Original Article

Synthesis and Anti-Inflammatory Activity Evaluation of 5-(1-Benzyl-1*H*-[1,2,3]Triazol-4-yl)-4-Phenyl-4*H*-[1,2,4]Triazole-3-Thiol Derivatives

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ABSTRACT

Context: Aims: In order to search new compounds with higher anti-inflammatory activities and lower toxicity. 1,2,3-triazole and 1,2,4-triazole derivatives were designed and synthesized, and then the anti-inflammatory activities (*in vitro*) were evaluated. **Settings and Design:** The triazole derivatives were designed on the basis of association principle. **Methods and Material:** 19 benzo[*d*]-5-(1-Benzyl-1H-[1,2,3]triazol-4-yl)-4-phenyl-4*H*-[1,2,4]triazole-3-thiol derivatives were synthesized and evaluated for their bioactivities *in vitro* as anti-inflammatory agents. **Statistical analysis used:** Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a least signifcant difference (LSD) test for multiple comparisons, using SPSS version 17.0 statistical software. **Results:** The results of this study indicated that compound 17(*1-Benzyl-4-[5-(4-chloro-phenylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]*-1H-[1,2,3] triazole) showed excellent inhibition on the expression of IL-6 among these compounds in LPS-induced RAW 264.7 macrophage cell. **Conclusion:** The findings of this study showed that compound 17 showed excellent inhibition on the expression of IL-6 in LPS-induced macrophage cells. Further studies are warranted to investigate the mechanism of anti-inflammation, in order to represent a novel strategy for the modulation of inflammatory responses.

Key words: Synthesize, Anti-inflammatory, RAW 264.7 cell, IL-6.

Key Messages: 19 new 5-(1-Benzyl-1*H*-[1,2,3]triazol-4-yl)-4-phenyl-4*H*-[1,2,4] triazole-3-thiol derivatives were evaluated for their anti-inflammatory activities (*in vitro*) in mouse RAW264.7 macrophage model induced by LPS. The results were that among these compounds, comound 17 showed excellent inhibition on the expression of IL-6 in LPS-induced inflammatory macrophages, which illustrated compound 17 possibily has certain inhibitory effect on inflammation.

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INTRODUCTION

Inflammation is a defense mechanism induced in the host in response to injury or infection.[1] There are many possible causes of inflammation, but the basic pathological changes are quite similar, such as tissue and cell degeneration, partial response for micro-vascular leakage of blood components, necrosis, hyperplasia, and repair.[2,3] The inflammatory response is characterized by redness, swelling, heat, and pain.[4] These symptoms present significant issues for managing many diseases such as arthritis, cancer, and diabetes.[5] Thus, inhibition and prevention of inflammatory processes are important research goals.

Non-steroidal anti-inflammatory drugs (NSAIDs) are the main available potent synthetic drugs in the treatment of inflammatory diseases.[6] NSAIDs reduce the pain and swelling associated with arthritis by blocking the metabolism of arachidonic

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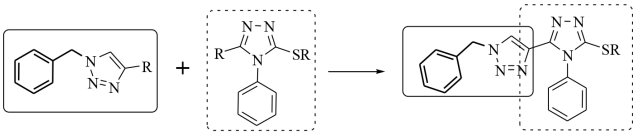
acid by cyclooxygenase enzyme (COX), thereby reduce the production of prostanoids (including prostaglandins, prostacyclins, and thromboxanes).[7] However, administration of NSAIDs in the long-term may lead to the gastrointestinal (GI) and cardiovascular (CV) events, renal toxicity, increased blood pressure, and deterioration of congestive heart failure among others.[8] Therefore, the discovery of new and safer anti-inflammatory drugs represents a challenge.[9]



It was reported that triazole compounds exhibited kinds of pharmacological abilities, such as anti-inflammatory, analgesic, antinociceptive, antiepileptic etc.[10-16] 1,2,3-Triazole and 1,2,4-triazole all possess anti-inflammatory activity.[11-13,16] In order to search new compounds with higher anti-inflammatory activities and lower toxicity, 1,2,3-triazole and 1,2,4-triazole derivatives were designed on the basis of association principle. All of these 5-(1-Benzyl-1*H*-[1,2,3]triazol-4-yl)-4-phenyl-4*H*-[1,2,4] triazole-3-thiol derivatives were synthesized, and then the anti-inflammatory activities (*in vitro*) were evaluated.

SUBJECTS AND METHODS

Chemistry

Materials and methods

Melting points (°C, uncorrected) were recorded on a Fisher-Johns apparatus. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a BRUKER-300 (Bruker Bioscience, Billerica, MA, USA) using TMS as interal standard (chemical shift in δ ppm). Solvent evaporations were performed under reduced pressure using a Buchi Rotary Evaporator. Thin layer chromatography was performed on Merch 5×10 cm plates precoated with silica gel GF254 using short wave-light (254 nm) to detect the UV absorbing. The major chemicals and reagents were purchased from (Aldrish Chemical Corporation). All other chemical were of analytical grade.

Chemistry synthesis

(Azidomethyl) benzene (24)[17]

Benzyl chloride (10 g, 0.08 mol) in DMF (40 mL), NaN_3 (5.5 g, 0.08 mol) was added and stirred at room temperature. The progress of the reaction was followed by thin layer chromatography. After 32 h, the reaction solution was poured into 200 mL deionized water. The aqueous phase was extracted with dichloromethane (CH_2Cl_2) (20 mL×3). The reservoirs were combined, then added anhydrous magnesium and sulfated for 24 h. Filtered, and methylene chloride was distilled off under reduced pressure to give 24 (85%) yield.

1-Benzyl-1H-[1,2,3]triazole-4-carboxylic acid methyl ester (23)[18]

(Azidomethyl) benzene (**24**, 9 g, 0.068 mol) in anhydrous ethanol (50 mL), methyl propiolate (5.88 g, 0.07 mol) was added and refluxed (80 °C). After 10 h, solvent was distilled off under reduced pressure to give **23** (82%) yield.

1-Benzyl-1H-[1,2,3]triazole-4-carboxylic acid hydrazide (22)[19]

1-Benzyl-1*H*-[1,2,3]triazole-4-carboxylic acid methyl ester (**23**, 12 g, 0.055 mol) in anhydrous ethanol (50 ml), hydrazine hydrate (80%, 20 mL) was added and refluxed (80°C). After 48 h, solvent was distilled off under reduced pressure, the precipitate was filtered and recrystallized from methanol and petroleum ether to give to **22** (80%) yield.

1-Benzyl-1H-[1,2,3]triazole-4-carboxylic acid N'-Phenylthioamino-hydrazide (21)

1-Benzyl-1*H*-[1,2,3]triazole-4-carboxylic acid hydrazide (**22**, 9g, 0.04mol) in EtOH (50 mL), phenyl isothiocyanate (5.6 g, 0.041 mol) was added and the reaction mixture was heated under reflux 1 h. On cooling the precipitated solid was fltered, died and recrystallized from EtOH to yield **21** (85%).

5-(1-Benzyl-1H-[1,2,3]triazol-4-yl)-4-phenyl-4H-[1,2,4] triazole-3-thiol (20)[20,21]

A mixture of thiosemicarbazide (11.9 g, 0.037 mol) in aquous sodium hydroxide solution (2M) was refluxed for 1 h. The reaction mixture was cooled, then adjusted to pH 6 with HCl (10%). The obtained solid was filtered, washed with water and recrystallized from EtOH to give **22** (90%).

Compound 1-19

5-(1-Benzyl-1*H*-[1,2,3]triazol-4-yl)-4-phenyl-4*H*-[1,2,4] triazole-3-thiol 20 (0.5g, 1.5mmol) and sodium carbonate (Na_2CO_3, 0.16 g, 1.51mmol) in absolute ethanol EtOH (15 mL), corresponding (1.51m mol) halogenated hydrocarbons was added portion wise and the reaction mixture was refluxed (80 °C) for 1-2 h. Ethanol was evaporated half, on cooling the precipitated solid was filtered, dried and recrystallized from EtOH to yield 1-19.

1-Benzyl-4-(4-phenyl-5-propylsulfanyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (1)

Yield: 56%. Mp: 162-164 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ (s, 1H, -CH), 7.53-7.50 (m, 3H, Ph-H), 7.39-7.26 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH), 3.24 (t, 2H, J = 7.5Hz, -$CH_2CH_2CH_3$), 1.84-1.72 (m, 2H, -$\underline{CH_2}CH_3$) , 1.01 (t, 3H, J = 6Hz, -CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.43, 147.70, 136.52, 133.81, 129.48, 129.21, 128.34, 127.65, 123.75, 77.05, 76.62, 54.33, 34.49, 22.69, 13.26.

1-Benzyl-4-(5-sec-butylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-1,2,3]triazole (2)

Yield: 60%. Mp: 132-133 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.52-7.47 (m, 3H, Ph-H), 7.39-7.27 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.85-3.74(m, 1H, S-CH), 1.82-1.58 (m, 2H, -$\underline{CH_2}CH_3$), 1.40 (d, 3H, J = 6.0Hz, -CH$\underline{CH_3}$), 0.97 (t, 3H, J = 7.5Hz, $CH_2\underline{CH_3}$). ^{13}C-NMR ($CDCl_3$, 75M) δ 136.57, 133.93, 133.81, 129.40, 129.21, 128.34, 127.77, 123.77, 77.04, 76.62, 54.33, 45.17, 29.68, 20.94, 11.27.

1-Benzyl-4-(5-isobutylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (3)

Yield: 49%. Mp: 162-163°C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.53-7.47 (m, 3H, Ph-H), 7.39-7.25 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.18 (d, 2H, J = 6.0Hz, S-CH_2), 2.08-1.94 (m, 1H, -$\underline{CH}(CH_3)CH_3$), 1.01 (d, 6H, J = 6.0Hz, -CH($\underline{CH_3}$)$_2$). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.68, 147.69, 136.52, 133.82, 129.48, 129.21, 128.34, 127.66, 123.76, 77.06, 76.63, 54.32, 41.03, 28.39, 21.78.

1-Benzyl-4-(5-pentylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (4)

Yield: 64%. Mp: 171-173 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.53-7.49 (m, 3H, Ph-H), 7.39-7.25 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.25 (t, 2H, J = 7.5Hz, -$CH_2CH_2CH_2CH_2CH_3$), 1.80-1.70 (m, 2H, -$CH_2CH_2CH_2CH_2CH_3$), 1.37-1.34 (m, 4H, -$CH_2CH_2CH_2CH_2CH_3$), 0.89 (t, 3H, J = 6.0Hz, -CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.47, 147.70, 136.55, 133.80, 129.47, 129.21, 128.34, 127.65, 123.72, 77.04, 76.62, 54.33, 32.58, 30.80, 28.93, 22.16, 13.19.

1-Benzyl-4-[5-(3-methyl-butylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-1H-1,2,3]triazole (5)

Yield: 71%. Mp: 163-164°C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, CH), 7.53-7.50 (m, 3H, Ph-H), 7.39-7.25 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.26 (t, 2H, J = 7.5Hz, S-CH_2), 1.77-1.58 (m, 3H, -CH_2CH-), 0.92 (d, 6H, J = 6.0Hz, CH(CH_3)$_2$). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.45, 147.70, 136.54, 133.80, 129.47, 129.21, 128.35, 127.64, 123,74, 77.05, 76.62, 54.33, 38.00, 30.75, 27.38, 22.18.

1-Benzyl-4-(5-hexylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (6)

Yield: 66%. Mp: 157-158°C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.53-7.50 (m, 3H, Ph-H), 7.39-7.25 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.25 (t, 2H, J = 7.5Hz, S-CH_2), 1.79-1.69 (m, 2H, S-CH_2CH_2), 1.30 (m, 2H, -$CH_2CH_2CH_2CH_3$), 1.29-1,28 (m, 4H, -$CH_2CH_2CH_3$), 0.88 (t, 3H, J = 6.0Hz, -CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.48, 147.69, 136.53, 133.80, 129.47, 129.21, 128.35, 127.65, 123.75, 77.05, 76.62, 54.33, 32.62, 31.25, 29.20, 28.34, 22.48, 14.00.

1-Benzyl-4-(5-cyclohexylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-1,2,3]triazole (7)

Yield: 58%. Mp: 182-183°C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.89 (s, 1H, -CH), 7.52-7.50 (m, 3H, Ph-H), 7.38-7.27 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.77 (s, 1H, S-CH), 2.12-2.09 (m, 2H, CYH-H), 1.74-1.71 (m, 2H, CYH-H), 1.63 (m, 1H, CYH-H), 1.59 (m, 4H, CYH-H), 1.52-1.24 (m, 2H, CYH-H). ^{13}C-NMR ($CDCl_3$, 75M) δ 152.74, 147.60, 136.52, 133.82, 129.41, 129.21, 128.35, 127.77, 123.85, 77.07, 76.64, 54.33, 46.54, 33.38, 25.82, 25.52.

1-Benzyl-4-(5-heptylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (8)

Yield: 56%. Mp: 162-163 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.90 (s, 1H, -CH), 7.52-7.50 (m, 3H, Ph-H), 7.38-7.26 (m, 7H, Ph-H), 5.49 (s, 2H, Ph-CH_2), 3,24 (t, 2H, J = 6.0Hz, S-CH_2), 2.04-1.98 (m, 2H, S-CH_2CH_2), 1.78-1.68 (m, 2H, S-$CH_2CH_2CH_2$), 1.39-1.26 (m, 6H, -$(CH_2)_3CH_3$), 0.87 (t, 3H, J = 6.0Hz, -CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 171.18, 153.51, 147.71, 136.47, 133.81, 133.77, 129.98, 129.21, 129.02, 128.35, 127.64, 123.80, 77.06, 76.63, 54.32, 32.62, 31.65, 29.23, 28.74, 28.63, 22.56, 20.96, 20.76, 14.06, 2.26.

1-Benzyl-4-(5-octylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (9)

Yield: 62%. Mp: 155-157 °C ^{1}H-NMR ($CDCl_3$, 300M) δ 7.89 (s, 1H, -CH), 7.53-7.49 (m, 3H, PH-H), 7.39-7.25 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.25 (t, 2H, J = 7.5Hz, S-CH_2), 1.79-1.69 (m, 2H, S-CH_2CH_2), 1.39-1.26 (s, 10H, -$(CH_2)_5CH_3$), 0.88 (t, 3H, J = 6.0Hz, -CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.51, 147.67, 136.49, 133.79, 129.97, 129.48, 129.22, 129.03, 128.35, 127.65, 123.78, 77.04,76.62, 54.33, 32.63, 31.77, 29.23, 29.12, 29.04, 28.68, 22.62, 14.09.

1-Benzyl-4-(5-nonylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (10)

Yield: 56%. Mp: 156-157 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.90 (s, 1H, -CH), 7.53-7.48 (m, 3H, Ph-H), 7.39-7.25 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.25 (t, 2H, J = 7.5Hz, S-CH_2), 1.79-1.69 (m, 2H, S-CH_2CH_2), 1.40-1.26 (s, 12H, -$(CH_2)_6CH_3$), 0.88 (t, 3H, J = 7.5Hz,

-CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.54, 147.64, 136.44, 133.77, 129.49, 129.22, 128.35, 127.64, 123.82, 77.03, 76.61, 54.34, 32.63, 31.83, 29.41, 29.23, 29.08, 28.68, 22.65, 14.11.

1-Benzyl-4-(5-decylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (11)

Yield: 66%. Mp: 149-150 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.89 (s, 1H, -CH), 7.53-7.51 (m, 3H, Ph-H), 7.39-7.28 (m, 7H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 3.25 (t, 2H, *J* = 7.5Hz, S-CH_2), 1.76-1.96 (m, 2H, S-CH_2CH_2), 1.39-1.26 (s, 14H, -$(CH_2)_7CH_3$), 0.89 (t, 3H, *J* = 7.5Hz,-CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 153.51, 147.67, 136.49, 133.79, 129.96, 129.48, 129.21, 129.02, 128.35, 127.65, 123.79, 77.04, 76.62, 54.33, 32.63, 31.88, 29.51, 29.46, 29.28, 29.24, 29.08, 28.68, 22.67, 14.12.

1-Benzyl-4-(5-benzylsulfanyl-4-phenyl-4H-[1,2,4]triazol-3-yl)-1H-[1,2,3]triazole (12)

Yield: 58 %. Mp: 161-162 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.89 (s, 1H, -CH), 7.48-7.45 (m, 3H, Ph-H), 7.39-7.16 (m, 12H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 4.47 (s, 2H, S-CH_2). ^{13}C-NMR ($CDCl_3$, 75M) δ 152.81, 147.81, 136.34, 133.76, 133.61, 129.96, 129.42, 129.23, 129.17, 129.05, 128.65,128.37, 127.75, 127.37, 123.82, 127.75, 127.61, 123.82, 77.05, 76.63, 54.35, 37.37.

1-Benzyl-4-[5-(3-methyl-benzylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-1H-[1,2,3]triazole (13)

Yield: 62%. Mp: 165-167 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.48-7.45 (m, 3H, Ph-H), 7.40-7.13 (m, 11H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 4.47 (s, 2H, S-CH_2), 2.31 (s, 3H, Ph-CH_3). ^{13}C-NMR ($CDCl_3$, 75M) δ 152.90, 147.79, 138.36, 136.46, 133.76, 133,64, 129.93, 129.88, 129.39, 123.23, 129.05, 128.53, 128.37, 127.63, 126.20, 123.77, 77.05, 76.62, 54.35, 37.38, 21.30.

1-Benzyl-4-[5-(2-fluoro-phenylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-1H-[1,2,3]triazole (14)

Yield: 66%. Mp: 155-157 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.49-7.42 (m, 3H, Ph-H), 7.39-6.99 (m, 11H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 4.51 (s, 2H, S-CH_2). ^{13}C-NMR ($CDCl_3$, 75M) δ 152.56, 147.96, 133.76, 131.46, 131.41, 129.98, 129.71, 129.61, 129.44, 129.23, 129.05, 128.36, 127.57, 124.23, 124.18, 123.76, 115.62, 115.34, 77.04, 76.62, 54.34, 30.45, 30.41.

1-Benzyl-4-[5-(3-fluoro-benzylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-1H-[1,2,3]triazole (15)

Yield: 72%. Mp: 145-147 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.51-7.47 (m, 3H, Ph-H), 7.39-7.37 (m, 3H, Ph-H), 7.28-6.96 (m, 3H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 4.45 (s, 2H, Ph-CH_2). ^{13}C-NMR ($CDCl_3$, 75M) δ 164.37, 161.10, 152.38, 147.95, 133.74, 133.54, 130.14, 130.04, 129.47, 129.23, 129.06, 128.36, 127.56, 124.86, 124.82, 123.78, 116.23, 115.94, 114.86,114.58, 77.05, 76.62, 54.35, 36.62.

1-Benzyl-4-[5-(2-chloro-benzylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-1H-[1,2,3]triazole (16)

Yield: 54%. Mp: 138-139 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.48-7.44 (m, 3H, Ph-H), 7.48-7.44 (m, 4H, Ph-H), 7.39-7.15 (m, 7H, Ph-H), 5.49 (s, 2H, Ph-CH_2), 4.59 (s, 2H, Ph-CH_2). ^{13}C-NMR ($CDCl_3$, 75M) δ 152.64, 147.98, 136.45, 134.52, 134.37, 133.76, 133.52, 131.48, 129.97,129.64, 129.43, 129.23, 129.05, 128.36, 127.58, 126.98, 123.73, 77.05, 76.63, 54.35, 34.99.

1-Benzyl-4-[5-(4-chloro-phenylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-1H-[1,2,3]triazole (17)

Yield: 56%. Mp: 197-198 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.89 (s, 1H, -CH), 7.49-7.47 (m, 3H, Ph-H), 7.40-7.18 (m, 11H, Ph-H), 5.50 (s, 2H, Ph-CH_2), 4.43 (s, 2H, Ph-CH_2). ^{13}C-NMR ($CDCl_3$, 75M) δ 136.31, 135.13, 133.72, 133.59, 133.51, 130.54, 130.05, 129.48, 129.24, 129.07, 128.76,128.37, 127.55, 123.84, 77.04, 76.62, 54.37, 36.39.

1-Benzyl-4-[5-(2,4-dichloro-benzylsulfanyl)-4-phenyl-4H-[1,2,4]triazol-3-yl]-1H-[1,2,3]Triazole (18)

Yield: 66%. Mp: 174-176 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.55-7.53 (m, 4H, Ph-H), 7.50-7.18 (m, 9H, Ph-CH_2), 5.50 (s, 2H, Ph-CH_2), 4.54 (s, 2H, Ph-CH_2). ^{13}C-NMR ($CDCl_3$, 75M) δ 136.31, 135.01, 134.33, 133.72, 133.42, 133.32, 130.06, 129.49, 129.42, 129.07, 128.36, 127.52,127.24, 123.79, 77.03, 76.61, 54.36, 34.09.

2-[5-(1-Benzyl-1H-[1,2,3]triazol-4-yl)-4-phenyl-4H-[1,2,4]triazol-3-ylsulfanylmethyl]-Benzonitrile (19)

Yield: 72%. Mp: 159-161 °C. ^{1}H-NMR ($CDCl_3$, 300M) δ 7.87 (s, 1H, -CH), 7.73-7.70 (m, 1H, Ph-H), 7.64-7.62 (m, 1H, Ph-H), 7.52-7.44 (m, 4H, Ph-H), 7.39-7.34 (m, 4H, Ph-H), 7.28-7.20 (m, 4H, Ph-H). ^{13}C-NMR ($CDCl_3$, 75M) δ 140.82, 133.00, 132.93, 130.82, 130,13, 129.55, 129.23, 129.06, 128.35, 138.21, 127.51, 117.17, 112.74, 102.61, 77.05, 76.63, 54.35, 34.86.

Pharmacology

Reagent and cell culture

Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), penicillin and streptomycin for cell culture was purchased from Invitrogen-Gibco (Grand Island, NY, USA). 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and lipopolysaccharide (LPS) (Escherichia coli 055:B5) were purchased from Sigma Chemical Co. (San Diego, CA, USA). Mouse TNF-α enzyme-linked

immunosorbent assay (ELISA) kits and Mouse IL-6 ELISA Kit were purchased from Biolegend (CA, USA). The mouse RAW264.7 macrophage cell line was obtained from the China Cell Line Bank (Beijing, China). RAW264.7 macrophages were incubated in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco®, Life Technologies, Carlsbad, CA, USA) supplemented with 10% heat-inactivated FBS (Gibco) and antibodies (100 U/ml penicillin and 100 μg/ml streptomycin), and incubated at 37°C with 5% CO_2.

In vitro study MTT assay for cytotoxicity and viability

Cell cytotoxicity and viability were determined by MTT reduction assay.

RAW264.7 cells were seeded into 96-well plates at a density of 5×10^5 cells per well in DMEM (Gibco) supplemented with 10% FBS and antibiotics (100 U/mL Penicillin and 100 μg/mL streptomycin) at 37°C in a humidified atmosphere containing 5% CO_2. All experiments were carried out 24 h after cells were seeded. Tested compounds were dissolved in DMSO and diluted with DMEM to the final concentrations. The cells were incubated with test compounds for 24 h before the MTT assay. A fresh solution of MTT (5 μg/mL) prepared in PBS was added to each well. The supernatant was removed after 4 h incubation and 100% DMSO was added to dissolve the formazan salt, and then analyzed in a multi-well-plate reader at 570 nm on a microplate reader (Thermo SCIENTIFIC, MA, USA). The cell viability in the control medium without any treatment was represented as 100% and the cell viability percentage of each well was calculated.

Measurement of cytokine production

The levels of TNF-α and IL-6 were measured using ELISA kits (Biolegend, USA) according to the manufacturer's instructions. Briefly, RAW264.7 cells (5×10^6 cells) were seeded into 96-well plates and incubated at 37°C in a humidified atmosphere containing 5% CO_2 overnight. The cells were pretreated with 30 μg/mL of compounds 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 14, 16, 17, 18, 19 or vehicle control for 1 h, then treated with LPS (1 μg/mL) for 23 h. Then, the supernatant were collected to determine the TNF-α and IL-6 levels by ELISA.

Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a least signifcant difference (LSD) test for multiple comparisons, using SPSS version 17.0 statistical software. $P<0.05$ was considered to indicate a statistically signifcant difference. All the results in this study are presented as the means ± SD.

RESULTS

Cytotoxicity and viability of all compounds at different densities

Cytotoxicity

The cytotoxic effects of all compounds were tested at the final concentration of 100 μg/mL. The macrophage cells were treated with compounds for 24 h and then measured by MTT. The preliminary screening results are shown in Figure 2A. In all 1-19 compounds, contrasting with the control group, compounds 12, 13, 16, 19 did not display cytotoxicity ($p>0.05$), compounds 14, 18 exhibited some cell toxicity ($p<0.05$), other compounds possessed significant effect on macrophage cells. The results show that alkyl-substituted compounds exhibited obvious cytotoxicity at concentration of 100 μg/mL. In these compounds phenyl-substituted, m-fluorophenyl and p-chlorophenyl have significant cytotoxicity (15, 17), o-fluorophenyl and 2,4-dichlorobenzene displayed moderate cytotoxicity (14, 18), no cytotoxicity was observed in compounds 12, 13, 16, 19.

Viability

To assess whether suitable concentration of those compounds has potential cell viability, RAW264.7 cells were treated with compounds at concentration of 30 μg/mL in the absence or presence of LPS. Twenty four h later the cell viability was measured by the MTT assay. The results showed that compounds 1, 5, 7, 9, 10 and 15 exhibited different degrees of cytotoxicity, while the others had no cell toxicity on RAW 264.7 cells at the concentration of 30 μg/mL (Figure 2B). LPS and the compounds exhibited no additive effects.

Figure 1 Cell cytotoxicity and cell viability of all compounds. (A) The cytotoxicity of compounds (100μg/mL) was tested in RAW264.7 cells. RAW264.7 macrophages were pre-treated with compounds (100μg/mL) for 1 hour and then treated with LPS (1μg/mL) for another 23 hours. MTT method was employed for cell cytotoxicity after a treatment period of 24 hours. The results were expressed as the percentage of control.*P<0.05; **P<0.01. (B) Effects of compounds on the cell viability of RAW264.7 macrophage cell. The cells were cultured with compounds (30μg/mL) in the absence or presence of 1μg/mL LPS for 24 h. Cell viability was determined by MTT analysis. Each bar represents mean ± SEM of three independent experiments. *P<0.05 vs. control, **P<0.01 vs. control; #P<0.05 vs. LPS, ##P<0.01 vs. LPS.

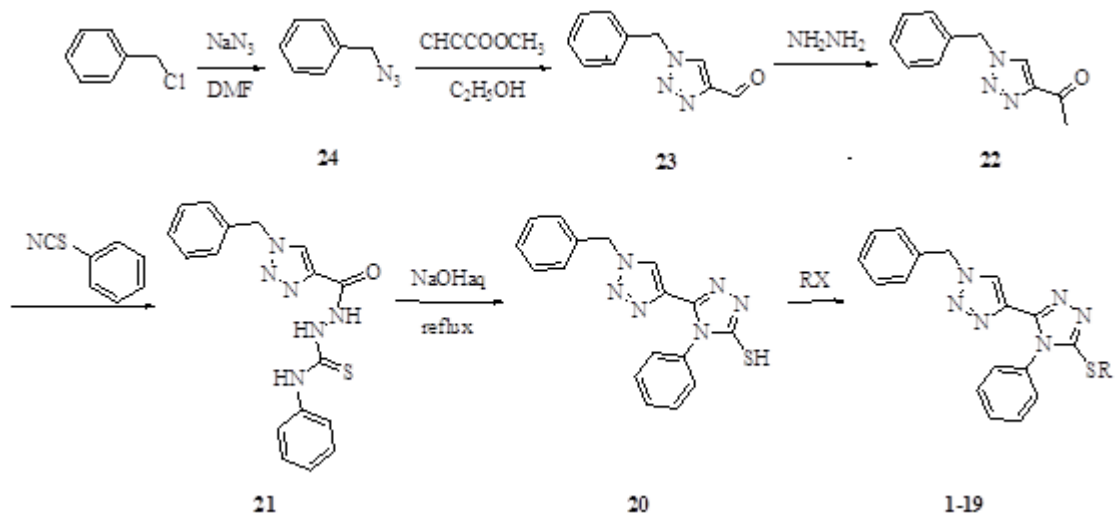


Figure 1: The synthesis route of compounds 1-19.

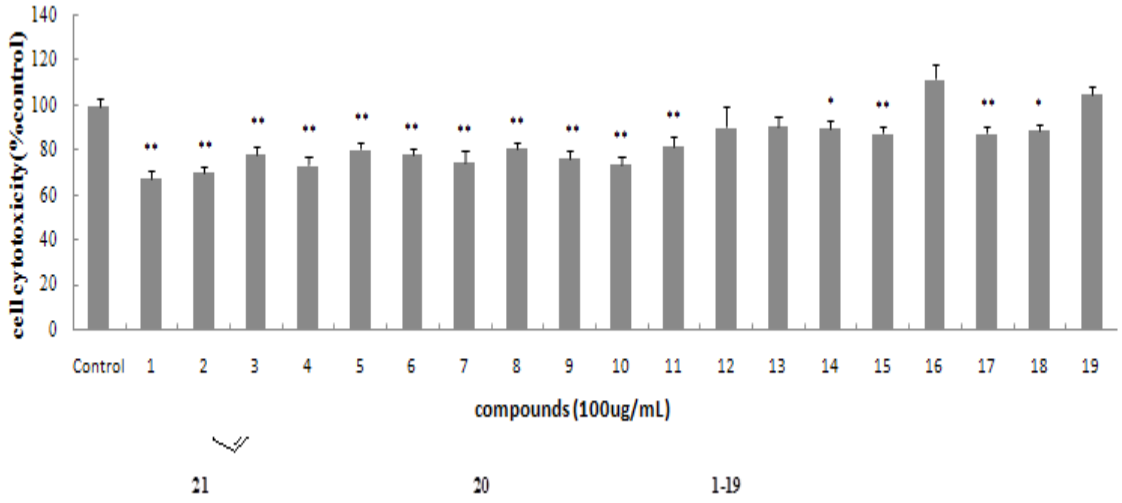


Figure 2 (A): Cytotoxicity of all compounds at the concentration of 100μg/mL.

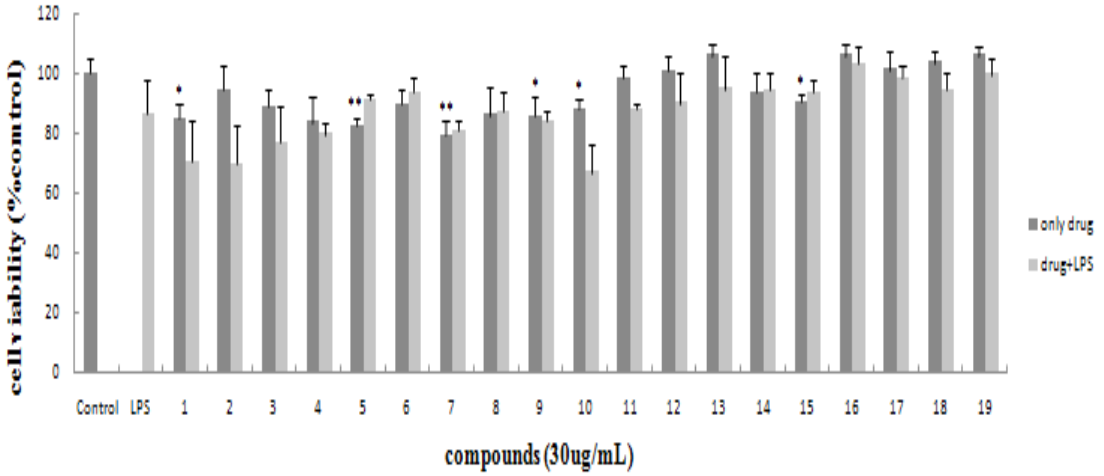


Figure 2(B): Viability of all compounds at concentration of 30μg/mL.

Cytokine-inhibitory activity of the nontoxic compounds

Sandwich ELISA was used to demonstrate the effect of the nontoxic compounds on cytokine production *in vitro*. The cultured macrophage stimulated by LPS, a major constituent of outer cell wall of Gram-negative bacteria, was used to establish the inflammatory cell model. The anti-inflammatory activity of compounds 2, 3, 4, 6, 8, 11-14, 16-19 was determined to evaluate the ability of inhibiting the TNF-α and IL-6 release stimulated by LPS in mouse RAW264.7 macrophages. The cells were pretreated with compounds (30 μg/mL) for 1 h and then treated with LPS (1 μg/mL) for 23 h. ELISA was employed to detect the cytokines in media. The result was shown in Figure 3. The levels of cytokine in the culture supernatants was markedly increased after treatment with LPS alone ($P < 0.01$). Compared with the LPS group, the production of TNF-α in all tested compounds and LPS-challenged cells were not significantly decreased ($P>0.05$) (Figure 3A). The pretreatment of compound 17 significantly decreased IL-6 expression ($P<0.01$), nevertheless compounds 6, 14, 18 increased IL-6 expression ($P<0.01$) (Figure 3B).

DISCUSSION

Chemistry

According to the literature,[21] from d to e, f to 1-19 is reacted at room temperature for 24h. The reaction was found to be very slow at room temperature in this study. But the reaction could finish at 80°C for an h or so, and the products were more pure. For the step of f⟶1-19, The basic conditions in the original literature are KOH ethanol solution. In this experiment, three alkaline substances NaOH, KOH and Na_2CO_3 were used for comparison. The results showed that the reaction time of Na_2CO_3 was the shortest and the product was most pure.

Pharmacology

The results of this study indicated that 17 showed excellent inhibition on the expression of IL-6 among these compounds in LPS-induced macrophages. The inflammatory response after challenge with LPS was associated with the release of pro-inflammatory cytokines and other inflammatory mediators, including TNF-α, IL-1, and IL-6 *et al.*[22] It was worth noting that TNF-α participates in the early development of inflammation.[23] Activated macrophage-derived pro-inflammatory cytokines acted as critical roles in inflammatory diseases.[24] IL-6 is an important cytokine released in many immunological and inflammatory responses.[25] Thus, TNF-α and IL-6 were significant targets in the treatment of inflammatory diseases to inhibit the pro-inflammatory mediator. In this study, we found that the cytokine level of TNF-α and IL-6 in supernatant was dramatically increased after LPS administration. The pretreatment of compound 17 significantly lowered IL-6 increments. This illustrated compound 17 possibly has certain inhibitory effect on inflammation.

CONCLUSION

We have presented 19 benzo[*d*]-5-(1-Benzyl-1H-[1,2,3] triazol-4-yl)-4-phenyl-4*H*-[1,2,4] triazole-3-thiol derivatives and evaluated their bioactivities *in vitro* as anti-inflammatory agents. The findings of this study showed that compound 17 showed excellent inhibition on the expression of IL-6 in LPS-induced macrophage cells. Further studies are warranted to investigate the mecha-

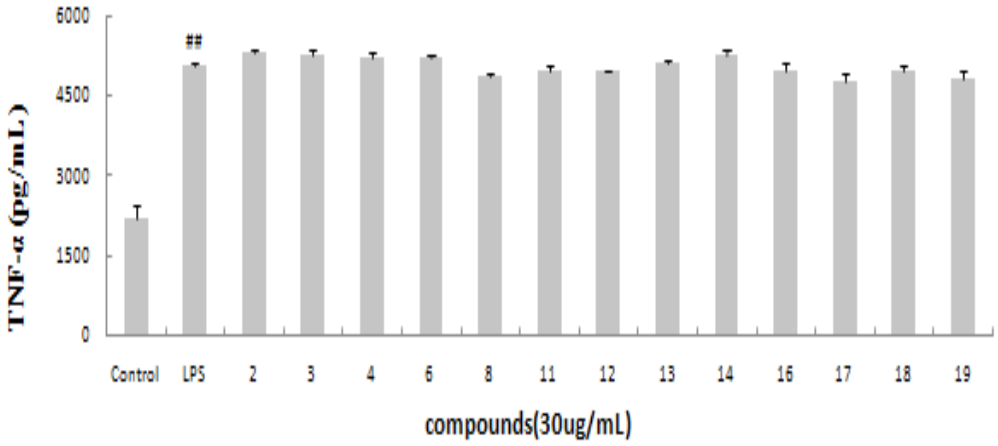


Figure 3(A): TNF-α inhibitory activity of the nontoxic compounds

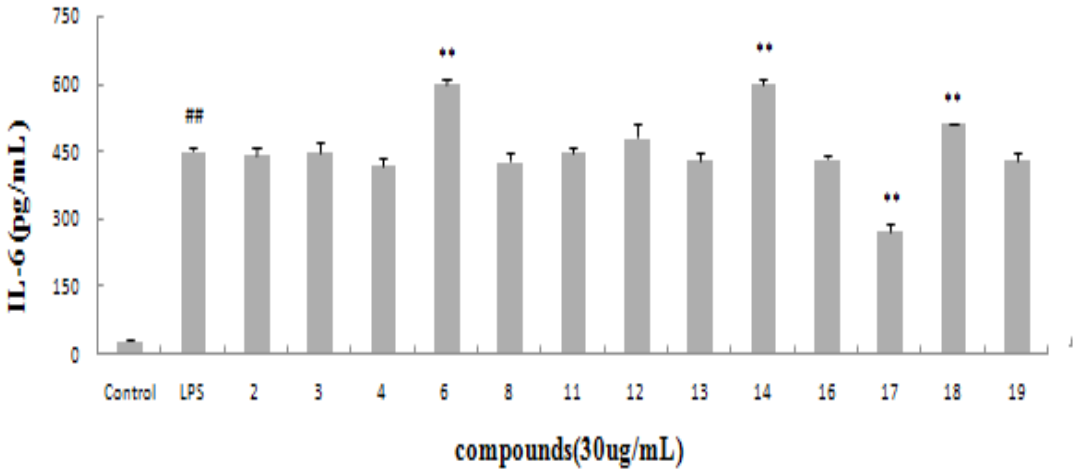


Figure 3(B): IL-6 inhibitory activity of the nontoxic compounds.

nism of anti-inflammatory, in order to represent a novel strategy for the modulation of inflammatory responses.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

NSAIDs: Non-steroidal Anti-Inflammatory Drugs; **COX:** Cyclooxygenase Enzyme; **GI:** Gastrointestinal; **CV:** Cardiovascular; **FBS:** Fetal Bovine Serum; **DMEM:** Dulbecco's Modified Eagle's Medium; **MTT:** 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide; **DMSO:** Dimethyl Sulfoxide; **LPS:** lipopolysaccharide.

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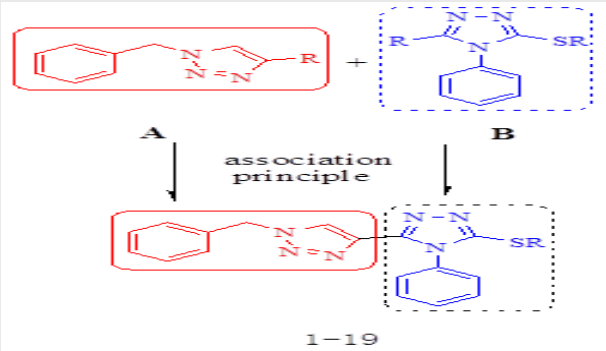
PICTORIAL ABSTRACT



In order to search new compounds with higher anti-inflammatory activities and lower toxicity, 1,2,3-triazole and 1,2,4-triazole derivatives were designed on the basis of association principle. Compound 17 (1-Benzyl-4-[5-(4-chloro-phenylsulfanyl)-4- phenyl-4H-[1,2,4]triazol-3-yl]-1H-[1,2,3] triazole) showed excellent inhibition on the expression of IL-6 among these compounds in LPS-induced RAW 264.7 macrophage cell.

Ms. CY Liu became the first master degree graduate student of Pro. CX Weiin Inner Mongolia University for the Nationalities in 2015, and she is engaged in pharmaceutical chemistry and pharmacology.

SUMMARY

19 new 5-(1-Benzyl-1H-[1,2,3]triazol-4-yl)-4-phenyl-4H-[1,2,4]triazole-3-thiol derivatives were evaluated for their anti-inflammatory activities (*in vitro*) in mouse RAW264.7 macrophage model induced by LPS. The results were that among these compounds, comound 17 showed excellent inhibition on the expression of IL-6 in LPS-induced inflammatory macrophages, which illustrated compound 17 possibly has certain inhibitory effect on inflammation.

About Authors

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