Bioorganic & Medicinal Chemistry Letters 20 (2010) 4479–4482

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and pharmacological evaluation of 1,1,3-substituted urea derivatives as potent TNF-α production inhibitors

Hiroshi Enomoto *, Ayako Sawa, Hiroshi Suhara, Noriyoshi Yamamoto, Hiroyuki Inoue, Chikako Setoguchi, Fumio Tsuji, Masahiro Okamoto, Yoshimasa Sasabuchi, Masato Horiuchi, Masakazu Ban

Nara Research & Development Center, Santen Pharmaceutical Co., Ltd, 8916-16 Takayama, Ikoma, Nara 630-0101, Japan

ARTICLE INFO

Article history:
Received 26 April 2010
Revised 2 June 2010
Accepted 7 June 2010
Available online 10 June 2010

Keywords:
Proinflammatory cytokine
TNF-α
Anti-TNF-α agent
SA13353
Urea
2,4-Dinitrobenzenesulfonamide
Manual synthesizer

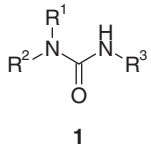
ABSTRACT

A three substituted urea derivative, **SA13353** (compound **1a**), exhibited potent inhibitory activity against lipopolysaccharide (LPS)-induced TNF-α production. We focused on the 1,1-substituted moiety (R^1 and R^2) of **SA13353** and investigated substituent effects of this moiety on LPS-induced TNF-α production by oral administration in rats. The synthesis of the urea derivatives was performed rapidly in a one-pot manner using a manual synthesizer. Several compounds containing hydrophobic substituents at this moiety showed more potent inhibitory activities than **SA13353**.

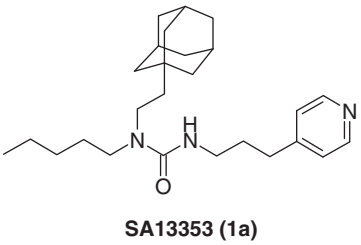
Cytokines are intercellular messengers responsible for host defense mechanisms such as inflammatory, immune and hematogenic responses. Although many of them are transient, they are produced by various cells and act as urgent response mediators in cases of invasive interventions. Disruption of this biological defense mechanism and continuous and excessive cytokine production contributes to pathogenesis of inflammatory diseases. One of the representatives of proinflammatory cytokines is tumor necrosis factor-α (TNF-α). The clinical success of TNF-α blocking biologics indicates that TNF-α plays an important role in the pathogenesis of inflammatory disease including rheumatoid arthritis, psoriasis and inflammatory bowel disease.[1] We have developed an orally active and low-molecular weight anti-TNF-α agent, the novel urea derivative **SA13353** (**1a**, Fig. 1).[2,3] This compound significantly inhibited lipopolysaccharide (LPS)-induced TNF-α production by oral administration in rats, and strongly reduced the hind-paw swelling and joint destruction associated with collagen-induced arthritis in rats.[2a] The mechanism of action of **SA13353** was initially not completely understood because this compound was originally discovered from in vivo screening using LPS-induced TNF-α production in rats. However, we recently discovered that **SA13353** is an orally active transient receptor potential vanilloid 1 (TRPV1) agonist and inhibits TNF-α production through the activation of capsaicin-sensitive afferent neurons.[2a] **SA13353** is now being evaluated in early phase II clinical trials in Japan.

SA13353 is a 1,1,3-substituted urea. In the present study, we focused on the 1,1-substituted moiety of urea (R^1 and R^2, Fig. 1) and investigated the substituent effects of this moiety to find more effective compounds than **SA13353** on LPS-induced TNF-α production by oral administration in rats.

The synthesis of **SA13353** is shown Scheme 1.[3] The tri-substituted urea, **SA13353**, was constructed from the primary amine **4** and the secondary amine **3**. 2-(1-Adamantyl)ethyl methanesulfonate (**2**) was reacted with an excess of pentylamine to give the secondary amine, 2-(1-adamantyl)-*N*-pentylethylamine (**3**). The primary amine, 3-(4-pyridyl)propylamine (**4**), was treated with 1,1′-carbonyldiimidazole to give the intermediate, 1-[3-(4-pyridyl)propylaminocarbon-



1

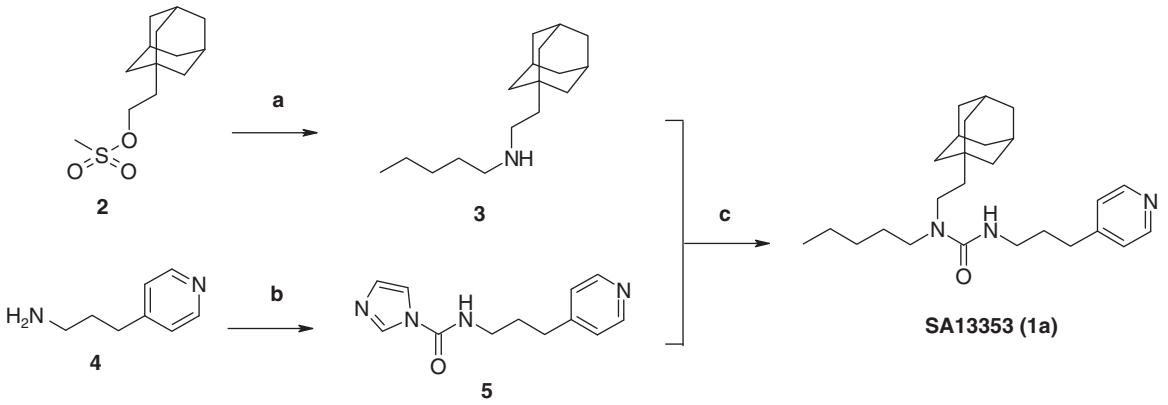


SA13353 (1a)

Figure 1. Chemical structures of **1** and **SA13353** (**1a**).

* Corresponding author. Tel.: +81 743 79 4527; fax: +81 743 79 4608.
E-mail address: hiroshi.enomoto@santen.co.jp (H. Enomoto).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.06.037



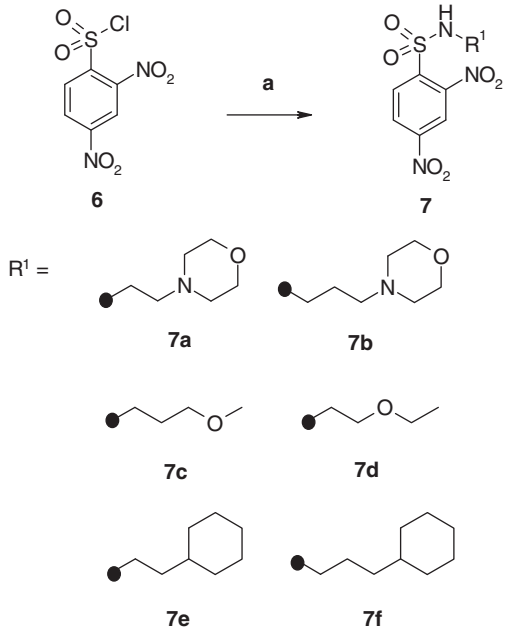
Scheme 1. Reagents and conditions: (a) pentylamine, K_2CO_3, NaI, EtOH, reflux, 17 h; (b) 1,1′-carbonyldiimidazole, THF, rt, 20 min; (c) reflux, 1 h.

yl]imidazole (**5**). The intermediate **5** without isolation was refluxed with the secondary amine **3** to provide **SA13353**. However, this synthetic route was not suitable for the efficient conversion of the 1,1-substituted moiety of urea because transformation of primary amines to the corresponding secondary amines requires excessive primary amines to avoid the formation of the undesired tertiary amines and quaternary ammonium salts.

A simple and effective method for the preparation of secondary amines was reported by Fukuyama et al.[4a,b] In this method, 2,4-dinitrobenzenesulfonamides[4b] which is prepared from primary amines and 2,4-dinitrobenzenesulfonyl chloride can be converted into *N,N*-di-substituted sulfonamides by alkylation using the Mitsunobu reaction[5] or conventional methods in excellent yield. The deprotection of *N,N*-di-substituted sulfonamides is accomplished by treatment with a variety of amines and thiol nucleophiles to give the desired secondary amines. Furthermore, treatment of 2,4-dinitrobenzenesulfonamides with either thioacids, hydroxamic acids, dithioacids or dithiocarbamic acids gives directly the corresponding amides, ureas, thioamides and thioureas, respectively.[4c,4d] As described above, synthesis of secondary amines by using 2,4-dinitrobenzenesulfonamides is applicable to preparation of not only diverse combinatorial libraries for exploring new bioactive molecules but also focused libraries in order to reveal structure–activity relationships.

We applied the synthesis of secondary amines using 2,4-dinitrobenzenesulfonamides to the replacement at the 1,1-substituted moiety (R^1 and R^2, Fig. 1) of the three substituted urea in order to elucidate the inhibitory activities on LPS-induced TNF-α production by oral administration in rats. Since **SA13353** consisted of hydrophobic substituents at R^1 and R^2, we chose amines containing hydrophilic substituents such as morpholine and alkoxy groups and a hydrophobic substituent such as the cyclohexyl group. Therefore, the corresponding 2,4-dinitrobenzenesulfonamides (**7a**–**7f**) were prepared by the reaction of six amines and 2,4-dinitrobenzenesulfonyl chloride (Scheme 2).[6]

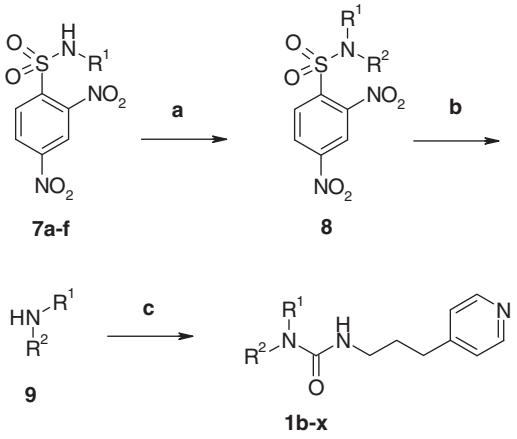
As a one-pot synthesis is an effective method to rapidly synthesize derivatives, we designed the reaction conditions of the one-pot, three step synthesis of urea derivatives containing alkylation of 2,4-dinitrobenzenesulfonamides, deprotection and urea formation by using the manual synthesizer Quest 210™ (Argonaut Technologies). Our strategy for the one-pot synthesis is described below. At the first step of the synthetic route, alkylation of the 2,4-dinitrobenzenesulfonamides is conducted under the Mitsunobu condition by using the polymer-supported triphenylphosphine because the separation of target materials and the by-product, triphenylphosphine oxide, seems to be complicated. At the second step, mercaptoacetic acid is used for the deprotection of 2,4-dinitrobenzenesulfonamides to give the secondary amines because



Scheme 2. Reagents and condition: (a) R^1NH_2, pyridine, CH_2Cl_2, −10 °C, 30 min.

the by-product, 2,4-dinitrophenylthioacetic acid, is easily removed by washing with aqueous basic solution. At the third step, the corresponding secondary amines are refluxed with 1-[3-(4-pyridyl)propylaminocarbonyl]imidazole (**5**)[7] to give ureas although the in situ synthesis of ureas has been reported.[4d] Therefore, the degrees of swelling of the polymer-supported triphenylphosphine were investigated in a few solvents in order to use the same solvent for three-step reaction.

The degrees of swelling of the resin against organic solvents were: tetrahydrofuran, chloroform > ethyl acetate ⩾ toluene. The resin was so swelled in the tetrahydrofuran and chloroform that the capacity of the reaction vessels of the Quest 210™ (10 mL) was not enough and the mixing effect seemed to be decreased. From the results of preliminary experiments, ethyl acetate was used for the reaction solvent, and the reactions were conducted under the conditions shown in Scheme 3.[8] In each vessel at Side A on a Quest 210™, a solution of sulfonamide, di-*t*-butyl azodicarboxylate (D-*t*-BAD), polymer-supported triphenylphosphine (PS-PPh_3) and alcohol (R^2-OH which was listed in Table 1) in ethyl acetate were mixed. After the reaction was complete as judged by TLC, the reaction mixture was treated with triethylamine and mercaptoacetic acid to give the secondary amine. Finally, reaction with



Scheme 3. Reagents and conditions: (a) D-*t*-BAD, PS-PPh_3, R^2OH, AcOEt, rt, overnight; (b) $HSCH_2COOH$, Et_3N, rt, 3 h; (c) compound **5**, 70 °C overnight.

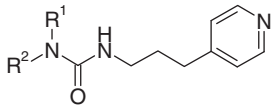
compound **5** provided the tri-substituted urea (**1b–1x**, Table 1) in 13–51% yield after standard work-up and column chromatography.

Table 1[9] outlines the tri-substituted ureas synthesized by employing the procedure as described above in Scheme 3. In the case of the morpholinoethyl group at the R^1 moiety, compounds **1b–1e** with hydrophobic groups introduced at the R^2 moiety demonstrated moderate inhibitory potency, while compounds **1f–1g** containing hydrophilic groups at the R^2 moiety reduced the potency. Compounds **1h–1k** having a morpholinopropyl group at the R^1 moiety and hydrophobic groups at the R^2 moiety showed also moderate inhibitory potency and the activities were similar to compounds **1b–1e**. In the case of the 3-methoxypropyl or 2-ethoxyethyl group at the R^1 moiety, compounds **1l–1q** showed no inhibitory activities or weak inhibitory activities in spite of containing hydrophobic groups at R^2. When the R^1 moiety was a 2-cyclohexylethyl group, the introduction of hydrophilic groups at the R^2 moiety showed weak or moderate inhibitory activities (**1s–1u**). However, introduction of a 2-cyclohexylethyl group made compound **1r** a more potent inhibitor relative to **SA13353**. Moreover, the compounds containing the 3-cyclohexylpropyl group at R^1 and hydrophobic groups at R^2 displayed an improvement in inhibitory activities (**1v–1x**). Among them, compound **1w** exhibited the most potent TNF-α inhibitory activity. As described above, hydrophobic substituents, such as a cycloalkylalkyl group at R^1 and R^2 made a significant contribution to the inhibition of TNF-α production. Structure–activity relationship (SAR) was examined further with 225 calculated molecular properties with MOE 2009.10 (Chemical Computing Group Inc., Montreal, H3A 2R7 Canada) to give significant correlations between logit-converted activity and PEOE_VSA_FPNEG ($r = 0.78$), RPC- ($r = 0.71$), Q_VSA_HYD ($r = 0.73$), SlogP_VSA8 ($r = 0.71$) or SlogP_VSA5 ($r = 0.71$). The results suggested that more the inhibitory activity was obtained with increasing hydrophobic or with decreasing polar nature of the molecules.

As shown in Figure 2, compound **1w** resulted in dose-dependent inhibition of LPS-induced TNF-α production, and the doses of 0.3, 1 and 3 mg/kg of **1w** were statistically significant compared to the vehicle. The ED_{50} value for **1w** was 0.34 mg/kg. The positive control, **SA13353** (3 mg/kg, po), significantly inhibited LPS-induced TNF-α production. Compound **1w** exhibited more potent inhibitory activity against TNF-α production than that of **SA13353** because the ED_{50} value for **SA13353** was 1.7 mg/kg.[2a]

In conclusion, further optimization of the 1,1-substituted moiety of **SA13353** was carried out. We demonstrated that the synthesis of a series of urea derivatives possessing a 3-(4-pyridyl)propyl group at the 3-substituted position (R^3, Fig. 1) of urea could be accomplished by a one-pot method using a manual synthesizer. Several com-

Table 1
Inhibitory effects of compounds **1a–1x** on LPS-induced TNF-α production in rats



Compounds	Sulfonamides	R^1	R^2	Yield (%)	Inhibition %[a]
SA13353 (**1a**)	—	2-(1-Adamantyl)ethyl	Pentyl	—	72 ± 8[b]
1b	**7a**	2-Morpholinoethyl	2-(1-Adamantyl)ethyl	45	45
1c	**7a**	2-Morpholinoethyl	2-Cyclohexylethyl	33	50
1d	**7a**	2-Morpholinoethyl	2-(Bicyclo[2.2.1]hept-2-yl)ethyl	36	55
1e	**7a**	2-Morpholinoethyl	Butyl	36	21
1f	**7a**	2-Morpholinoethyl	2-(7-Aza-bicyclo[2.2.1]hept-7-yl)ethyl	13	8
1g	**7a**	2-Morpholinoethyl	2-(4-Tetrahydropyranyl)ethyl	14	NI
1h	**7b**	3-Morpholinopropyl	2-(1-Adamantyl)ethyl	27	45
1i	**7b**	3-Morpholinopropyl	2-Cyclohexylethyl	25	43
1j	**7b**	3-Morpholinopropyl	2-(Bicyclo[2.2.1]hept-2-yl)ethyl	13	44
1k	**7b**	3-Morpholinopropyl	Butyl	17	32
1l	**7c**	3-Methoxypropyl	2-Cyclohexylethyl	13	NI
1m	**7c**	3-Methoxypropyl	2-(Bicyclo[2.2.1]hept-2-yl)ethyl	39	10
1n	**7c**	3-Methoxypropyl	Butyl	22	21
1o	**7d**	2-Ethoxyethyl	2-Cyclohexylethyl	41	4
1p	**7d**	2-Ethoxyethyl	2-(Bicyclo[2.2.1]hept-2-yl)ethyl	38	NI
1q	**7d**	2-Ethoxyethyl	Butyl	34	NI
1r	**7e**	2-Cyclohexylethyl	2-Cyclohexylethyl	18	91
1s	**7e**	2-Cyclohexylethyl	2-(2-Methoxyethoxy)ethyl	27	14
1t	**7e**	2-Cyclohexylethyl	2-(7-Aza-bicyclo[2.2.1]hept-7-yl)ethyl	51	48
1u	**7e**	2-Cyclohexylethyl	2-(4-Tetrahydropyranyl)ethyl	28	38
1v	**7f**	3-Cyclohexylpropyl	Cyclohexylmethyl	40	71
1w	**7f**	3-Cyclohexylpropyl	2-Cyclohexylethyl	46	92
1x	**7f**	3-Cyclohexylpropyl	2-Cyclopentylethyl	18	87

[a] Values are expressed as the concentration of TNF-α in the serum of the compounds administration group divided by it of the non-administration group. The compounds were administrated orally at 2 mg/kg. Values are means of five rats. (NI = not inhibit).
[b] The value is expressed as mean ± SD of 13 tests.

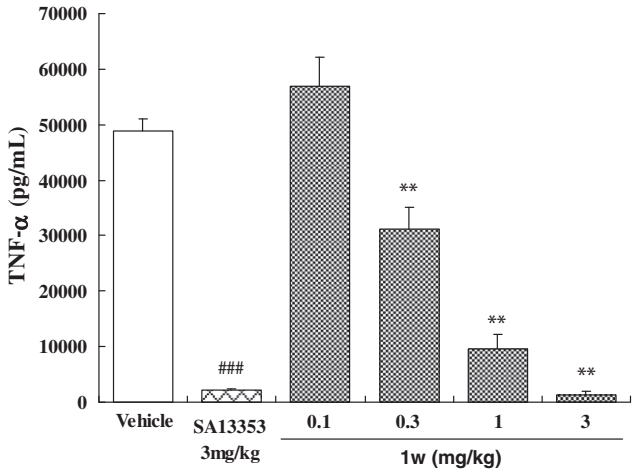


Figure 2. Effects of compound **1w** on LPS-induced TNF-α production in rats. Compound **1w** or **SA13353** was administered orally 30 min prior to LPS injection. Serum was collected 1.5 h after LPS injection. Results are expressed as the mean ± SEM of five rats per group. ### p <0.001, Student's t-test versus vehicle group. ** p <0.01, Dunnett's t-test versus vehicle group.

pounds, especially containing hydrophobic substituents such as 2-cyclohexyethyl or 3-cyclohexylpropyl groups at R^1 and R^2, showed more potent inhibitory activities against LPS-induced TNF-α production than **SA13353**. Among them, compound **1w** exhibited the most potent inhibitory activity. Based on the results described above, these novel three substituted urea derivatives may prove to be potential inhibitors for treatment of diseases mediated by TNF-α.

References and notes

1. (a) Williams, R. O.; Paleolog, E.; Feldmann, M. *Curr. Opin. Pharmacol.* **2007**, *7*, 412; (b) Bradly, Jr., Jr. *J. Pathol.* **2008**, *214*, 149.
2. (a) Murai, M.; Tsuji, F.; Nose, M.; Seki, I.; Oki, K.; Setoguchi, C.; Suhara, H.; Sasano, M.; Aono, H. *Eur. J. Pharmacol.* **2008**, *588*, 309; (b) Tsuji, F.; Murai, M.; Oki, K.; Seki, I.; Ueda, K.; Inoue, H.; Nagelkerken, L.; Sasano, M.; Aono, H. *Eur. J. Pharmacol.* **2010**, *627*, 332; (c) Ueda, K.; Tsuji, F.; Hirata, T.; Ueda, K.; Murai, M.; Aono, H.; Takaoka, M.; Matsumura, Y. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 202; (d) Tsuji, F.; Murai, M.; Oki, K.; Inoue, H.; Sasano, M.; Tanaka, H.; Inagaki, N.; Aono, H. *J. Pharmacol. Sci.* **2010**, *112*, 487.
3. Ban, M.; Suhara, H.; Horiuchi, M.; Yamamato, N.; Enomoto, H.; Inoue, H. U.S. Patent 7,098,226, 2006.
4. (a) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373; (b) Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831; (c) Messeri, T.; Sternbach, D. D.; Tomkinson, N. C. O. *Tetrahedron Lett.* **1998**, *39*, 1669; (d) Messeri, T.; Sternbach, D. D.; Tomkinson, N. C. O. *Tetrahedron Lett.* **1998**, *39*, 1673.
5. Mitsunobu, O. *Synthesis* **1981**, 1.
6. The corresponding experimental procedure to Scheme 2 is shown below. Synthesis of compound **7a**; a solution of 4-(2-aminoethyl)morpholine (5.0 ml, 38 mmol) in dichloromethane (20 ml) was slowly added to a solution of 2,4-dinitrobenzenesulfonyl chloride (10 g, 38 mmol) and pyridine (4.0 ml, 49 mmol) in dichloromethane (180 ml) dropwise at −10 °C over 15 min, and the mixture was stirred at −10 °C for 30 min. After the reaction mixture was concentrated under reduced pressure, water (300 ml) was added to the residue and the whole solution was extracted with ethyl acetate (700 ml). The organic layer was dried over anhydrous magnesium sulfate. After the solvent was evaporated under reduced pressure, the resultant solid was washed with diethylether to give the title compound (11 g) as a brown solid (yield 80 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.37 (t, J = 4.6 Hz, 4H), 2.51–2.54 (m, 2H), 3.19–3.22 (m, 2H), 3.66 (t, J = 4.6 Hz, 4H), 8.38 (d, J = 8.5 Hz, 1H), 8.56 (dd, J = 8.5, 2.2 Hz, 1H), 8.68 (d, J = 2.2 Hz, 1H); IR (KBr, cm^{-1}) 3299, 2820, 1543, 1356, 1172; MS (ESI, Pos.) 361 $[M+H]^+$, (ESI, Neg.) 359 $[M-H]^-$.
7. The experimental procedure of synthesis of compound **5** is shown below. 3-(4-Pyridyl)propylamine (**4**, Scheme 1) (5.0 g, 37 mmol) was slowly added to a solution of 1,1′-carbonyldiimidazole (8.9 g, 55 mmol) in anhydrous tetrahydrofuran (40 ml) dropwise at 0 °C over 30 min, and the mixture was stirred at room temperature for 30 min. After the reaction mixture was concentrated under reduced pressure, saturated ammonium chloride solution (40 ml) was added to the residue and the mixture was extracted with chloroform (120 ml). The organic layer was washed with brine and dried over anhydrous sodium sulfate. After the solvent was evaporated under reduced pressure, the resultant solid was washed with diethylether to give the title compound (7.4 g) as a colorless solid (yield 88 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.96–2.03 (m, 2H), 2.71 (t, J = 7.4 Hz, 2H), 3.45 (td, J = 7.4, 6.0 Hz, 2H), 7.05 (dd, J = 1.5, 1.0 Hz, 1H), 7.11–7.15 (m, 3H), 7.40 (dd, J = 1.5, 1.0 Hz, 1H), 8.15 (t, J = 1.0 Hz, 1H), 8.47 (dd, J = 4.5, 1.5 Hz, 2H); IR (KBr, cm^{-1}) 3443, 3203, 3114, 1719, 1552, 1290; MS (ESI, Pos.) 231 $[M+H]^+$, (ESI, Neg.) 229 $[M-H]^-$.
8. The corresponding experimental procedure to Scheme 3 is shown below. Synthesis of compound **1s**; In a reaction vessel at Side A on a Quest 210™, the solution mixture of *N*-(2-cyclohexylethyl)-2,4-dinitrobenzenesulfonamide (**7e**) (0.18 g, 0.5 mmol), di-*t*-butyl azodicarboxylate (0.23 g, 1.0 mmol), triphenylphosphine polystyrene (1 g/1 mmol, 100–200 mesh) (1.0 g, 1.0 mmol), diethylene glycol methyl ether (89 μl, 0.75 mmol) in ethyl acetate (4 ml) was mixed at room temperature for overnight. Then triethylamine (0.21 ml, 1.5 mmol) and mercaptoacetic acid (0.10 ml, 1.5 mmol) were added to the reaction mixture. The solution was mixed at room temperature for 3 h. 1-[3-(4-Pyridyl)propylaminocarbonyl]imidazole (**5**) (0.12 g, 0.5 mmol) was added to the mixture and mixed at 70 °C overnight. The reaction mixture was filtrated to remove the triphenylphosphine polystyrene. At the same time, the filtrate was transferred from the vessel at Side A to a vessel at Side B by using a Teflon tube, and was extracted with 2 M hydrochloric acid (3 ml). After the organic layer was removed, 4 M sodium hydroxide solution (5 ml) was added to the aqueous layer. The solution was extracted with chloroform (3 ml × 2). The solution was dried over anhydrous magnesium sulfate. After the solvent was evaporated under reduced pressure, the residue was purified by silica gel column chromatography (NH silica gel, 50% AcOEt in hexane) to give the title compound (53 mg) as a slightly brown oil (yield 27 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89–0.96 (m, 2H), 1.12–1.27 (m, 4H), 1.41–1.45 (m, 2H), 1.68–1.71 (m, 5H), 1.80–1.87 (m, 2H), 2.65 (t, J = 7.9 Hz, 2H), 3.19–3.28 (m, 4H), 3.31 (s, 3H), 3.34 (t, J = 4.8 Hz, 2H), 3.49–3.51 (m, 2H), 3.58–3.62 (m, 4H), 5.57 (s, 1H), 7.13 (dd, J = 4.5, 1.7 Hz, 2H), 8.48 (dd, J = 4.5, 1.7 Hz, 2H); IR (KBr, cm^{-1}) 3355, 2852, 1603, 1533, 1103; MS (ESI, Pos.) 392 $[M+H]^+$, (ESI, Neg.) 390 $[M-H]^-$.
9. LPS-induced TNF-α production in rats; Male normal rats (Lewis, seven weeks old) were fasted overnight and intravenously injected with lipopolysaccharide (LPS, *Escherichia coli* serotype 055:B5) dissolved in saline (100 μg/kg). The compounds were administered orally 30 min prior to LPS injection. Serum was collected 1.5 h after LPS injection since TNF-α levels were maximal at this time. The amounts of TNF-α in serum were measured by enzyme-linked immunosorbent assay (ELISA).