), 153.00 (C-6). IR (KBr, cm^{-1}): 1,539 (C=N), 1,222, 1,030 (C–O–C), 1,112 (C–N). ESI-HRMS calcd. for $C_{18}H_{17}N_4O^+$ ($[M + H]^+$): 305.1397; found: 305.1401.

9-p-Tolyl-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene ***(6b)*** Yield: 23.42 %; m.p.: 184 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.27 (s, 3H, $–CH_3$), 2.94 (t, 2H, $J = 7.5$ Hz, H-5), 3.14 (t, 2H, $J = 7.5$ Hz, H-4), 4.64 (s, 2H, H-10), 5.37 (s, 2H, $–CH_2O–$), 6.78–7.10 (m, 6H, H–Ar), 8.54 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.55 (CH_3), 20.59 (C-3), 25.78 (C-4), 50.63 (C-12), 80.36 (C-11), 114.55 (C-5), 117.64 (C-16), 118.85 (C-14, C-18), 120.37 (C-7), 125.52 (C-9), 127.52 (C-8), 129.91 (C-10), 131.73 (C-15, C-17), 137.31 (C-1), 145.62 (C-13), 150.31 (C-2), 152.99 (C-6). IR (KBr, cm^{-1}): 1,539 (C=N), 1,223, 1,030 (C–O–C), 1,114 (C–N). ESI-HRMS calcd. for $C_{19}H_{19}N_4O^+$ ($[M + H]^+$): 319.1553; found: 319.1553.

9-(4-Methoxy-phenyl)-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene ***(6c)*** Yield: 19.14 %; m.p.: 150 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.95 (t, 2H, $J = 7.5$ Hz, H-5), 3.15 (t, 2H, $J = 7.5$ Hz, H-4), 3.75 (s, 3H, $–OCH_3$), 4.59 (s, 2H, H-10), 5.32 (s, 2H, $–CH_2O–$), 6.79–7.09 (m, 6H, H–Ar), 8.53 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.59 (C-3), 25.78 (C-4), 51.10 (C-12), 55.50 (OCH_3), 81.05 (C-11), 114.53 (C-5), 114.58 (C-15, C-17), 117.61 (C-7), 120.34 (C-9), 120.90 (C-14, C-18), 125.53 (C-8), 127.52 (C-10), 137.30 (C-1), 141.81(C-13), 150.30 (C-2), 152.99 (C-6), 155.30 (C-16). IR (KBr, cm^{-1}): 1,539 (C=N), 1,223, 1,031 (C–O–C), 1,114 (C–N). ESI-HRMS calcd. for $C_{19}H_{19}N_4O_2^+$ ($[M + H]^+$): 335.1503; found: 335.1504.

9-(4-Fluoro-phenyl)-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene ***(6d)*** Yield: 18.81 %; m.p.: 148 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.97 (t, 2H, $J = 7.5$ Hz, H-5), 3.16 (t, 2H, $J = 7.5$ Hz, H-4), 4.63 (s, 2H, H-10), 5.34 (s, 2H, $–CH_2O–$), 6.81–7.08 (m, 6H,

H–Ar), 8.55 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.59 (C-3), 25.81 (C-4), 51.08 (C-12), 80.55 (C-11), 114.49 (C-5), 116.09 (C-14, C-18), 117.74 (C-7), 120.03 (C-15, C-17), 120.78 (C-9), 124.87 (C-8), 125.71 (C-10), 127.71 (C-1), 137.27 (C-13), 144.47 (C-16), 150.31 (C-2), 152.86 (C-6). IR (KBr, cm^{-1}): 1,539 (C=N), 1,222, 1,002 (C–O–C), 1,130 (C–N). ESI-HRMS calcd. for $C_{18}H_{16}FN_4O^+$ ($[M + H]^+$): 323.1303; found: 323.1304.

*9-(4-Chloro-phenyl)-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene (**6e**)* Yield: 20.58 %; m.p.: 145 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.97 (t, 2H, $J = 7.5$ Hz, H-5), 3.17 (t, 2H, $J = 7.5$ Hz, H-4), 4.65 (s, 2H, H-10), 4.90 (s, 2H, –CH_2O–), 6.82–7.25 (m, 6H, H–Ar), 8.54 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.57 (C-3), 25.80 (C-4), 50.85 (C-12), 79.78 (C-11), 114.48 (C-5), 116.07 (C-7), 117.81 (C-9), 119.95 (C-14, C-18), 125.75 (C-16), 127.80 (C-8), 129.34 (C-15, C-17), 137.25 (C-10), 143.09 (C-1), 146.62 (C-13), 150.31 (C-2), 152.80 (C-6). IR (KBr, cm^{-1}): 1,539 (C=N), 1,222, 1,002 (C–O–C), 1,116 (C–N). ESI-HRMS calcd. for $C_{18}H_{16}ClN_4O^+$ ($[M + H]^+$): 339.1007; found: 339.1022.

*9-Benzyl-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene (**6f**)* Yield: 11.76 %; m.p.: 131 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.99 (t, 2H, $J = 7.5$ Hz, H-5), 3.17 (t, 2H, $J = 7.5$ Hz, H-4), 3.92 (s, 2H, –CH_2–Ar), 4.00 (s, 2H, H-10), 4.92 (s, 2H, –CH_2O–), 6.82 (s, 1H, H-6), 6.99 (s, 1H, H-11), 7.25–7.37 (m, 5H, H–Ar), 8.52 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.49 (C-3), 25.67 (C-4), 49.14 (CH_2), 55.41 (C-12), 82.45 (C-11), 115.64 (C-5), 116.98 (C-7), 119.50 (C-9), 125.36 (C-8), 127.17 (C-16), 127.48 (C-10), 128.46 (C-15, C-17), 128.82 (C-14, C-18), 137.55 (C-13), 137.68 (C-1), 150.15 (C-2), 152.72 (C-6). IR (KBr, cm^{-1}): 1,537 (C=N), 1,250, 1,029 (C–O–C), 1,111 (C–N). ESI-HRMS calcd. for $C_{19}H_{19}N_4O^+$ ($[M + H]^+$): 319.1553; found: 319.1555.

*9-(4-Methyl-benzyl)-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene (**6g**)* Yield: 12.38 %; m.p.: 136 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.72 (s, 3H, –CH_3), 3.01 (t, 2H, $J = 6.0$ Hz, H-5), 3.20 (t, 2H, $J = 6.0$ Hz, H-4), 3.89 (s, 2H, –CH_2–Ar), 4.00 (s, 2H, H-10), 4.93 (s, 2H, –CH_2O–), 6.83 (s, 1H, H-6), 6.97 (s, 1H, H-11), 7.20–7.27 (m, 4H, H–Ar), 8.51 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.63 (CH_3), 21.14 (C-3), 25.80 (C-4), 49.11 (CH_2), 55.25 (C-12), 82.53 (C-11), 115.40 (C-5), 111.91 (C-7), 119.58 (C-9), 125.40 (C-8), 127.30 (C-10), 128.48 (C-13), 128.88 (C-14, C-18), 129.24 (C-15, C-17), 134,53 (C-16), 137.32 (C-1), 150.31 (C-2), 152.91 (C-6). IR (KBr, cm^{-1}): 1,537 (C=N), 1,250, 1,029 (C–O–C), 1,111 (C–N). ESI-HRMS calcd. for $C_{20}H_{21}N_4O^+$ ($[M + H]^+$): 333.1710; found: 333.1700.

*9-(4-Methoxy-benzyl)-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene (**6h**)* Yield: 16.13 %; m.p.: 138 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.01 (t, 2H, $J = 7.5$ Hz, H-5), 3.21 (t, 2H, $J = 7.5$ Hz, H-4), 3.84 (s, 3H, –OCH_3), 3.87 (s, 2H, –CH_2–Ar), 4.00 (s, 2H, H-10), 4.92 (s, 2H, –CH_2O–), 6.83–7.29 (m, 6H, H–Ar), 8.52 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.67 (C-3), 25.83 (C-4), 49.06 (CH_2), 54.90 (OCH_3), 55.31 (C-12), 82.36 (C-11), 113.93 (C-15, C-17), 115.34 (C-5), 117.19 (C-7), 119.56 (C-9), 125.45 (C-8), 127.32 (C-10), 129.59 (C-13), 130.15 (C-14, C-18), 137.29 (C-1), 150.35 (C-2), 152.91 (C-6), 159.13 (C-16). IR (KBr, cm^{-1}): 1,537 (C=N), 1,249, 1,029 (C–O–C), 1,111 (C–N). ESI-HRMS calcd. for $C_{20}H_{21}N_4O_2^+$ ($[M + H]^+$): 349.1659; found: 349.1657.

*9-(4-Fluoro-benzyl)-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene (**6i**)* Yield: 15.88 %; m.p.: 146 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.01 (t, 2H, $J = 7.5$ Hz, H-5), 3.20 (t, 2H, $J = 7.5$ Hz, H-4), 3.90 (s, 2H, –CH_2–Ar), 4.00 (s, 2H, H-10), 4.90 (s, 2H, –CH_2O–), 6.83 (s, 1H, H-6), 6.99 (s, 1H, H-11), 7.03–7.36 (m, 4H, H–Ar), 8.51 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.66 (C-3), 25.84 (C-4), 49.21 (CH_2), 54.80 (C-12), 82.37 (C-11), 115.33 (C-5), 115.55 (C-15, C-17), 117.29 (C-7), 119.38 (C-9), 125.55 (C-8), 127.44 (C-10), 130.40 (C-14, C-18), 130.50 (C-13), 133.31 (C-1), 137.28 (C-2), 150.25 (C-6), 152.80 (C-16). IR (KBr, cm^{-1}): 1,537 (C=N), 1,250, 1,030 (C–O–C), 1,116 (C–N). ESI-HRMS calcd. for $C_{19}H_{18}FN_4O^+$ ($[M + H]^+$): 337.1459; found: 337.1455.

*9-(4-Chloro-benzyl)-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene (**6j**)* Yield: 10.18 %; m.p.: 130 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.00 (t, 2H, $J = 7.5$ Hz, H-5), 3.19 (t, 2H, $J = 7.5$ Hz, H-4), 3.90 (s, 2H, –CH_2–Ar), 3.99 (s, 2H, H-10), 4.90 (s, 2H, –CH_2O–), 6.83 (s, 1H, H-6), 6.98 (s, 1H, H-11), 7.28–7.33 (m, 4H, H–Ar), 8.51 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.62 (C-3), 25.80 (C-4), 49.24 (CH_2), 54.85 (C-12), 82.45 (C-11), 115.37 (C-5), 117.26 (C-7), 119.33 (C-9), 125.52 (C-8), 127.43 (C-10), 128.71 (C-15, C-17), 130.16 (C-14, C-18), 133.35 (C-16), 136.16 (C-13), 137.31 (C-1), 150.32 (C-2), 152.78 (C-6). IR (KBr, cm^{-1}): 1,537 (C=N), 1,250, 1,030 (C–O–C), 1,114 (C–N). ESI-HRMS calcd. for $C_{19}H_{18}ClN_4O^+$ ($[M + H]^+$): 353.1164; found: 353.1163.

*4-(5,10-Dihydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracen-9-ylmethyl)-benzonitrile (**6k**)* Yield: 11.26 %; m.p.: 152 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.00 (t, 2H, $J = 6.0$ Hz, H-5), 3.17 (t, 2H, $J = 6.0$ Hz, H-4), 3.99 (s, 2H, –CH_2–Ar), 4.00 (s, 2H, H-10), 4.90 (s, 2H, –CH_2O–), 6.84 (s, 1H, H-6), 6.94 (s, 1H, H-11), 7.49–7.62 (m, 4H,

H–Ar), 8.51 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.60 (C-3), 25.80 (C-4), 49.54 (CH_2), 55.22 (C-12), 82.60 (C-11), 111.49 (C-16), 115.37 (C-5), 117.41 (C-7), 118.73 (CN), 119.09 (C-9), 125.26 (C-8), 127.61 (C-10), 128.95 (C-13), 129.28 (C-14, C-18), 132.40 (C-15, C-17), 143.36 (C-1), 150.15 (C-2), 152.69 (C-6). IR (KBr, cm^{-1}): 1,539 (C=N), 1,250, 1,039 (C–O–C), 1,140 (C–N). ESI-HRMS calcd. for $C_{20}H_{18}N_5O^+$ ($[M + H]^+$): 344.1506; found: 344.1499.

*9-Phenethyl-5,8,9,10-tetrahydro-4H-7-oxa-2,3,9,11b-tetraaza-cyclopenta[a]anthracene (**6l**)* Yield: 22.03 %; m.p.: 154 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.90 (t, 2H, $J = 7.5$ Hz, H-5), 2.96–3.07 (m, 4H, $-(CH_2)_2N$), 3.17 (t, 2H, $J = 7.5$ Hz, H-4), 4.08 (s, 2H, H-10), 4.93 (s, 2H, $-CH_2O-$), 6.78 (s, 1H, H-6), 7.00 (s, 1H, H-11), 7.21–7.32 (m, 5H, H–Ar), 8.53 (s, 1H, –N=CH–). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.67 (C-3), 25.81 (C-4), 34.88 (CH_2), 50.01 (CH_2), 53.14 (C-12), 82.86 (C-11), 115.23 (C-5), 117.21 (C-9), 119.73 (C-7), 125.38 (C-8), 126.31 (C-16), 127.34 (C-10), 128.45 (C-14, C-18), 128.65 (C-15, C-17), 137.29 (C-13), 139.47 (C-1), 150.63 (C-2), 152.97 (C-6). IR (KBr, cm^{-1}): 1539 (C=N), 1,222, 1,032 (C–O–C), 1,111 (C–N). ESI-HRMS calcd. for $C_{20}H_{21}N_4O^+$ ($[M + H]^+$): 333.1710; found: 333.1712.

Pharmacology

In vivo study

Kunming mice (mean body weight, 20–25 g; 10 animals per group) were purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University (Yanji, China). Animals were housed at a constant room temperature with a 12–12 h light–dark cycle and fed a standard rodent diet and water. Mice were acclimatized to the laboratory for ≥7 days before experimentation.

Xylene-induced ear-edema model with intraperitoneally administered compounds

In this screening, all novel compounds were evaluated with regard to anti-inflammatory activity via an in vivo inhibition assay of xylene-induced ear edema (Sowemimo *et al*., 2013) in mice. All freshly prepared test compounds and ibuprofen (dissolved in DMSO) were administered to mice via the intraperitoneal route at a dose of 100 mg/kg (0.05 mL/20 g body weight). Control mice received DMSO (0.05 mL/20 g body weight) only. Thirty minutes after administration, mice were used in the xylene-induced ear-edema test (20 L xylene was daubed by a micropipette on the surface of the right ear of each mouse). After preventing the mice from struggling for 30 min, a cylindrical tissue plug (7 mm diameter) was excised from the ears of treated and untreated mice. Edema was quantified by measuring the difference in weight between the plugs from the treated and untreated mice. Anti-inflammatory activity was expressed as the percent reduction in edema in comparison with the DMSO-treated control group. The NSAID ibuprofen was tested in parallel as a reference drug.

Xylene-induced ear-edema model with orally administered compounds

Test compounds (**3g**, **6d,** and ibuprofen) were homogenized with 0.5 % sodium carboxymethylcellulose (CMC-Na) and administered via the oral route to mice at 100 mg/kg (0.4 mL/20 g body weight). Control mice received 0.5 % CMC-Na (0.4 mL/20 g body weight). To explore the peak activity of the test compounds, edema was quantified at different intervals (1, 2, 3, 4, and 5 h). In a separate experiment, compounds **3g**, **6d**, and ibuprofen were homogenized with 0.5 % CMC-Na and administered orally to mice (at a lower dose of 50 mg/kg; 0.4 mL/20 g body weight). Control mice received 0.5 % CMC-Na (0.4 mL/20 g body weight) and edema was quantified at the peak activity time point of 3 h.

Assay of hydrochloride salts in the xylene-induced ear-edema model via the intraperitoneal route

Compounds **3g** (hydrochloride salt) and **6d** (hydrochloride salt) were homogenized with physiological (0.9 %) saline and administered to mice via the intraperitoneal route at a dose of 100 mg/kg. Control mice received an equal volume of physiological saline via the intraperitoneal route.

In vitro study

DMSO, LPS (*Escherichia coli* lipopolysaccharide; 055:B5), and 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin for cell culture were obtained from Invitrogen–Gibco (Grand Island, NY, USA). Mouse TNF-α and IL-6 ELISA kits were purchased from Biolegend (San Diego, CA, USA).

Culture and treatment of cells

The RAW264.7 mouse macrophage cell line was purchased from China Cell Line Bank (Beijing, China). Cells were cultured in DMEM (Invitrogen–Gibco) supplemented with 10 % heat-inactivated FBS (Invitrogen–Gibco), at 37 °C under a humidified atmosphere of 5 % CO_2 in air. In all experiments, cells were incubated in the presence or

absence of various concentration of the test compounds, which were added 1 h before LPS (1 μg/mL) treatment.

MTT assay for cell viability

MTT was used to evaluate cell viability. RAW264.7 cells were mechanically scraped. Cells (100 μL) were plated at 4×10^5 cells/mL in 96-well plates in a 37 °C, 5 % CO_2 incubator for 1 h. Cells were treated with 50 μL of different concentrations of the test compounds (0–50 μg/mL) for 1 h, followed by stimulation with 50 μL LPS (4 μg/mL). After 18 h of LPS stimulation, 20 μL MTT (5 mg/mL) was added to each well, and the cells were incubated for an additional 4 h. The supernatants were removed and resolved with DMSO (150 μL/well). The optical density of the samples was measured at 570 nm on a Microplate Reader (Tecan, Groedia, Austria).

Measurement of pro-inflammatory cytokine (TNF-α and IL-6) production

Compound **6d** (hydrochloride salt) was dissolved in distilled water before treatment. RAW264.7 cells (4×10^5 cells/mL) were seeded into 24-well plates and pretreated with 10, 20, and 40 μg/mL **6d** hydrochloride salt for 1 h before treatment with 1 μg/mL LPS for 24 h. Cell-free supernatants were collected and analyzed for levels of TNF-α and IL-6 using ELISA kits and following the manufacturer's instructions (Biolegend).

Statistical analyses

Data are represented as the mean ± SEM. Statistical analyses for comparisons of the control and experimental groups were performed using one-way analysis of variance (ANOVA) followed by the Dunnett's test using GraphPad Prism version 5 (GraphPad, San Diego, CA, USA). Results of $P < 0.05$ were considered to be statistically significant.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Nos. 81160382 and 81360468) and the National Science and Technology Major Project of China (No. 2012ZX09103-101-044).

Conflict of interest The authors declare that they have no conflict of interest with respect to this study.

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