

Synthesis and Biological Evaluation of Novel 5-Benzylidenethiazolidine-2, 4-dione Derivatives for the Treatment of Inflammatory Diseases

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1: Synthesis and ^1H NMR, MS analysis of 1A-V and 2A-V;

Chemical reagents of analytical grade were purchased from Chengdu Changzheng Chemical Factory (Sichuan, P. R. China). The final compounds were synthesized using an EYELA Personal Organic Synthesizer with ChemiStation PPS-CTRL and PPW-20A (Tokyo, Rikakikai) using a 5-well liquid-phase reaction block. TLC was performed on 0.20 mm silical gel 60 F₂₅₄ plates (Qingdao Ocean Chemical Factory, Shangdong, China). Hydrogen Nuclear magnetic resonance spectra (^1H NMR) were recorded at 400 MHz on a Varian spectrometer (Varian, Palo Alto, CA, USA) model Gemini 400 and reported in parts per million. Chemical shifts (δ) are quoted in ppm relative to tetramethylsilane (TMS) as an internal standard, where (δ) TMS = 0.00 ppm. The multiplicity of the signal is indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, defined as all multipeak signals where overlap or complex coupling of signals makes definitive descriptions of peaks difficult. Mass Spectra (MS) were measured by Q-TOF Premier mass spectrometer utilizing electrospray ionization (ESI) (Micromass, Manchester, UK). Room temperature (RT) is within the range 20-25 °C.

General procedure for synthesis of 2-chloro-N-substituted-acetamide (1A-V).

2-Chloroacetyl chloride (24 mmol) was slowly added dropwise to a mixture of R-NH₂ (20 mmol) and Et₃N (24 mmol, 3.3 mL) in anhydrous CH₂Cl₂ (20 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for an additional 20 hours. After the solvent was removed under reduced pressure, the residue was washed

with ice water (3×20 mL) and the precipitate was separated by filtration. The crude product was purified by crystallization from a mixture solvent of Et₂O / petroleum.

2-chloro-N-phenylacetamide (1A).

Yield: 92 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.23 (s, 1H), 7.56 - 7.54 (m, 2H), 7.39 - 7.34 (m, 2H), 7.20 - 7.16 (m, 1H), 4.20 (s, 2H); MS (ESI), m/z: 170.01 [M + H]⁺.

2-chloro-N-p-tolylacetamide (1B).

Yield: 99.3 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.19 (s, 1H), 7.42 (d, 2H, *J* = 8.4 Hz), 7.16 (d, 2H, *J* = 8.0 Hz), 4.18 (s, 2H), 2.33 (s, 3H); MS (ESI), m/z: 182.04 [M + H]⁻.

2-chloro-N-(4-methoxyphenyl) acetamide (1C).

Yield: 99.4 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.15 (s, 1H), 7.46 - 7.44 (m, 2H), 6.92 - 6.89 (m, 2H), 4.19 (s, 2H), 3.81 (s, 3H); MS (ESI), m/z: 197.78 [M + H]⁻.

2-chloro-N-(3,4-dimethoxyphenyl) acetamide (1D).

Yield: 85.0 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.13 (s, 1H), 7.26 (m, 1H, *J* = 8.4 Hz), 6.95 - 6.92 (m, 1H), 6.81 (d, 1H, *J* = 8.4 Hz), 4.16 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H); MS (ESI), m/z: 227.99 [M + H]⁻.

2-chloro-N-(4-(trifluoromethyl)phenyl) acetamide (1E).

Yield: 88.5 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.37 (s, 1H), 7.74 - 7.33 (m, 4H), 4.27 (s, 2H); MS (ESI), m/z: 236.10 [M + H]⁻.

N-(4-acetylphenyl)-2-chloroacetamide (1F).

Yield: 97.9 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 8.40 (s, 1H), 7.98 (d, 2H, J = 8.8 Hz), 7.68 (d, 2H, J = 8.8 Hz), 4.22 (s, 2H), 2.59 (s, 3H); MS (ESI), m/z: 210.10 [M + H]⁺.

2-chloro-N-(3-fluorophenyl) acetamide (1G).

Yield: 90.2 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 8.27 (s, 1H), 7.52 (d, 1H, J = 10.6 Hz), 7.31 (q, 1H, J = 8.0 Hz), 7.19 (d, 1H, J = 8.4 Hz), 6.90 - 6.86 (m, 1H), 4.19 (s, 2H); MS (ESI), m/z: 186.03 [M + H]⁺.

2-chloro-N-(4-fluorophenyl) acetamide (1H).

Yield: 86.7 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 8.24 (s, 1H), 7.540 - 7.12 (m, 4H), 4.23 (s, 2H); MS (ESI), m/z: 186.13 [M + H]⁺.

2-chloro-N-(3-chlorophenyl)acetamide (1I). Yield: 100 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 8.29 (s, 1H), 7.67 (s, 1H), 7.40 (d, 1H, J = 8.0 Hz), 7.27 (t, 1H, J = 8.0 Hz), 7.15 (d, 1H, J = 8.0 Hz), 4.19 (s, 2H); MS (ESI), m/z: 202.05 [M + H]⁺.

2-chloro-N-(4-chlorophenyl)acetamide (1J).

Yield: 91.6 %; light yellow solid; ^1H NMR (400 MHz, CDCl_3): δ 8.31 (s, 1H), 7.98 - 7.24 (m, 4H), 4.17 (s, 2H); MS (ESI), m/z: 202.02 [M + H]⁺.

N-(4-bromophenyl)-2-chloroacetamide (1K).

Yield: 88.5 %; grey solid; ^1H NMR (400 MHz, CDCl_3): δ 8.21 (s, 1H), 7.50 - 7.24 (m, 4H), 4.17 (s, 2H); MS (ESI), m/z: 245.97 [M + H]⁺.

2-chloro-N-(2, 4-dichlorophenyl)acetamide (1L).

Yield: 99.4 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 8.89 (s, 1H), 8.35 (d, 1H, J = 8.8 Hz), 7.42 (d, 1H, J = 2.4 Hz), 7.30 - 7.26 (m, 1H), 4.23 (s, 2H); MS (ESI), m/z:

236.02 [M + H]⁺.

2-chloro-N-(3, 5-dichlorophenyl)acetamide (1M).

Yield: 100 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.72 (s, 1H), 7.74 - 7.71 (m, 3H), 4.24 (s, 2H); MS (ESI), m/z: 236.02 [M + H]⁺.

2-chloro-N-(3-chloro-4-fluorophenyl)acetamide (1N).

Yield: 99.3 % ; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.22 (s, 1H), 7.75 - 7.73 (m, 1H), 7.39 - 7.37 (m, 1H), 7.13 (t, 1H, *J* = 8.4 Hz), 4.20 (s, 2H); MS (ESI), m/z: 219.85 [M + H]⁺.

2-chloro-N-(3, 4-difluorophenyl)acetamide (1O).

Yield: 94.1%; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.24 (s, 1H), 8.34 - 8.32 (m, 2H), 7.68 - 7.62 (m, 1H), 7.18 - 7.10 (m, 1H), 4.205 (s, 2H); MS (ESI), m/z: 203.93 [M + H]⁺.

2-chloro-N-methyl-N-phenylacetamide (1P).

Yield: 92.6 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 7.47 - 7.43 (m, 2H), 7.41 - 7.37 (m, 1H), 7.24 (d, 2H, *J* = 7.6 Hz), 3.84 (s, 2H), 3.31 (s, 3H); MS (ESI), m/z: 182.09 [M + H]⁺.

2-chloro-N-cyclohexylacetamide (1Q).

Yield: 87.8 %; light yellow solid; ¹H NMR (400 MHz, CDCl₃): δ 6.44 (s, 1H), 4.03 (s, 2H), 3.83 - 3.76 (m, 1H), 1.95 - 1.91 (m, 2H), 1.76 - 1.71 (m, 2H), 1.66 - 1.61 (m, 1H); MS (ESI), m/z: 198.13 [M + H]⁺.

N-benzyl-2-chloroacetamide (1R).

Yield: 96.7 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.72 (s, 1H), 7.32 - 7.28

(m, 2H), 7.25 - 7.23 (m, 3H), 4.18 (s, 2H); MS (ESI), m/z: 206.06 [M + H]⁺.

2-chloro-N-(pyridin-2-yl) acetamide (1S).

Yield: 89.3 %; yellow solid; ¹H NMR (400 MHz, CDCl₃): δ 8.94 (s, 1H), 8.34 - 8.32 (m, 2H), 8.21 (d, 1H, *J* = 4.4 Hz), 7.78 - 7.73 (m, 1H), 7.13 - 7.10 (m, 1H), 4.21 (s, 2H); MS (ESI), m/z: 314.07 [M + H]⁻.

2-chloro-N-(5-methylpyridin-2-yl) acetamide (1T).

Yield: 87.4 %; yellow solid; ¹H NMR (400 MHz, CDCl₃): δ 9.00 (s, 1H), 8.13 - 8.10 (m, 2H), 7.59 - 7.56 (dd, 1H, *J* = 8.4 Hz), 4.20 (m, 2H), 2.33 (s, 3H); MS (ESI), m/z: 184.98 [M + H]⁺.

2-chloro-N-(N.d.phthalen-1-yl) acetamide (1U).

Yield: 94.3 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.77 (s, 1H), 7.99 (d, 1H, *J* = 7.6 Hz), 7.88 (t, 2H, *J* = 9.6 Hz), 7.76 (d, 1H, *J* = 8.4 Hz), 7.60 - 7.49 (m, 3H), 4.35 (s, 2H); MS (ESI), m/z: 218.03 [M + H]⁻.

2-chloro-N-(N.d.phthalen-2-yl) acetamide (1V).

Yield: 90.4 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 8.40 (s, 1H), 8.22 (s, 1H), 7.84 - 7.79 (m, 3H), 7.52 - 7.42 (m, 3H), 4.25 (s, 2H); MS (ESI), m/z: 217.99 [M + H]⁻.

General procedure for synthesis of 2-(4-formylphenoxy)-N-substituted-phenyl-acetamide and special aldehyde intermediates (2A-V).

4-hydroxybenzaldehyde (1.34 g, 11 mmol), anhydrous K₂CO₃ (2.76 g, 20 mmol) and the 2-chloro-N-substituted-acetamide (10 mmol) were dissolved in anhydrous acetone (30 ml), and then KI (166 mg, 1 mmol) were added into the solution. The reaction

mixture was refluxed for 24 hours and then cooled to room temperature. Then the K_2CO_3 solid was filtered and the acetone solution was removed under reduced pressure to obtain the crude products. The residue was purified by silica gel column chromatography (eluent: ethyl acetate/petroleum=1/1.5) to give the appropriate aldehyde product.

2-(4-formylphenoxy)-N-phenylacetamide (2A).

Yield: 88.6%; light yellow solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.17 (s, 1H), 7.94 – 7.90 (m, 2H), 7.59 (d, 2H, J = 7.6 Hz), 7.39 – 7.35 (m, 2H), 7.20 – 7.16 (m, 1H), 7.15 – 7.12 (m, 2H), 4.71 (s, 2H); MS (ESI), m/z: 254.09 [M + H]⁺.

2-(4-formylphenoxy)-N-p-tolylacetamide (2B).

Yield: 85.3 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.12 (s, 1H), 7.92 – 7.90 (m, 2H), 7.46 (d, 2H, J = 8.4 Hz), 7.18 – 7.11 (m, 4H), 4.69 (s, 2H), 2.38 (s, 3H); MS (ESI), m/z: 268.14 [M + H]⁺.

2-(4-formylphenoxy)-N-(4-methoxyphenyl) acetamide (2C).

Yield: 84.6 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.09 (s, 1H), 7.93 – 7.90 (m, 2H), 7.50 – 7.46 (m, 2H), 7.14 – 7.11 (m, 2H), 6.92 – 6.88 (m, 2H), 4.70 (s, 2H), 3.81 (s, 3H); MS (ESI), m/z: 284.12 [M + H]⁺.

N-(3, 4-dimethoxyphenyl)-2-(4-formylphenoxy) acetamide (2D).

Yield: 91.0 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.13 (s, 1H), 7.93 – 7.90 (m, 2H), 7.35 – 7.34 (m, 1H), 7.15 – 7.11 (m, 2H), 7.00 – 6.97 (m, 1H), 6.85 – 6.82 (m, 1H), 4.69 (s, 2H), 3.87 (d, 6H, J = 7.6 Hz); MS (ESI), m/z: 328.11 [M + H]⁺.

2-(4-formylphenoxy)-N-(4-(trifluoromethyl)phenyl) acetamide (2E).

Yield: 87.3 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.34 (s, 1H), 7.92 (d, 2H, J = 8.8 Hz), 7.75 – 7.62 (m, 4H), 7.14 (d, 2H, J = 8.8 Hz), 4.72 (s, 2H); ES (ESI), m/z: 322.11 [M + H] $^+$.

N-(4-acetylphenyl)-2-(4-formylphenoxy) acetamide (2F).

Yield: 84.9 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.95 (s, 1H), 8.40 (s, 1H), 7.99 (d, 2H, J = 8.8 Hz), 7.93 (d, 2H, J = 8.8 Hz), 7.72 (d, 2H, J = 8.8 Hz), 7.14 (d, 2H, J = 8.4 Hz), 4.73 (s, 2H), 2.60 (s, 3H); MS (ESI), m/z: 296.13 [M + H] $^+$.

N-(3-fluorophenyl)-2-(4-formylphenoxy) acetamide (2G).

Yield: 85.3 %; white solid ; ^1H NMR (400 MHz, CDCl_3): δ 9.95 (s, 1H), 8.23 (s, 1H), 7.94 – 7.90 (m, 2H), 7.59 – 7.55 (dt, 1H, J = 10.4 Hz), 7.35 – 7.29 (m, 1H), 7.25 – 7.22 (m, 1H), 7.15 – 7.11 (m, 1H), 6.91 – 6.86 (m, 1H), 4.71 (s, 2H); MS (ESI), m/z: 272.10 [M + H] $^+$.

N-(4-fluorophenyl)-2-(4-formylphenoxy) acetamide (2H).

Yield: 81.4 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.18 (s, 1H), 7.92 (d, 2H, J = 8.4 Hz), 7.57 – 7.54 (m, 2H), 7.13 (d, 2H, J = 8.4 Hz), 7.07 (t, 2H, J = 8.4 Hz), 4.71 (s, 2H); MS (ESI), m/z: 272.05 [M + H] $^+$.

N-(3-chlorophenyl)-2-(4-formylphenoxy)acetamide (2I). Yield: 75.0 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.19 (s, 1H), 7.94 – 7.91 (m, 2H), 7.71 (t, 1H, J = 2.0 Hz), 7.46 – 7.44 (m, 1H), 7.31 – 7.27 (m, 1H), 7.17 – 7.11 (m, 3H), 4.70 (s, 2H); MS (ESI), m/z: 288.17 [M + H] $^+$.

N-(4-chlorophenyl)-2-(4-formylphenoxy) acetamide (2J).

Yield: 99.0 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.22 (s, 1H), 7.94 – 7.90 (m, 2H), 7.57 – 7.54 (m, 2H), 7.35 – 7.32 (m, 2H), 7.13 (d, 2H, J = 8.8 Hz), 4.70 (s, 2H); MS (ESI), m/z: 288.11 [M + H] $^+$.

N-(4-bromophenyl)-2-(4-formylphenoxy) acetamide (2K).

Yield: 91.0 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.20 (s, 1H), 7.92 (d, 2H, J = 8.8 Hz), 7.52 – 7.47 (m, 4H), 7.12 (d, 2H, J = 8.8 Hz), 4.70 (s, 2H); MS (ESI), m/z: 332.02 [M + H] $^+$.

N-(2, 4-dichlorophenyl)-2-(4-formylphenoxy) acetamide (2L).

Yield: 82.1 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.95 (s, 1H), 8.20 (s, 1H), 7.94 (d, 2H, J = 8.4 Hz), 7.61 (d, 2H, J = 8.8 Hz), 7.12 – 7.11 (m, 3H), 4.71 (s, 2H); MS (ESI), m/z: 322.07 [M + H] $^+$.

N-(3, 5-dichlorophenyl)-2-(4-formylphenoxy) acetamide (2M).

Yield: 79.3 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.95 (s, 1H), 8.22 (s, 1H), 7.93 (d, 2H, J = 8.8 Hz), 7.59 (d, 2H, J = 2.0 Hz), 7.14 (t, 1H, J = 3.2 Hz), 7.13 (d, 2H, J = 8.8 Hz), 4.71 (s, 2H); MS (ESI), m/z: 322.03 [M + H] $^+$.

N-(3-chloro-4-fluorophenyl)-2-(4-formylphenoxy) acetamide (2N)

Yield: 69.3 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.95 (s, 1H), 8.19 (s, 1H), 7.93 – 7.90 (m, 2H), 7.79 – 7.77 (m, 1H), 7.45 – 7.41 (m, 1H), 7.16 – 7.11 (m, 3H), 4.70 (s, 2H); MS (ESI), m/z: 306.09 [M + H] $^+$.

N-(3, 4-difluorophenyl)-2-(4-formylphenoxy) acetamide (2O).

Yield: 79.6 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H), 8.20 (s, 1H), 7.94 – 7.90 (m, 2H), 7.72 – 7.67 (m, 1H), 7.20 – 7.15 (m, 2H), 7.13 – 7.10 (m, 2H),

4.70 (s, 2H); MS (ESI), m/z: 290.14 [M + H]⁺.

2-(4-formylphenoxy)-N-methyl-N-phenylacetamide (2P).

Yield: 82.6 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 9.87 (s, 1H); 7.79 (d, 2H, *J* = 8.4 Hz), 7.50 – 7.40 (m, 3H), 7.27 (t, 2H, *J* = 3.6 Hz), 6.88 (d, 2H, *J* = 8.8 Hz), 4.50 (s, 2H), 3.34 (s, 3H); MS (ESI), m/z: 292.07 [M + H]⁺.

N-cyclohexyl-2-(4-formylphenoxy) acetamide (2Q).

Yield: 80.3 %; light yellow solid; ¹H NMR (400 MHz, CDCl₃): δ 9.92 (s, 1H), 7.90 – 7.87 (m, 2H), 7.07 – 7.04 (m, 2H), 6.34 (d, 1H, *J* = 7.2 Hz), 4.55 (s, 2H), 3.92 – 3.84 (m, 1H), 1.95 – 1.91 (m, 2H), 1.75 – 1.71 (m, 2H), 1.70 – 1.61 (m, 1H), 1.45 – 1.34 (m, 2H), 1.24 – 1.13 (m, 3H); MS (ESI), m/z: 260.2 [M + H]⁺.

N-benzyl-2-(4-formylphenoxy) acetamide (2R).

Yield: 90.2 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 9.91 (s, 1H), 7.88 – 7.85 (m, 2H), 7.37 – 7.26 (m, 4H), 7.03 (d, 2H, *J* = 8.8 Hz), 6.84 (s, 1H), 4.63 (s, 2H), 4.56 (d, 2H, *J* = 6.0 Hz); MS (ESI), m/z: 268.28 [M + H]⁺.

2-(4-formylphenoxy)-N-(pyridine-2-yl) acetamide (2S).

Yield: 89.6%; white solid; ¹H NMR (400 MHz, CDCl₃): δ 9.94 (s, 1H), 8.92 (s, 1H), 8.33 (dd, 1H, *J* = 8.8 Hz), 8.30 (d, 1H, *J* = 8.4 Hz), 7.93 – 7.90 (m, 2H), 7.80 – 7.75 (m, 1H), 7.15 – 7.11 (m, 3H), 4.73 (s, 2H); MS (ESI), m/z: 257.06 [M + H]⁺.

2-(4-formylphenoxy)-N-(5-methylpyridin-2-yl) acetamide (2T).

Yield: 88.9 %; white solid; ¹H NMR (400 MHz, CDCl₃): δ 9.93 (s, 1H), 8.97 (s, 1H), 8.20 (d, 1H, *J* = 8.8 Hz), 8.14 (s, 1H), 7.92 – 7.89 (m, 1H), 7.60 (dd, 1H, *J* = 8.4 Hz), 7.16 – 7.13 (m, 2H), 4.72 (s, 2H), 2.34 (s, 3H); MS (ESI), m/z: 269.05 [M + H]⁺.

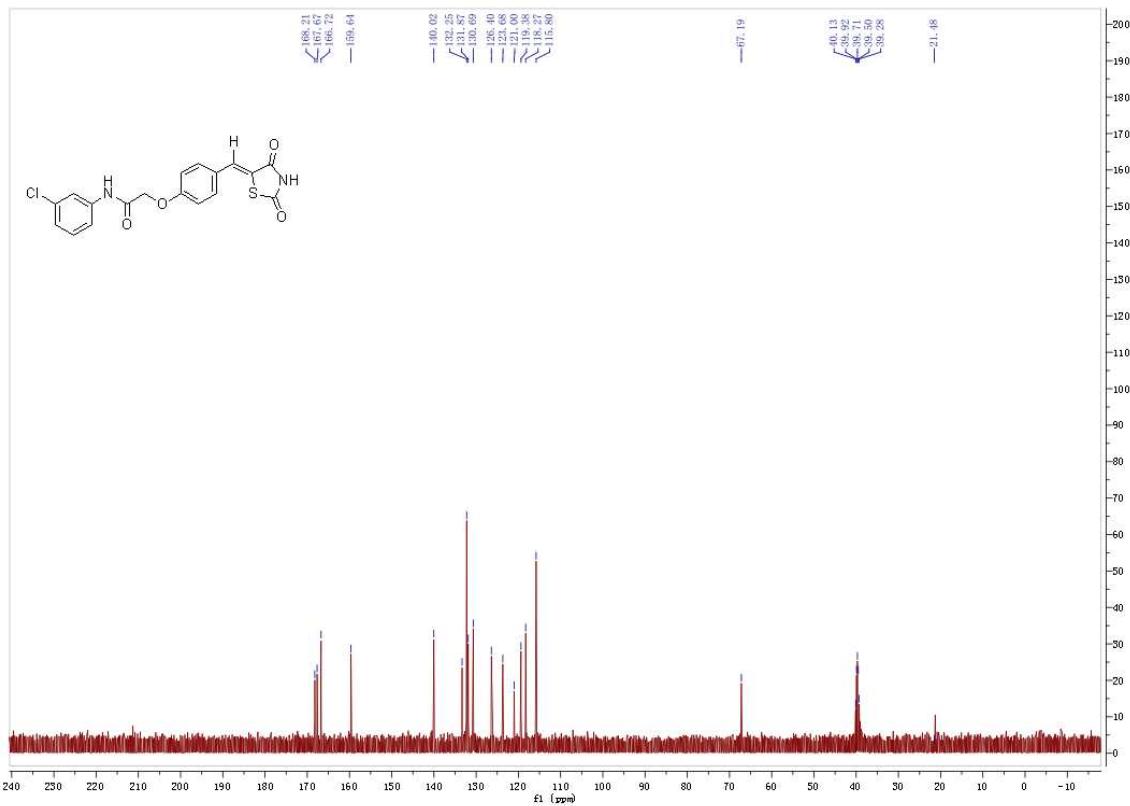
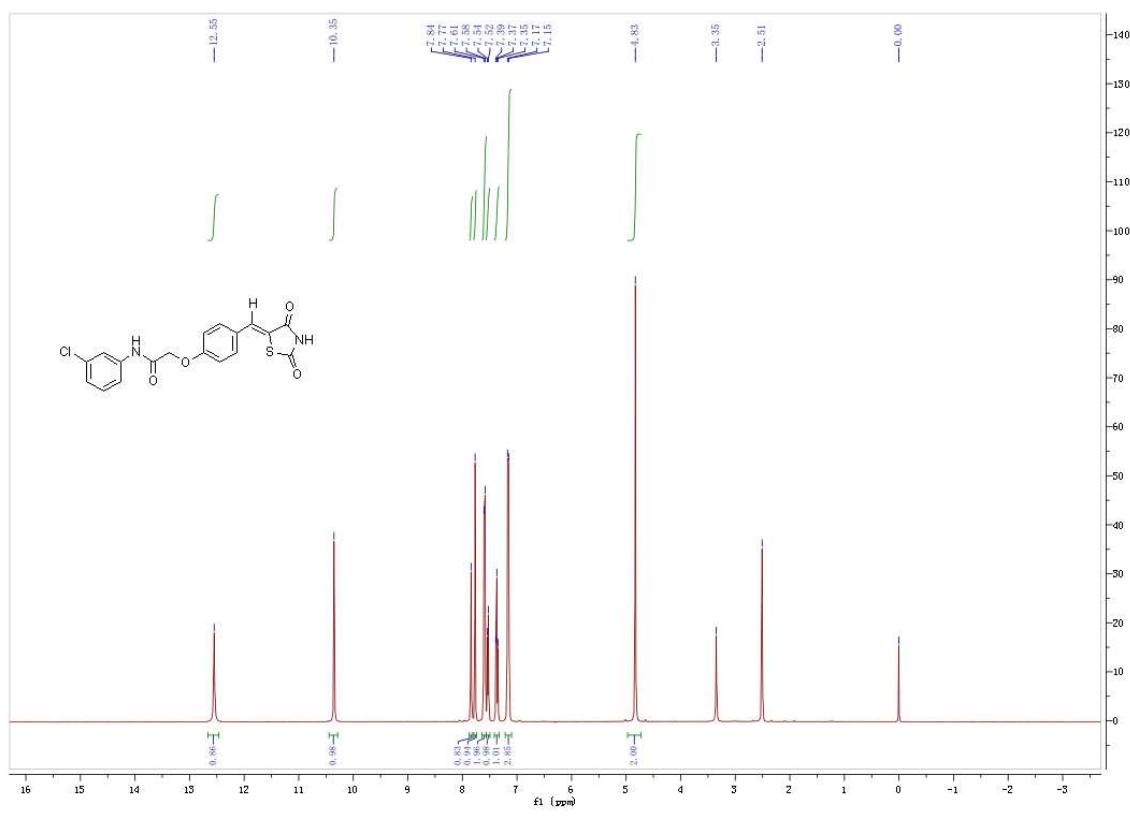
2-(4-formylphenoxy)-N-(N.d.phthalen-1-yl) acetamide (2U).

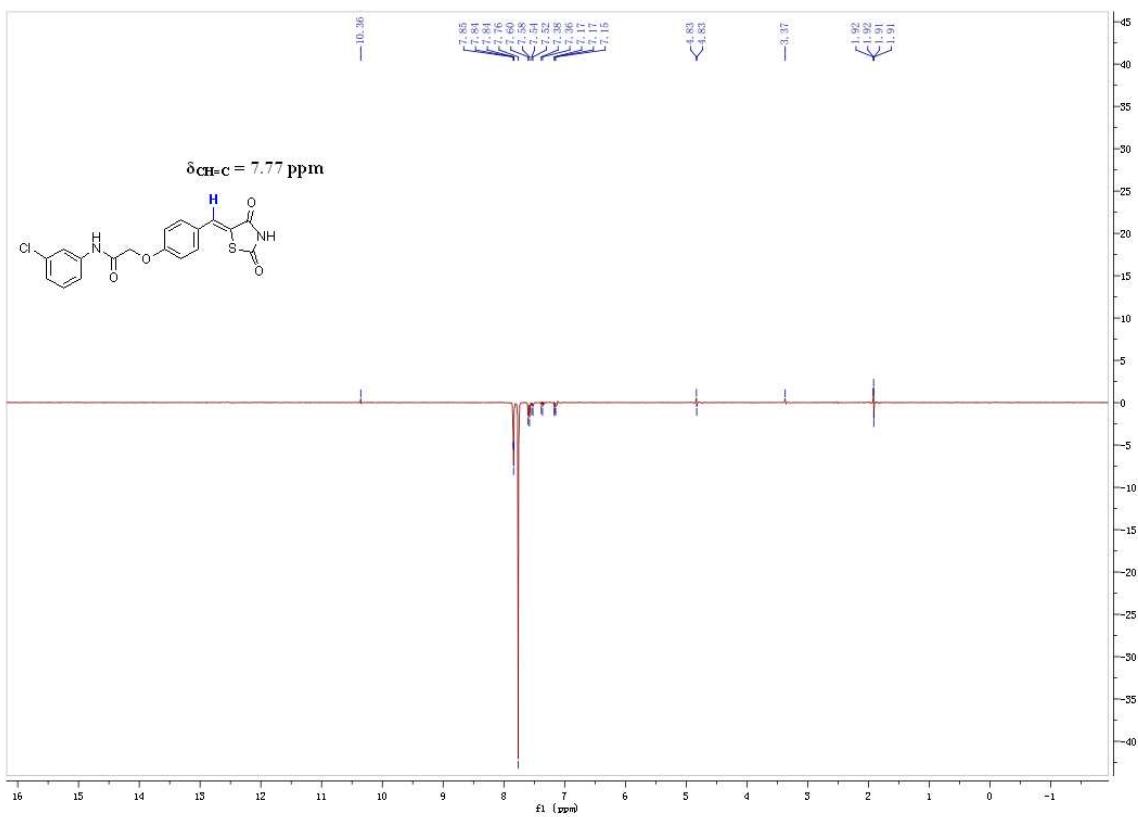
Yield: 86.5 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.96 (s, 1H); 8.69 (s, 1H), 8.03 (d, 1H, J = 7.2 Hz), 7.96 – 7.96 (m, 2H), 7.917 – 7.88 (m, 1H), 7.76 – 7.75 (m, 2H), 7.54 – 7.49 (m, 3H), 7.20 (d, 2H, J = 8.8 Hz), 4.85 (s, 2H); MS (ESI), m/z: 304.16 [M + H] $^+$.

2-(4-formylphenoxy)-N-(N.d.phthalen-2-yl) acetamide (2V).

Yield: 81.9 %; white solid; ^1H NMR (400 MHz, CDCl_3): δ 9.94 (s, 1H); 8.36 (s, 1H), 8.26 (d, 1H, J = 1.6 Hz), 7.92 (d, 2H, J = 4.8 Hz), 7.856 – 7.79 (m, 1H), 7.56 – 7.54 (dd, 1H, J = 7.2 Hz), 7.51 – 7.42 (m, 2H), 7.14 (d, 2H, J = 8.8 Hz), 4.74 (s, 2H); MS (ESI), m/z: 304.21 [M + H] $^+$.

2: ^1H NMR, ^{13}C NMR and NOEDS of 3I





3: HPLC analysis of 3A-V;

All compounds were supplied as 1 mg/mL in DMSO or methanol with 10 µL injected on a partial loop fill at a flow rate of 1 mL/min for 30 min. **Solvent A:** 75 % methanol; **Solvent B:** 25 % water with 0.5 % trifluoroacetic acid.

Compds	T _R (min)	UV-vis (nm)	
3A	5.076	340.4	237.2
3B	6.433	344.0	239.6
3C	4.749	344.0	241.9
3D	4.040	345.2	241.9
3E	9.331	344.0	243.1
3F	4.251	344.0	286.9
3G	5.594	344.0	238.4
3H	4.952	342.8	237.2
3I	7.289	344.0	239.6
3J	7.047	344.0	243.1
3K	7.869	344.0	245.5
3L	12.562	341.6	238.4
3M	14.792	344.0	243.1
3N	3.810	342.8	238.4
3O	3.352	342.8	236.0
3P	3.085	344.0	230.1

3Q	3.357	341.6	234.9
3R	2.839	328.5	236.0
3S	2.672	342.8	233.7
3T	2.777	342.8	236.0
3U	3.222	340.4	218.4
3V	3.933	344.0	284.5
			240.7

3: Docking results of all molecules with Murine iNOS.

According to the 3D structure of murine iNOS(PDB ID: 1r35),¹ all molecules were docked to the active site of iNOS by the aid of a protein-ligand docking program FRED. At the exhaustive searching stage, chemgauss score² was employed. Then the docked structures were performed an solid body optimization with piecewise linear potential.³ Finally, a full coordinate refinement of the optimized structures within the site was done through the Merck Molecular Mechanics Force Field. The poses of the refined structures were assessed by using zapbind scoring function⁴ which was based on PB electrostatic calculations in combination with an area contact term. As shown in **Table**, three of the four effective inhibitors (**3I**, **3M**, **3O**, **3U**) are among the top five. **3I** was the top one of consensus structures which were selected using Chemgauss and zapbind scoring functions.

Compd	Exp.	Zapbind score	Consensus score
3R	41.6	-10.09	20
3M	72.0	-3.47	22
3I	80.9	-2.37	10
3U	70.9	-1.67	22
3G	41.5	-0.11	16
3H	57.9	0.81	16
3E	26.6	7.38	11
3L	10.0	13.87	14

3Q	47.8	23.94	26
3V	45.1	26.72	15
3A	55.3	33.90	20
3O	83.9	34.45	20
3P	51.0	42.00	28
3F	10.2	44.22	28
3K	23.7	53.34	16
3B	38.4	54.12	18
3N	36.0	56.51	17
3S	14.6	57.44	31
3J	9.7	58.42	22
3C	0.6	60.44	19
3T	12.5	60.83	33
3D	16.2	73.87	38

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