European Journal of Medicinal Chemistry 45 (2010) 4807–4812

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and anti-inflammatory activity evaluation of some novel 6-alkoxy (phenoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amine derivatives

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ARTICLE INFO

Article history:
Received 21 May 2010
Received in revised form
24 July 2010
Accepted 28 July 2010
Available online 6 August 2010

Keywords:
3-Amine triazole
Phthalazine
[1,2,4]Triazolo[3,4-*a*]phthalazine-3-amine
Anti-inflammatory

ABSTRACT

Starting from phthalic anhydride, several new 6-alkoxy(phenoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amine derivatives were synthesized as potent anti-inflammatory agent. The study showed that the compounds **6h** (6-(2-chlorophenoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amine) and **6s** (6-(4-amino-phenoxy)-[1,2,4] triazolo[3,4-*a*]phthalazine-3-amine) exhibited the highest anti-inflammatory activity (81% and 83% inhibition, respectively, at 0.5 h after i.p. administration) which were slightly more potent than the reference drug Ibuprofen (61%). Furthermore, the peak activity of **6h** and **6s** was observed at the 3 h after p.o. administration, and they exhibited stronger anti-inflammatory activity than Ibuprofen at the dose of 50 mg/kg at the peak time.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are useful tools in the treatment of acute and chronic inflammation [1], pain [2], and fever [3]. However, long-term clinical usage of NSAIDs is associated with significant side effects of gastrointestinal lesions, bleeding, and nephrotoxicity [4,5]. Therefore the discovery of new and safer anti-inflammatory drugs represents a challenging goal for such a research area [6]. As resistance to anti-inflammatory drugs is widespread, there is an increasing need for identification of novel structure leads that may be of use in designing new, potent and less toxic anti-inflammatory agents.

In the previous studies [7–12], the pharmacological activities of several 4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinolin compounds have been evaluated, among these compounds, 7-alkoxy-4,5-dihydro[1,2,4]triazolo[4,3-*a*]quinolin-1-amine (Fig. 1. **I**) was identified to possess anti-inflammatory activity. Analysed the structure of compound **I**, 3-amine triazole was assumed to be the main pharmacological moiety playing anti-inflammatory activity. So, on the basic of compound **I**, made the quinoline ring substituted with phthalazine ring and retained 3-amine triazole moiety, designed a series of 6-alkoxy(phenoxy)-[1,2,4]triazolo [3,4-*a*]phthalazine-3-amine derivatives. Quinoline ring and phthalazine ring could be regarded as generalized Bioisosterism [13,14]. Bioisosterism, can be defined as the property by which the substituents, or groups with similar physical or chemical properties, impart similar biological properties to a chemical compound is a useful strategy for both medicinal chemistry and rational design of new drugs. Herein, we report the synthesis of 6-alkoxy(phenoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amine derivatives and evaluation of their experimental anti-inflammatory activity.

2. Chemistry

Compounds were prepared according to Scheme 1. On the basis of the previous studies carried out in our laboratory, we designed and prepared 6-alkoxy(phenoxy)-[1,2,4]triazolo[3,4-*a*] phthalazine-3-amine derivatives (**6a**–**u**). The target compounds **6a**–**u** were synthesized according to Scheme 1. The starting material phthalic anhydride reacted with hydrazine hydrate in ethanol to yield 2,3-dihydrophthalazine-1,4-dione (compound **2**), which reacted further with the refluxing phosphorus oxychloride ($POCl_3$) to yield 1,4-dichlorophthalazine (compound **3**) [15]. Compound **3** reacted further with hydrazine hydrate in THF to produce compound **4** (1-hydrazine-4-chrorophthalazin) [16]. Then, 6-chlorom-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amine **5** was prepared by cyclising compound **4** with cyanogene bromide

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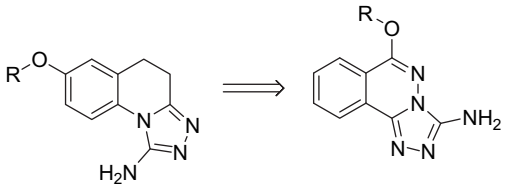
doi:10.1016/j.ejmech.2010.07.049



I **title compound**

Fig. 1. Structure of compound I and title compound.

in the presence of Na_2CO_3 [17]. Finally, compound **5** reacted with appropriate alkanol and substituted phenol to produce the target compounds, 6-alkoxy(phenoxy)-[1,2,4]triazolo[3,4-*a*] phthalazine-3-amine derivatives (**6a**–**u**). The compounds synthesized were characterized by IR, ^{1}H NMR, MS, and elemental analysis.

3. Pharmacology

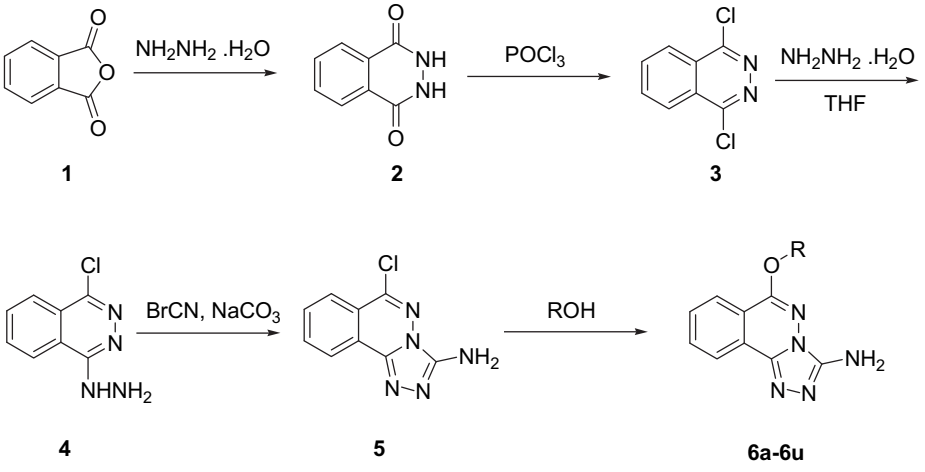
All the synthesized compounds were evaluated the anti-inflammatory activity via the method of the xylene-induced ear edema in Kunming mice, 20–25 g body weight, 10 animals per group purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University [18]. Firstly, the test compounds were evaluated their intraperitoneal (i.p.) administration anti-inflammatory activities at a dose of 100 mg/kg. Then, the outstanding compounds (**6h**, **6s**) were administered orally (p.o.) to mice and quantified their anti-inflammatory activities at different intervals (1 h, 2 h, 3 h, 4 h, 5 h and 24 h) under dose of 200 mg/kg. The peak activity of **6h** and **6s** was observed at the 3 h after p.o. administration. At last, the anti-inflammatory activities of the two compounds were investigated at different p.o. doses (50, 100, 200 mg/kg) at the peak time and made comparison with Ibuprofen.

4. Results and discussion

In the primary screening, all the synthesized compounds was tested in the xylene-induced ear edema test in mice. These compounds were evaluated for anti-inflammatory activity through monitoring their ability to inhibit xylene-induced ear edema, and made comparison with vehicle (DMSO) and Ibuprofen. As shown in Table 1, most of the tested compounds exhibited anti-inflammatory activity at a dose of 100 mg/kg administered intraperitoneally (i.p.), especially, the compounds **6h** (6-(2-chlorophenoxy)-[1,2,4]triazolo [3,4-*a*]phthalazine-3-amine) and **6s** (6-(4-aminophenoxy)-[1,2,4] triazolo[3,4-*a*]phthalazine-3-amine) showed the highest anti-inflammatory inhibition rate, 81% and 83%, respectively.

Among these alkyloxy substituted compounds, compounds **6d**, **6f** and **6g** possessed similar anti-inflammatory activity with Ibuprofen, but compounds **6a** and **6b** did not exhibited anti-inflammatory activity compared with the vehicle group, suggesting that appropriate length of the alkyl chain at C-6 position, or appropriate lipophilic property, was essential to the anti-inflammatory activity of these compounds [19,20]. All the fourteen aryl-substituted derivatives **6h**–**u** exhibited anti-inflammatory inhibition to a certain extent (38.4%–83.0%). Among them, compounds **6i**, **6j**, **6o**, **6p**, **6t** and **6u** exhibited similar anti-inflammatory activity with Ibuprofen, the compounds **6h** and **6s** were slightly more potent than the reference drug Ibuprofen. Comparison of the halogen substituted derivatives indicated that different halogen atoms contributed to the anti-inflammatory activity in the order of Cl > F = Br; but bichloride **6k** did not showed strong anti-inflammatory activity. Furthermore, the position of substituted group on the phenyl ring greatly influenced the anti-inflammatory activity with an activity order of *o* > *m* > *p*, compared with the non-substituted phenyl derivative **6u**, compounds containing electron donor group on the phenyl ring did not appeared to be more potent in the anti-inflammatory activity, only one derivative **6s** (*p*-amino substituted) showed increased activity.

Based on the results of primary screening, two outstanding derivatives **6h** and **6s** were chosen to be evaluated in the further



1 **2** **3** **4** **5** **6a-6u**

R:

a: -CH_3	h: -C_6H_4(*o*-Cl)	o: -C_6H_4(*o*-CH_3)
b: -C_2H_5	i: -C_6H_4(*m*-Cl)	p: -C_6H_4(*m*-CH_3)
c: *i*-C_3H_7	j: -C_6H_4(*p*-Cl)	q: -C_6H_4(*p*-CH_3)
d: *n*-C_4H_9	k:- C_6H_3(2,4-Cl_2)	r: -C_6H_4(*o*-NH_2)
e: *n*-C_6H_{13}	l: -C_6H_4(*p*-F)	s: -C_6H_4(*p*-NH_2)
f: *n*-C_7H_{15}	m: -C_6H_4(*p*-Br)	t: -C_6H_4(*m*-NH_2)
g: *n*-C_8H_{17}	n: -C_6H_4(*p*-OCH_3)	u: -C_6H_5

Scheme 1. Synthesis of compounds **6a**–**u**.

Table 1
Anti-inflammatory activity of compounds **6a**–**u** administrated i.p.

Comp.	R	Dose (mg/kg)	Number of mice	Edema mean ± S.D. (mg)	Inhibition rate (%)
DMSO	–	–	10	13.36 ± 2.84	–
Ibuprfen	–	100	10	5.19 ± 1.67*	61.2
6a	*n*-CH_3	100	10	9.09 ± 2.63	–
6b	*n*-C_2H_5	100	10	10.30 ± 2.66	–
6c	*i*-C_3H_7	100	10	8.10 ± 5.57*	39.4
6d	*n*-C_4H_9	100	10	4.33 ± 2.35*	67.6
6e	*n*-C_6H_{13}	100	10	8.09 ± 2.27	39.5
6f	*n*-C_7H_{15}	100	10	4.59 ± 2.52*	65.7
6g	*n*-C_8H_{17}	100	10	5.62 ± 4.43*	58.0
6h	-C_6H_4(*o*-Cl)	100	10	2.51 ± 2.15*	81.2
6i	-C_6H_4(*p*-Cl)	100	10	4.16 ± 2.86*	68.8
6j	-C_6H_4(*m*-Cl)	100	10	3.34 ± 2.12*	75.0
6k	-C_6H_3(*2,4*-Cl_2)	100	10	7.96 ± 3.90*	40.4
6l	-C_6H_4(*p*-F)	100	10	6.84 ± 3.04*	48.8
6m	-C_6H_4(*p*-Br)	100	10	6.90 ± 3.36*	48.4
6n	-C_6H_4(*p*-OCH_3)	100	10	7.48 ± 1.92*	44.0
6o	-C_6H_4(*o*-CH_3)	100	10	4.24 ± 2.92*	68.3
6p	-C_6H_4(*m*-CH_3)	100	10	4.28 ± 2.92*	68.0
6q	-C_6H_4(*p*-CH_3)	100	10	6.31 ± 3.03*	52.7
6r	-C_6H_4(*o*-NH_2)	100	10	8.23 ± 3.05*	38.4
6s	-C_6H_4(*p*-NH_2)	100	10	2.28 ± 0.82*	83.0
6t	-C_6H_4(*m*-NH_2)	100	10	4.28 ± 1.69*	68.0
6u	-C_6H_5	100	10	6.03 ± 3.18*	55.0

*$p < 0.01$ compared with vehicle group.

screening where the dose was 200 mg/kg by oral administration, but multiple intervals (1 h, 2 h, 3 h, 4 h, 5 h and 24 h) for xylene application were assessed. The results were shown in Table 2. As the interval lengthened, the anti-inflammatory activity of compounds **6h** and **6s** first increased and then declined; the peak activity was observed at the 3 h interval. Both compound **6h** and **6s** exhibited higher anti-inflammatory inhibition rate from 1 to 5 h in the test. Notably, compound **6h** possessed more potential activity than Ibuprofen at the 4 h interval, and showed no difference with Ibuprofen at the other time interval. Comparing **6s** and Ibuprofen, compound **6s** showed stronger anti-inflammatory activity than Ibuprofen at the 2–4 h interval, and showed similar activity level as the reference drug at other time points.

In succession, the ear inflammation inhibition rate of compounds **6h**, **6s** and reference drug Ibuprofen at lower doses (100 mg/kg and 50 mg/kg) administered 3 h before xylene application were evaluated and compared (Table 3). Both the two compounds showed similar effects as Ibuprofen at the dose of 100 mg/kg; but they exhibited stronger anti-inflammatory activity than Ibuprofen at the dose of 50 mg/kg, at which dose Ibuprofen did not showed any anti-inflammatory activity after 3 h administration p.o. in the test. A conclusion of compounds **6h**, **6s** possessing a slightly long-term anti-inflammatory action in animal test could be indicated by the present result.

Table 2
Anti-inflammatory activity of compounds **6h** and **6s** administered orally at different times before xylene application.

Time (h)	Dose (mg/kg)	Inhibition (%)		
		6h	**6s**	Ibuprofen
1	200	52.70	60.46	29.63
2	200	59.05	81.29**	38.58
3	200	75.05	84.35**	64.58
4	200	64.11*	80.58**	36.33
5	200	57.17	55.87	23.39
24	200	8.24	18.92	13.18

*$p < 0.05$, **$p < 0.01$ compared with Ibuprofen at the corresponding time.

Table 3
Anti-inflammatory activity of compounds **6h** and **6s** administered orally at different doses.

Time (h)	Dose (mg/kg)	Inhibition (%)		
		6h	**6s**	Ibuprofen
3	200	75.05	84.35*	64.58
3	100	46.70	37.86	26.93
3	50	30.80*	22.81*	–

*: $p < 0.01$ compared with Ibuprofen at the corresponding dose.
–: no anti-inflammatory activity.

5. Conclusion

A new series of anti-inflammatory compounds, 6-alkoxy(phenoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amines, were synthesized and their anti-inflammatory activities were evaluated by an *in vivo* test. Two compounds **6h** (6-(2-chlorophenoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amine) and **6s** (6-(4-aminophenoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine-3-amine) showed promising anti-inflammatory activity (81% and 83% inhibition, respectively, at 0.5 h after i.p. administration) which were slightly more potent than the reference drug Ibuprofen (61%).

6. Experimental protocols

6.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. ^{1}H NMR spectra's were measured on an AV-300 (Bruker, Fällanden, Switzerland), and all chemical shifts were reported in parts per million relative to tetramethylsilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, Santa Clara, USA). Elemental analyses were performed on a 204Q CHN (PerkinElmer, Fremont, USA). Major chemicals were purchased from Aldrich Chemical Corporation (Shanghai, China). All other chemicals were of analytical grade.

6.1.1. Synthesis of 2,3-dihydrophthalazine-1,4-dione (**2**)

The starting compound phthalic anhydride **1** (50 g, 337.8 mmol) was dissolved in ethanol (500 mL) in 30 min, then to this mixture was added hydrazine hydrate (20.3 g, 405.4 mmol) dropwise under ice-bath. The reaction mixture was stirred at room temperature (r.t.) for 1 h then made cool in the ice-bath. The white solid was collected through filtration and dried in a vacuum to give the compound 2,3-dihydrophthalazine-1,4-dione 43.0 g. M.p. 181–183 °C, yield = 78.5%. ^{1}H NMR (DMSO, 300 MHz) δ 7.59–7.63 (m, 2H, H-6, H-7), 8.14 (d, 2H, J = 7.9 Hz, H-5, H-8), 9.04 (s, 2H, –CO–NH–NH–CO–).

6.1.2. Synthesis of 1,4-dichlorophthalazine (**3**)

Compound **2** (8.5 g, 52.5 mmol) was dissolved in phosphorus oxychloride (45 mL) and stirred under reflux for 4 h. Then the solvent was removed under vacuum. The residue was dissolved in dichloromethane (200 mL) and stirred rapidly, and the solution was neutralized by the addition of solid and aqueous sodium hydrogen carbonate (cautiously). When effervescence had ceased, the organic layer was separated and the aqueous layer was extracted with dichloromethane (2 × 200 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated; the residue was purified by silica gel column chromatography with ethyl acetate: petroleum ether (1:8) and gained 8.5 g. M.p. 192–194 °C, yield = 81.2%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.59 (d, J = 7.9 Hz, 2H, H-5, H-8), 7.90–7.94 (m, 2H, H-6, H-7).

*6.1.3. Synthesis of 1-hydrazine-4-chrorophthalazin (**4**)*

A solution of compound **3** (5 g, 25.1 mmol) in THF (60 mL) was added dropwise to a solution of hydrazine hydrate (6.28 g, 125.6 mmol) in THF (10 mL) at room temperature. The mixture was stirred and heated at 60 °C for 1 h, then half of the solvent was removed under reduced pressure and the solution was poured into petroleum ether. The precipitate was filtered and washed with petroleum ether, and then kept below 0 °C. The compounds obtained were pure enough for the following step.

*6.1.4. Synthesis of 6-chloro-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**5**)*

In a three-neck round-bottomed flask with thermometer, compound **4** (2.2 g, 11.3 mmol) was dissolved in dioxane (60 mL), and the solution was treated with Na_2CO_3 (1.2 g, 11.3 mmol) in H_2O (20 mL). Then cyanogene bromide (1.3 g, 12.5 mmol) in dioxane (20 mL) was added dropwise to the mixture under ice-bath and kept the reaction temperature below 10 °C, after stirring for 2 h, the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (dichloromethane-methanol 10:1). M.p. 218–220 °C, yield = 30%. ^{1}H NMR (DMSO, 300 MHz) δ 6.67 (s, 2H, $-NH_2$), 7.86 (t, 1H, J = 7.7 Hz, H-8), 8.03 (t, 1H, J = 7.6 Hz, H-9) 8.18 (d, 1H, J = 8.0 Hz, H-10), 8.34 (d, 1H, J = 7.9 Hz, H-7).

*6.1.5. General procedure for synthesis of 6-alkoxy(phenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine derivatives (**6a**–**u**)*

Compound **5** (0.4 g, 1.83 mmol), NaOH (80 mg, 2.01 mmol), and alkanol (15 mL) were reacted together by stirring and refluxing for approximately 5 h. After the alkanol was removed under reduced pressure, the solid residue was purified by silica gel chromatography (dichloromethane:methanol, 10:1) to obtain compounds **6a**–**g**.

A mixture of appropriate substituted phenol (2.01 mmol), NaOH (80 mg, 2.01 mmol) in DMF (20 mL) was stirred at 50 °C for 15 min, then to this mixture was added compound **5** (0.4 g, 1.83 mmol), the mixture was stirred at 120 °C for approximately 5 h. After the DMF was removed under reduced pressure, the solid residue was purified by silica gel chromatography (dichloromethane:methanol, 10:1) to obtain compounds **6h**–**u**. The yield, melting point data of each compound were given below.

*6.1.5.1. 6-Methoxy-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6a**).* M.p. 198–200 °C, yield = 70%. IR (KBr) cm^{-1}: 3422, 3059, 1640, 1239, 1045. ^{1}H NMR (DMSO, 300 MHz) δ 4.14 (s, 3H, $-OCH_3$), 6.24 (s, 2H, $-NH_2$), 7.74 (t, 1H, J = 7.7 Hz, H-8), 7.90 (t, 1H, J = 7.6 Hz, H-9) 8.07 (d, 1H, J = 8.0 Hz, H-10), 8.26 (d, 1H, J = 7.7 Hz, H-7). MS m/z 216 (M+). *Anal.* Calcd. for $C_{10}H_9N_5O$: C 55.81, H 4.22, N 32.54. Found: C 55.56, H 4.48, N 32.78.

*6.1.5.2. 6-Ethoxy-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6b**).* M.p. 196–198 °C, yield = 73%. IR (KBr) cm^{-1}: 3408, 3069, 1641, 1230, 1048. ^{1}H NMR (DMSO, 300 MHz) δ 1.48 (t, 3H, J = 7.0 Hz, $-OCH_3$), 4.55(q, 2H, J = 7.0 Hz, $-OCH_2-$), 6.28 (s, 2H, $-NH_2$), 7.76 (t, 1H, J = 7.6 Hz, H-8), 7.93 (t, 1H, J = 7.4 Hz, H-9) 8.07 (d, 1H, J = 8.0 Hz, H-10), 8.25 (d, 1H, J = 7.9 Hz, H-7). ^{13}C NMR (DMSO, 300 MHz) δ 22.05, 71.25, 118.34, 121.99, 125.08, 125.51, 130.10, 134.18, 138.29, 151,17, 155,71. MS m/z 230 (M+). *Anal.* Calcd. for $C_{11}H_{11}N_5O$: C 57.63, H 4.84, N 30.55. Found: C 57.49, H 5.10, N 30.81.

*6.1.5.3. 6-Isopropoxy-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6c**).* M.p. 176–178 °C, yield = 80%. IR (KBr) cm^{-1}: 3407, 3066, 1633, 1234, 1051. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.51 (d, 6H, J = 6.1 Hz, $-CH(CH_3)_2$), 5.02 (s, 2H, $-NH_2$), 5.38–5.46 (m, 1H, $-OCH-$), 7.67 (t, 1H, J = 7.7 Hz, H-8), 7.83 (t, 1H, J = 7.6 Hz, H-9) 8.12 (d, 1H, J = 8.0 Hz, H-10), 8.45 (d, 1H, J = 7.9 Hz, H-7). ^{13}C NMR (DMSO, 300 MHz) δ 22.05, 71.26, 118.34, 121.99, 125.08, 125.51, 130.09, 134.16, 138.30, 151,17, 155,71. MS m/z 244 (M+). *Anal.* Calcd. for $C_{12}H_{13}N_5O$: C 59.25, H 5.39, N 28.79. Found: C 59.40, H 5.64, N 29.07.

*6.1.5.4. 6-Butoxy-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6d**).* M.p. 160–162 °C, yield = 78%. IR (KBr) cm^{-1}: 3414, 3073, 1644, 1240, 1047. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.05 (t, 3H, J = 7.4 Hz, $-CH_3$), 1.52–1.65 (m, 2H, $-CH_2-$), 1.87–1.96 (m, 2H, $-CH_2-$), 4.48 (t, 2H, J = 6.5 Hz, -OCH_2-), 4.91 (s, 2H, $-NH_2$), 7.68 (t, 1H, J = 7.7 Hz, H-8), 7.84 (t, 1H, J = 7.2 Hz, H-9) 8.12 (d, 1H, J = 8.0 Hz, H-10), 8.45 (d, 1H, J = 7.9 Hz, H-7). MS m/z 258 (M+). *Anal.* Calcd. for $C_{13}H_{15}N_5O$: C 60.69, H 5.88, N 27.22. Found: C 60.82, H 5.98, N 27.45.

*6.1.5.5. 6-(Hexyloxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6e**).* M.p. 130–132 °C, yield = 76%. IR (KBr) cm^{-1}: 3425, 3072, 1642, 1241, 1047. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95 (t, 3H, J = 6.5 Hz, $-CH_3$), 1.34–1.41 (m, 4H, $-(CH_2)_2-$), 1.49–1.59 (m, 2H, $-CH_2-$), 1.87–1.96 (m, 2H, $-CH_2-$), 4.46 (t, 2H, J = 6.5 Hz, $-OCH_2-$), 5.40 (s, 2H, $-NH_2$), 7.68 (t, 1H, J = 7.5 Hz, H-8), 7.83 (t, 1H, J = 7.4 Hz, H-9) 8.11 (d, 1H, J = 8.1 Hz, H-10), 8.43 (d, 1H, J = 7.8 Hz, H-7). MS m/z 286 (M+). *Anal.* Calcd. for $C_{15}H_{19}N_5O$: C 63.14, H 6.71, N 25.54. Found: C 63.40, H 6.90, N 25.20.

*6.1.5.6. 6-(Heptyloxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6f**).* M.p. 114–116 °C, yield = 72%. IR (KBr) cm^{-1}: 3412, 3062, 1634, 1240, 1052. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, 3H, J = 6.5 Hz, $-CH_3$), 1.34–1.43 (m, 6H, $-(CH_2)_3-$), 1.50–1.55 (m, 2H, $-CH_2-$), 1.89–1.98 (m, 2H, $-CH_2-$), 4.48 (t, 2H, J = 6.5 Hz, $-OCH_2-$), 5.02 (s, 2H, $-NH_2$), 7.69 (t, 1H, J = 7.5 Hz, H-8), 7.84 (t, 1H, J = 7.7 Hz, H-9) 8.13 (d, 1H, J = 8.1 Hz, H-10), 8.45 (d, 1H, J = 7.8 Hz, H-7). MS m/z 300 (M+). *Anal.* Calcd. for $C_{16}H_{21}N_5O$: C 64.19, H 7.07, N 23.39. Found: C 64.31, H 7.12, N 23.42.

*6.1.5.7. 6-(Octyloxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6g**).* M.p. 98–100 °C, yield = 70%. IR (KBr) cm^{-1}: 3416, 3064, 1651, 1246, 1056. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.90 (t, 3H, J = 6.9 Hz, $-CH_3$), 1.32–1.54 (m, 10H, $-(CH_2)_5-$), 1.88–1.95 (m, 2H, $-CH_2-$), 4.48 (t, 2H, J = 6.5 Hz, $-OCH_2-$), 5.09 (s, 2H, $-NH_2$), 7.68 (t, 1H, J = 7.7 Hz, H-8), 7.83 (t, 1H, J = 7.7 Hz, H-9) 8.12 (d, 1H, J = 7.8 Hz, H-10), 8.46 (d, 1H, J = 8.1 Hz, H-7). MS m/z 314 (M+). *Anal.* Calcd. for $C_{17}H_{23}N_5O$: C 65.15, H 7.40, N 22.35. Found: C 65.30, H 7.58, N 22.52.

*6.1.5.8. 6-(2-Chlorophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6h**).* M.p. 278–280 °C, yield = 85%. IR (KBr) cm^{-1}: 3418, 3080, 1630, 1230, 1056. ^{1}H NMR (DMSO, 300 MHz) δ 6.03 (s, 2H, $-NH_2$), 7.38–7.68 (m, 4H, Ar-H), 7.88 (t, 1H, J = 7.7 Hz, H-8), 8.03 (t, 1H, J = 7.6 Hz, H-9) 8.30 (d, 1H, J = 8.0 Hz, H-10), 8.35 (d, 1H, J = 7.9 Hz, H-7). MS m/z 312 (M+), 314 (M+3). *Anal.* Calcd. for $C_{15}H_{10}ClN_5O$: C 57.79, H 3.23, N 22.47. Found: C 57.91, H 3.34, N 22.78.

*6.1.5.9. 6-(4-Chlorophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6i**).* M.p. 204–206 °C, yield = 87%. IR (KBr) cm^{-1}: 3407, 3083, 1635 1227, 1052. ^{1}H NMR (DMSO, 300 MHz) δ 6.38 (s, 2H, $-NH_2$), 7.63 (m, 4H, Ar-H), 7.83 (t, 1H, J = 7.7 Hz, H-8), 8.00 (t, 1H, J = 7.6 Hz, H-9) 8.25 (d, 1H, J = 8.0 Hz, H-10), 8.33 (d, 1H, J = 7.9 Hz, H-7). ^{13}C NMR (DMSO, 300 MHz) δ 91.23, 115.97, 116.44, 116.98, 122.50, 123.62, 123.99, 125.76, 126.34, 130.81, 134.63, 138.06, 151.28, 158.39, 175.57. MS m/z 312 (M+), 314 (M+3). *Anal.* Calcd. for $C_{15}H_{10}ClN_5O$: C 57.79, H 3.23, N 22.47. Found: C 57.92, H 3.38, N 22.62.

*6.1.5.10. 6-(3-Chlorophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6j**).* M.p. 247–249 °C, yield = 90%. IR (KBr) cm^{-1}: 3409, 3078, 1627, 1241, 1054. ^{1}H NMR (DMSO, 300 MHz) δ 6.13 (s, 2H, $-NH_2$), 7.37–7.62 (m, 4H, Ar-H), 7.83 (t, 1H, J = 7.7 Hz, H-8), 8.01

(t, 1H, J = 7.6 Hz, H-9) 8.23 (d, 1H, J = 8.0 Hz, H-10), 8.33 (d, 1H, J = 7.9 Hz, H-7). MS m/z 312 (M+), 314 (M + 3). *Anal.* Calcd. for $C_{15}H_{10}ClN_5O$: C 57.79, H 3.23, N 22.47. Found: C 57.70, H 3.40, N 22.22.

*6.1.5.11. 6-(2,4-Dichlorophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6k**).* M.p. 232–234 °C, yield = 82%. IR (KBr) cm^{-1}: 3407, 3075, 1631, 1233, 1049. ^1H NMR (DMSO, 300 MHz) δ 6.13 (s, 2H, $-NH_2$), 7.53–7.84 (m, 3H, Ar-H), 7.86 (t, 1H, J = 7.7 Hz, H-8), 8.05 (t, 1H, J = 7.6 Hz, H-9) 8.30 (d, 1H, J = 7.9 Hz, H-10), 8.34 (d, 1H, J = 7.9 Hz, H-7). MS m/z 346 (M+), 348 (M+3). *Anal.* Calcd. for $C_{15}H_9Cl_2N_5O$: C 52.04, H 2.62, N 20.23. Found: C 52.20, H 2.78, N 20.54.

*6.1.5.12. 6-(4-Fluorophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6l**).* M.p. 238–240 °C, yield = 81%. IR (KBr) cm^{-1}: 3420, 3068, 1620, 1240, 1056. ^1H NMR (DMSO, 300 MHz) δ 6.06 (s, 2H, $-NH_2$), 7.29–7.55 (m, 4H, Ar-H), 7.84 (t, 1H, J = 7.7 Hz, H-8), 8.00 (t, 1H, J = 7.6 Hz, H-9) 8.27 (d, 1H, J = 8.0 Hz, H-10), 8.33 (d, 1H, J = 7.9 Hz, H-7). ^{13}C NMR (DMSO, 300 MHz) δ 90.87, 116.61, 116.92, 117.96, 122.17, 123.39, 123.51, 125.44, 125.74, 130.40, 134.81, 138.65, 151.10, 158.23, 179.40. MS m/z 296 (M+). *Anal.* Calcd. for $C_{15}H_{10}FN_5O$: C 61.02, H 3.41, N 23.72. Found: C 60.89, H 3.27, N 23.79.

*6.1.5.13. 6-(4-Bromophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6m**).* M.p. 218–220 °C, yield = 75%. IR (KBr) cm^{-1}: 3408, 3083, 1622, 1255, 1058. ^1H NMR (DMSO, 300 MHz) δ 6.11 (s, 2H, $-NH_2$), 7.45–7.68 (m, 4H, Ar-H), 7.83 (t, 1H, J = 7.7 Hz, H-8), 8.00 (t, 1H, J = 7.6 Hz, H-9) 8.25 (d, 1H, J = 8.0 Hz, H-10), 8.32 (d, 1H, J = 7.9 Hz, H-7). MS m/z 355 (M+). *Anal.* Calcd. for $C_{15}H_{10}BrN_5O$: C 50.58, H 2.83, N 19.66. Found: C 50.30, H 3.12, N 19.49.

*6.1.5.14. 6-(4-Methoxyphenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6n**).* M.p. 224–226 °C, yield = 82%. IR (KBr) cm^{-1}: 3398, 3078, 1635, 1254, 1046. ^1H NMR (DMSO, 300 MHz) δ 3.80 (s, 3H, $-OCH_3$), 6.00 (s, 2H, $-NH_2$), 7.00–7.41 (m, 4H, Ar-H), 7.83 (t, 1H, J = 7.3 Hz, H-8), 8.00 (t, 1H, J = 7.2 Hz, H-9) 8.27 (d, 1H, J = 7.9 Hz, H-10), 8.32 (d, 1H, J = 7.8 Hz, H-7). ^{13}C NMR (DMSO, 300 MHz) δ 69.41, 115.07, 118.09, 122.15, 122.53, 125.38, 125.77, 129.02, 130.26, 130.40, 134.36, 134.72, 138.67, 146.55, 15.06, 157.08. MS m/z 3.08 (M+). *Anal.* Calcd. for $C_{16}H_{13}N_5O_2$: C 62.53, H 4.26, N 22.79. Found: C 62.78, H 4.29, N 22.91.

*6.1.5.15. 6-(2-Methylphenoxyl)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6o**).* M.p. 265–267 °C, yield = 85%. IR (KBr) cm^{-1}: 3413, 3068, 1623, 1239, 1052. ^1H NMR (DMSO, 300 MHz) δ 2.24 (s, 3H, $-CH_3$), 5.93 (s, 2H, $-NH_2$), 7.23–7.39 (m, 4H, Ar-H), 7.85 (t, 1H, J = 7.7 Hz, H-8), 8.02 (t, 1H, J = 7.6 Hz, H-9) 8.33 (t, 2H, H-7, H-10). MS m/z 292 (M+). *Anal.* Calcd. for $C_{16}H_{13}N_5O$: C 65.97, H 4.50, N 24.04. Found: C 65.70, H 4.75, N 24.23.

*6.1.5.16. 6-(4-Methylphenoxyl)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6p**).* M.p. 166–168 °C, yield = 80%. IR (KBr) cm^{-1}: 3409, 3070, 1640, 1247, 1054. ^1H NMR (DMSO, 300 MHz) δ 2.35 (s, 3H, $-CH_3$), 6.01 (s, 2H, $-NH_2$), 7.26–7.35 (m, 4H, Ar-H), 7.82 (t, 1H, J = 7.7 Hz, H-8), 7.99 (t, 1H, J = 7.6 Hz, H-9) 8.24 (d, 1H, J = 8.0 Hz, H-10), 8.32 (d, 1H, J = 7.9 Hz, H-7). MS m/z 292 (M+). *Anal.* Calcd. for $C_{16}H_{13}N_5O$: C 65.97, H 4.50, N 24.04. Found: C 66.12, H 4.23, N 24.09.

*6.1.5.17. 6-(3-Methylphenoxyl)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6q**).* M.p. 250–252 °C, yield = 86%. IR (KBr) cm^{-1}: 3421, 3056, 1637, 1258, 1048. ^1H NMR (DMSO, 300 MHz) δ 2.36 (s, 3H, $-CH_3$), 6.06 (s, 2H, $-NH_2$), 7.10–7.39 (m, 4H, Ar-H), 7.83 (t, 1H, J = 8.1 Hz, H-8), 8.01 (t, 1H, J = 7.5 Hz, H-9) 8.22 (d, 1H, J = 8.0 Hz, H-10), 8.33 (d, 1H, J = 7.8 Hz, H-7). MS m/z 292 (M+). *Anal.* Calcd. for $C_{16}H_{13}N_5O$: C 65.97, H 4.50, N 24.04. Found: C 65.73, H 4.21, N 24.10.

*6.1.5.18. 6-(2-Aminophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6r**).* M.p. 300–302 °C, yield = 81%. IR (KBr) cm^{-1}: 3413, 3074, 1634, 1248, 1049. ^1H NMR (DMSO, 300 MHz) δ 5.13 (s, 2H, Ar-NH_2), 5.86 (s, 2H, $-NH_2$), 6.58–7.18 (m, 4H, Ar-H), 7.83 (t, 1H, J = 7.5 Hz, H-8), 7.99 (t, 1H, J = 7.5 Hz, H-9) 8.26 (d, 1H, J = 8.0 Hz, H-10), 8.32 (d, 1H, J = 7.9 Hz, H-7). MS m/z 293 (M+). *Anal.* Calcd. for $C_{15}H_{12}N_6O$: C 61.64, H 4.14, N 28.75. Found: C 61.69, H 4.01, N 28.60.

*6.1.5.19. 6-(4-Aminophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6s**).* M.p. 248–250 °C, yield = 78%. IR (KBr) cm^{-1}: 3411, 3083, 1633, 1232, 1047. ^1H NMR (DMSO, 300 MHz) δ 5.10 (s, 2H, Ar-NH_2), 5.96 (s, 2H, $-NH_2$), 6.61–7.11 (m, 4H, Ar-H), 7.82 (t, 1H, J = 7.6 Hz, H-8), 7.99 (t, 1H, J = 7.5 Hz, H-9) 8.25 (d, 1H, J = 8.0 Hz, H-10), 8.31 (d, 1H, J = 7.8 Hz, H-7). MS m/z 293 (M+). *Anal.* Calcd. for $C_{15}H_{12}N_6O$: C 61.64, H 4.14, N 28.75. Found: C 61.78, H 4.01, N 28.52.

*6.1.5.20. 6-(3-Aminophenoxy)-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6t**).* M.p. 266–268 °C, yield = 84%. IR (KBr) cm^{-1}: 3415, 3076, 1630, 1235, 1054. ^1H NMR (DMSO, 300 MHz) δ 5.31 (s, 2H, Ar-NH_2), 6.12 (s, 2H, $-NH_2$), 6.45–7.10 (m, 4H, Ar-H), 7.83 (t, 1H, J = 7.7 Hz, H-8), 8.01 (t, 1H, J = 7.6 Hz, H-9) 8.18 (d, 1H, J = 8.0 Hz, H-10), 8.34 (d, 1H, J = 7.9 Hz, H-7). MS m/z 293 (M+). *Anal.* Calcd. for $C_{15}H_{12}N_6O$: C 61.64, H 4.14, N 28.75. Found: C 61.79, H 4.32, N 28.92.

*6.1.5.21. 6-Phenoxy-[1,2,4]triazolo[3,4-a]phthalazine-3-amine (**6u**).* M.p. 212–214 °C, yield = 73%. IR (KBr) cm^{-1}: 3410, 3067, 1630, 1244, 1049. ^1H NMR (DMSO, 300 MHz) δ 6.05 (s, 2H, $-NH_2$), 7.28–7.52 (m, 5H, Ar-H), 7.84 (t, 1H, J = 7.7 Hz, H-8), 8.01 (t, 1H, J = 7.6 Hz, H-9) 8.26 (d, 1H, J = 8.1 Hz, H-10), 8.34 (d, 1H, J = 7.9 Hz, H-7). MS m/z 278 (M+). *Anal.* Calcd. for $C_{15}H_{11}N_5O$: C 64.97, H 4.00, N 25.26. Found: C 64.81, H 3.85, N 25.50.

6.2. Pharmacology

The anti-inflammatory activity was evaluated by an *in vivo* inhibition assay by monitoring xylene-induced ear edema in mice [18]. In the primary screening, all tested compounds were freshly prepared (dissolved with DMSO) prior to administered i.p. at a dose of 100 mg/kg to mice and at a concentration of 0.05 mL/20 g of mice weight. Control mice received the vehicle only (DMSO, 0.05 mL/20 g of mice weight). 0.5 h after administration i.p., animals were used in the xylene-induced ear edema test. In the latter evaluation, tested compounds (**6h**, **6s** and Ibuprofen) were homogenized with 0.5% sodium carboxymethylcellulose (CMC-Na) and administered orally to mice at a concentration of 0.2 mL/10 g mice weight. Control mice received the vehicle only (0.5% CMC-Na, 0.2 mL/10 g). At a specified later time, 20 μL xylene was applied to the surface of the right ear of each mouse by a micropipette. Mice were sacrificed 30 min later and a cylindrical plug (7 mm diameter) was excised from each of the treated and untreated ears. Edema was quantified by the difference in weight between the two plugs. The anti-inflammatory activity was expressed as percent edema reduction as compared to the CMC-Na administered control group. The NSAID drug Ibuprofen was tested in parallel as an activity reference. Edema values, expressed as mean ± standard deviation, were compared statistically using one-way-ANOVA followed by Dunnet's test. A level of $p < 0.05$ was adopted as the test of significance.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 30760290 and No. 30860340).

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2010.07.049.

References

[1] B.L. Sng, S.A. Schug, Ann. Acad. Med. Singap. 38 (2009) 960–966.
[2] (a) H.P. Zahradnik, A. Hanjalic-Beck, K. Groth, Contraception 3 (2010) 185–196; (b) R. Chou, G.J. Fanciullo, P.G. Fine, J.A. Adler, J.C. Ballantyne, P. Davies, et al., J. Pain 10 (2009) 113–130; (c) F.W. Kraemer, J.B. Rose, Anesthesiol. Clin. 2 (2009) 241–268.
[3] R. Eccles, J. Clin. Pharm. Ther. 31 (2006) 309–319.
[4] P. Rathee, H. Chaudhary, S. Rathee, D. Rathee, V. Kumar, K. Kohli, Inflamm. Allergy Drug Targets 3 (2009) 229–235.
[5] D. Mukherjee, S.E. Nissen, E.J. Topol, JAMA 286 (2001) 954–959.
[6] J.R. Van, R.M. Botting, Inflamm. Res. 44 (1995) 1–10.
[7] L.J. Cui, Z.F. Xie, H.R. Piao, G. Li, K.Y. Chai, Z.S. Quan, Biol. Pharm. Bull. 7 (2005) 1216–1220.
[8] Z.F. Xie, K.Y. Chai, H.R. Piao, K.C. Kwak, Z.S. Quan, Bioorg. Med. Chem. Lett. 21 (2005) 4803–4805.
[9] X.Y. Sun, Y.Z. Jin, F.N. Li, G. Li, K.Y. Chai, Z.S. Quan, Arch. Pharm. Res. 12 (2006) 1080–1085.
[10] Y.Z. Jin, L.P. Guan, L.M. Zhao, H.R. Piao, Z.S. Quan, Chin. J. Org. Chem. 12 (2007) 1567–1572.
[11] X.Y. Sun, C.X. Wei, K.Y. Chai, H.R. Piao, Z.S. Quan, Arch. Pharm. 341 (2008) 288–293.
[12] X.S. Cui, J. Chen, K.Y. Chai, J.S. Lee, Z.S. Quan, Med. Chem. Res. 1 (2009) 49–58.
[13] L.M. Lima, E.J. Barreiro, Curr. Med. Chem. 1 (2005) 23–49.
[14] A. Burger, Prog. Drug Res. 37 (1991) 287–371.
[15] J.S. Leslie, S. Francine, A.J. Richard, J.R. Austin, W.C. Robert, W.M. Kevin, et al., J. Med. Chem. 47 (2004) 3642–3657.
[16] X.Y. Sun, L.P. Guan, L. Zhang, C.X. Wei, H.R. Piao, Z.S. Quan, J. Braz, Chem. Soc. 20 (2009) 826–831.
[17] T. Ramalingam, M.S. Murty, Y.V.D. Nageswar, P.B.J. Sattur, Heterocyclic.Chem. 27 (1990) 981–982.
[18] G.L. Chen, W. Shan, Y.L. Wu, L.X. Ren, J.H. Dong, Z.Z. Ji, Chem. Pharm. Bull. 53 (2005) 1587–1590.
[19] R.A. Kroll, E.A. Neuwelt, Neurosurgery 42 (1998) 1083–1099.
[20] W.M. Pardridge, NeuroRx 2 (2005) 3–14.