

SUPPORTING INFORMATION

Cinnoline Derivatives as Human Neutrophil Elastase Inhibitors

Maria Paola Giovannoni^a, Igor A. Schepetkin^b, Letizia Crocetti^a, Giovanna Ciciani^a, Agostino Cilibrizzi^c, Gabriella Guerrini^a, Andrei I. Khlebnikov^d, Mark T. Quinn^b, Claudia Vergelli^a

^a*NEUROFARBA, Sezione di Farmaceutica e Nutraceutica, Università degli Studi di Firenze, Via Ugo Schiff 6, 50019 Sesto Fiorentino, Italy.*

^b*Department of Immunology and Infectious Diseases, Montana State University, Bozeman, MT 59717, USA.*

^c*Department of Chemistry, Imperial College London, South Kensington, London SW7 2AZ, UK.*

^d*Department of Biotechnology and Organic Chemistry, Tomsk Polytechnic University, Tomsk 634050, Russia and Department of Chemistry, Altai State Technical University, Barnaul, Russia.*

Contents

- 1) Characterization of derivatives **10c, 11b,c, 16b,c, 17b,c, 18b-g, 21b-d, 24**
- 2) Figure S1, S2, S3, S4, S5, S6
- 3) Elemental analyses for all new compounds (Elemental analyzer CHN/S, 2400 series 2 Perkin Elmer)

1) Characterization of derivatives **10c**, **11b,c**, **16b,c**, **17b,c**, **18b-g**, **21b-d**, **24**

3-Propylcinnolin-4(1H)-one (10c). Yield = 20%; mp = 178-185 °C dec (EtOH). ¹H NMR (CDCl₃) δ 1.04 (t, 3H, CH₃, *J* = 7.4 Hz), 1.77 (sex, 2H, CH₂CH₂CH₃, *J* = 7.4 Hz), 2.83 (t, 2H, CH₂CH₂CH₃, *J* = 7.6 Hz), 7.32-7.39 (m, 2H, Ar), 7.69 (t, 1H, Ar, *J* = 7.8 Hz), 8.30 (d, 1H, Ar, *J* = 8.4 Hz). ESI-MS calcd. for C₁₁H₁₂N₂O, 188.23; found: *m/z* 189.07 [M + H]⁺.

3-Cyclopropyl-1-(3-methylbenzoyl)cinnolin-4(1H)-one (11b). Yield = 31%; mp = 116-120 °C (EtOH). ¹H NMR (CDCl₃) δ 1.14-1.20 (m, 2H, CH₂ cyclopropyl), 1.53-1.59 (m, 2H, CH₂ cyclopropyl), 2.28-2.36 (m, 1H, CH cyclopropyl), 2.53 (s, 3H, CH₃), 7.52 (t, 1H, Ar, *J* = 8.0 Hz), 7.58 (d, 1H, Ar, *J* = 7.6 Hz), 7.71 (t, 1H, Ar, *J* = 7.4 Hz), 7.76-7.83 (m, 2H, Ar), 8.18 (s, 2H, Ar), 8.54 (d, 1H, Ar, *J* = 8.4 Hz). ¹³C NMR (CDCl₃) δ 9.92 (CH₂), 10.47 (CH₃), 21.32 (CH), 29.69 (CH₂), 119.98 (CH), 120.82 (C), 127.77 (CH), 127.87 (C), 128.92 (CH), 129.49 (CH), 129.74 (CH), 131.08 (CH), 131.36 (CH), 135.35 (CH), 139.01 (C), 143.97 (C), 150.71 (C), 151.00 (C), 163.99 (C). IR= 1618 cm⁻¹ (C=O amide), 1736 cm⁻¹ (C=O). ESI-MS calcd. for C₁₉H₁₆N₂O₂, 304.34; found: *m/z* 305.12 [M + H]⁺.

1-(3-Methylbenzoyl)-3-propylcinnolin-4(1H)-one (11c). Yield = 17%; oil. ¹H NMR (CDCl₃) δ 1.03 (t, 3H, CH₃, *J* = 7.4 Hz), 1.98 (sex, 2H, CH₂CH₂CH₃, *J* = 7.4 Hz), 2.53 (s, 3H, CH₃), 3.18 (t, 2H, CH₂CH₂CH₃, *J* = 7.6 Hz), 7.52 (t, 1H, Ar, *J* = 7.8 Hz), 7.59 (d, 1H, Ar, *J* = 7.2 Hz), 7.73 (t, 1H, Ar, *J* = 7.4 Hz), 7.83 (t, 2H, Ar, *J* = 8.0 Hz), 8.14 (s, 2H, Ar), 8.60 (d, 1H, Ar, *J* = 8.4 Hz). ¹³C NMR (CDCl₃) δ 14.03 (CH₃), 21.33 (CH₃), 22.32 (CH₂), 32.87 (CH₂), 120.39 (CH), 121.12 (C), 127.69 (CH), 127.80 (C), 128.96 (CH), 129.86 (CH), 129.90 (CH), 131.03 (CH), 131.32 (CH), 135.41 (CH), 139.05 (C), 144.02 (C), 150.97 (C), 151.32 (C), 164.10 (C). ESI-MS calcd. for C₁₉H₁₈N₂O₂, 306.36; found: *m/z* 307.14 [M + H]⁺.

3-Bromo-1-(3-methylbenzoyl)cinnolin-4(1H)-one (16b). Yield = 65%; mp = 157-162 °C dec (EtOH). ¹H NMR (CDCl₃) δ 2.48 (s, 3H, CH₃), 7.44 (t, 1H, Ar, *J* = 7.6 Hz), 7.49 (d, 1H, Ar,

$J = 7.2$ Hz), 7.59-7.69 (m, 2H, Ar), 7.71 (s, 1H, Ar), 7.85 (t, 1H, Ar, $J = 8.0$ Hz), 8.42 (d, 1H, Ar, $J = 8.0$ Hz), 8.48 (d, 1H, Ar, $J = 8.8$ Hz). IR = 1662 cm^{-1} (C=O amide), 1720 cm^{-1} (C=O). ESI-MS calcd. for $\text{C}_{16}\text{H}_{11}\text{BrN}_2\text{O}_2$, 343.17; found: m/z 344.00 $[\text{M} + \text{H}]^+$.

3-Iodo-1-(3-methylbenzoyl)cinnolin-4(1H)-one (16c). Yield = 16%; mp = 185-186 °C dec (EtOH). ^1H NMR (CDCl_3) δ 2.48 (s, 3H, CH_3), 7.44 (t, 1H, Ar, $J = 7.4$ Hz), 7.49 (d, 1H, Ar, $J = 7.6$ Hz), 7.59-7.65 (m, 2H, Ar), 7.71 (s, 1H, Ar), 7.86 (t, 1H, Ar, $J = 8.0$ Hz), 8.40 (d, 1H, Ar, $J = 8.0$ Hz), 8.47 (d, 1H, Ar, $J = 8.4$ Hz). IR = 1654 cm^{-1} (C=O amide), 1715 cm^{-1} (C=O). ESI-MS calcd. for $\text{C}_{16}\text{H}_{11}\text{IN}_2\text{O}_2$, 390.18; found: m/z 390.99 $[\text{M} + \text{H}]^+$.

3-Bromocinnolin-4-yl 3-methylbenzoate (17b). Yield = 16%; mp = 133-138 °C (EtOH). ^1H NMR (CDCl_3) δ 2.53 (s, 3H, CH_3), 7.51-7.61 (m, 2H, Ar), 7.81-7.93 (m, 3H, Ar), 8.15 (s, 2H, Ar), 8.60-8.70 (m, 1H, Ar). IR = 1744 cm^{-1} (C=O ester). ESI-MS calcd. for $\text{C}_{16}\text{H}_{11}\text{BrN}_2\text{O}_2$, 343.17; found: m/z 344.00 $[\text{M} + \text{H}]^+$.

3-Iodocinnolin-4-yl 3-methylbenzoate (17c). Yield = 16%; mp = 163-166 °C dec (EtOH). ^1H NMR (CDCl_3) δ 2.53 (s, 3H, CH_3), 7.53 (t, 1H, Ar, $J = 7.8$ Hz), 7.60 (d, 1H, Ar, $J = 7.2$ Hz), 7.81 (t, 1H, Ar, $J = 7.6$ Hz), 7.87 (d, 1H, Ar, $J = 8.4$ Hz), 7.95 (t, 1H, Ar, $J = 7.8$ Hz), 8.17 (d, 2H, Ar, $J = 6.8$ Hz), 8.64 (d, 1H, Ar, $J = 8.4$ Hz). IR = 1744 cm^{-1} (C=O ester). ESI-MS calcd. for $\text{C}_{16}\text{H}_{11}\text{IN}_2\text{O}_2$, 390.18; found: m/z 390.99 $[\text{M} + \text{H}]^+$.

1-Acetylcinnolin-4(1H)-one (18b). Yield = 31%; mp = 132-144 °C dec (EtOH). ^1H NMR (CDCl_3) δ 2.79 (s, 3H, CH_3), 7.55 (t, 1H, Ar, $J = 7.6$ Hz), 7.80-7.85 (m, 2H, Ar), 8.31 (d, 1H, Ar, $J = 7.6$ Hz), 9.03 (d, 1H, Ar, $J = 8.8$ Hz). IR = 1648 cm^{-1} (C=O amide), 1734 cm^{-1} (C=O). ESI-MS calcd. for $\text{C}_{10}\text{H}_8\text{N}_2\text{O}_2$, 188.18; found: m/z 189.06 $[\text{M} + \text{H}]^+$.

1-Propionylcinnolin-4(1H)-one (18c). Yield = 48%; mp = 87-90 °C (EtOH). ^1H NMR (CDCl_3) δ 1.33 (t, 3H, CH_3 , $J = 7.2$ Hz), 3.22 (q, 2H, CH_2 , $J = 7.2$ Hz), 7.54 (t, 1H, Ar, $J =$

7.6 Hz), 7.79-7.84 (m, 2H, Ar), 8.31 (d, 1H, Ar, $J = 8.0$ Hz), 9.04 (d, 1H, Ar, $J = 8.8$ Hz). IR = 1645 cm^{-1} (C=O amide), 1739 cm^{-1} (C=O). ESI-MS calcd. for $\text{C}_{11}\text{H}_{10}\text{N}_2\text{O}_2$, 202.21; found: m/z 203.08 $[\text{M} + \text{H}]^+$.

1-Butyrylcinnolin-4(1H)-one (18d). Yield = 68%; mp = 95-98 °C dec (EtOH). ^1H NMR (CDCl_3) δ 1.09 (t, 3H, CH_3 , $J = 7.6$ Hz), 1.85 (sex, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$, $J = 7.4$ Hz), 3.17 (t, 2H, $\text{CH}_2\text{CH}_2\text{CH}_3$, $J = 7.4$ Hz), 7.54 (t, 1H, Ar, $J = 7.6$ Hz), 7.79-7.84 (m, 2H, Ar), 8.31 (d, 1H, Ar, $J = 8.0$ Hz), 9.02 (d, 1H, Ar, $J = 8.8$ Hz). IR = 1647 cm^{-1} (C=O amide), 1740 cm^{-1} (C=O). ESI-MS calcd. for $\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_2$, 216.24; found: m/z 217.09 $[\text{M} + \text{H}]^+$.

1-Pentanoylcinnolin-4(1H)-one (18e). Yield = 25%; oil. ^1H NMR (CDCl_3) δ 1.00 (t, 3H, CH_3 , $J = 7.4$ Hz), 1.48 (sex, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$, $J = 7.4$ Hz), 1.79 (quin, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$, $J = 7.6$ Hz), 3.17 (t, 2H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3$, $J = 7.4$ Hz), 7.52 (t, 1H, Ar, $J = 7.6$ Hz), 7.76-7.82 (m, 2H, Ar), 8.29 (d, 1H, Ar, $J = 8.4$ Hz), 8.99 (d, 1H, Ar, $J = 9.2$ Hz). ESI-MS calcd. for $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_2$, 230.26; found: m/z 231.11 $[\text{M} + \text{H}]^+$.

1-(Cyclopropanecarbonyl)cinnolin-4(1H)-one (18f). Yield = 41%; mp = 137-138 °C (EtOH). ^1H NMR (CDCl_3) δ 1.16-1.22 (m, 2H, CH_2 cyclopropyl), 1.30-1.36 (m, 2H, CH_2 cyclopropyl), 3.17 (sep, 1H, CH cyclopropyl, $J = 4.2$ Hz), 7.53 (t, 1H, Ar, $J = 7.6$ Hz), 7.79 (t, 1H, Ar, $J = 8.0$ Hz), 7.90 (s, 1H, Ar), 8.32 (d, 1H, Ar, $J = 8.0$ Hz), 8.87 (d, 1H, Ar, $J = 8.8$ Hz). IR = 1651 cm^{-1} (C=O amide), 1719 cm^{-1} (C=O). ESI-MS calcd. for $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_2$, 214.22; found: m/z 215.08 $[\text{M} + \text{H}]^+$.

1-(Cyclopentanecarbonyl)cinnolin-4(1H)-one (18g). Yield = 50%; oil. ^1H NMR (CDCl_3) δ 1.70-1.77 (m, 2H, CH_2 cyclopent), 1.79-1.85 (m, 2H, CH_2 cyclopent), 1.91-1.99 (m, 2H, CH_2 cyclopent), 2.06-2.12 (m, 2H, CH_2 cyclopent), 4.04 (quin, 1H, CH cyclopent, $J = 8.0$ Hz), 7.53 (t, 1H, Ar, $J = 7.6$ Hz), 7.79 (t, 1H, Ar, $J = 8.8$ Hz), 7.84 (s, 1H, Ar), 8.31 (d, 1H, Ar, J

= 8.0 Hz), 8.96 (d, 1H, Ar, $J = 8.8$ Hz). ESI-MS calcd. for $C_{14}H_{14}N_2O_2$, 242.27; found: m/z 243.11 $[M + H]^+$.

6-Chloro-1-(3-methylbenzoyl)cinnolin-4(1H)-one (21b). Yield = 30%; mp = 120-128 °C (EtOH). 1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 7.53 (t, 1H, Ar, $J = 7.8$ Hz), 7.59 (d, 1H, Ar, $J = 7.6$ Hz), 7.86 (d, 1H, Ar, $J = 8.8$ Hz), 8.04 (s, 1H, Ar), 8.13 (s, 2H, Ar), 8.60 (d, 1H, Ar, $J = 8.8$ Hz), 9.49 (s, 1H, Ar). ESI-MS calcd. for $C_{16}H_{11}ClN_2O_2$, 298.72; found: m/z 300.05 $[M + H]^+$.

6-Bromo-1-(3-methylbenzoyl)cinnolin-4(1H)-one (21c). Yield = 33%; mp = 110-112 °C dec (EtOH). 1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 7.53 (t, 1H, Ar, $J = 7.8$ Hz), 7.59 (d, 1H, Ar, $J = 7.6$ Hz), 8.00 (d, 1H, Ar, $J = 7.6$ Hz), 8.13 (s, 2H, Ar), 8.23 (d, 1H, Ar, $J = 1.2$ Hz), 8.51 (d, 1H, Ar, $J = 8.8$ Hz), 9.49 (s, 1H, Ar). IR = 1654 cm^{-1} (C=O amide), 1734 cm^{-1} (C=O). ESI-MS calcd. for $C_{16}H_{11}BrN_2O_2$, 343.17; found: m/z 344.00 $[M + H]^+$.

6-Iodo-1-(3-methylbenzoyl)cinnolin-4(1H)-one (21d). Yield = 11%; mp = 120-125 °C (EtOH). 1H NMR ($CDCl_3$) δ 2.53 (s, 3H, CH_3), 7.53 (t, 1H, Ar, $J = 7.8$ Hz), 7.59 (d, 1H, Ar, $J = 7.6$ Hz), 8.13 (s, 2H, Ar), 8.17 (dd, 1H, Ar, $J = 9.0$ Hz, $J = 1.4$ Hz), 8.34 (d, 1H, Ar, $J = 8.8$ Hz), 8.47 (s, 1H, Ar), 9.48 (s, 1H, Ar). ^{13}C NMR ($CDCl_3$) δ 21.37 (CH_3), 99.26 (C), 121.83 (C), 127.58 (C), 127.80 (CH), 129.00 (CH), 129.52 (CH), 131.05 (CH), 131.16 (CH), 135.69 (CH), 139.12 (C), 139.30 (CH), 140.14 (CH), 144.16 (C), 150.87 (C), 163.50 (C). IR = 1577 cm^{-1} (C=O amide), 1750 cm^{-1} (C=O). ESI-MS calcd. for $C_{16}H_{11}IN_2O_2$, 390.18; found: m/z 390.99 $[M + H]^+$.

3-Methyl-N-[1-(3-methylbenzoyl)-4-oxo-1,4-dihydrocinnolin-6-yl]-benzamide (24). Yield = 20%; mp = 198-203 °C (EtOH). 1H NMR ($DMSO-d_6$) δ 2.40 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.44 (d, 2H, Ar, $J = 5.2$ Hz), 7.61 (t, 1H, Ar, $J = 7.6$ Hz), 7.69 (d, 1H, Ar, $J = 8.0$ Hz), 7.75-7.80 (m, 2H, Ar), 8.08-8.13 (m, 2H, Ar), 8.40 (dd, 1H, Ar, $J = 9.2$ Hz, $J = 2$ Hz), 8.57-8.62

(m, 2H, Ar), 9.48 (s, 1H, Ar), 10.88 (exch br s, 1H, NH). ESI-MS calcd. for $C_{24}H_{19}N_3O_3$, 397.43; found: m/z 398.15 $[M + H]^+$.

2) Figure S1, S2, S3, S4, S5, S6

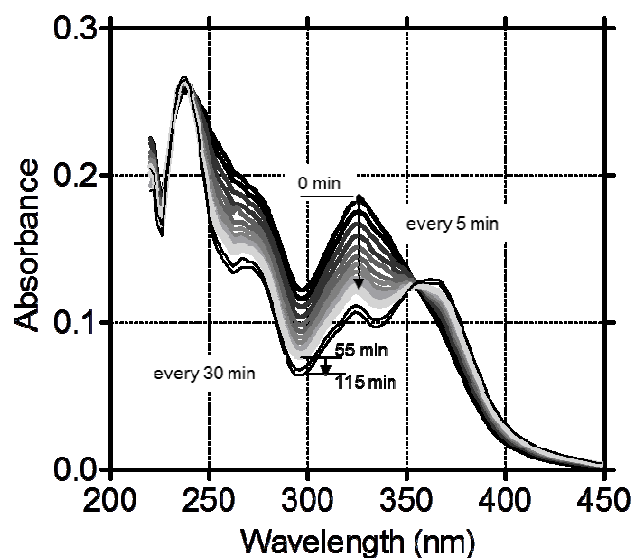


Figure S1. Analysis of compound hydrolysis in aqueous solution. The changes in absorbance spectra of compound **21a** (20 μ M) were monitored over time with 5-min (first 12 spectra; from 0 to 55 min) and 30-min (last 2 spectra; from 55 to 115 min) intervals in 0.05 M phosphate buffer (pH 7.5, 25 $^{\circ}$ C). Representative scans are from two independent experiments.

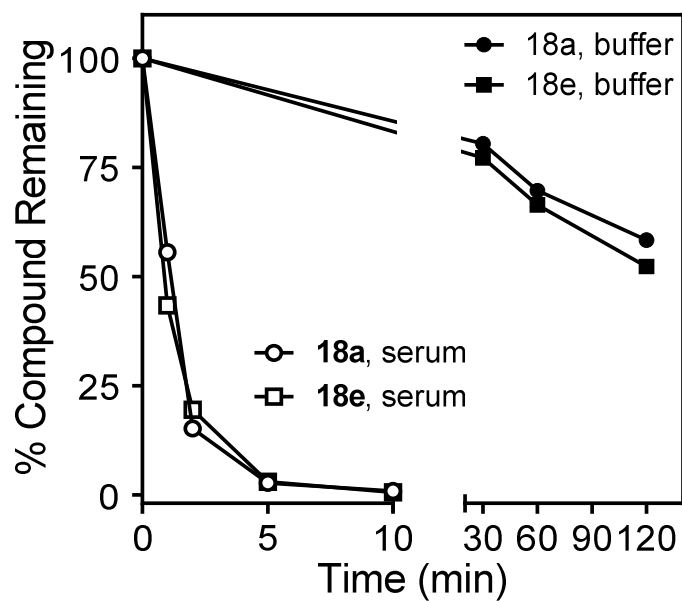


Figure S2. Analysis of compound stability in serum and buffer. The indicated compounds were added to human serum or phosphate buffer (0.05 M, pH 7.3), and the samples were incubated at 25°C. At the indicated times, the samples were analyzed by analytical RP-HPLC. Representative time-course curves are from two independent experiments.

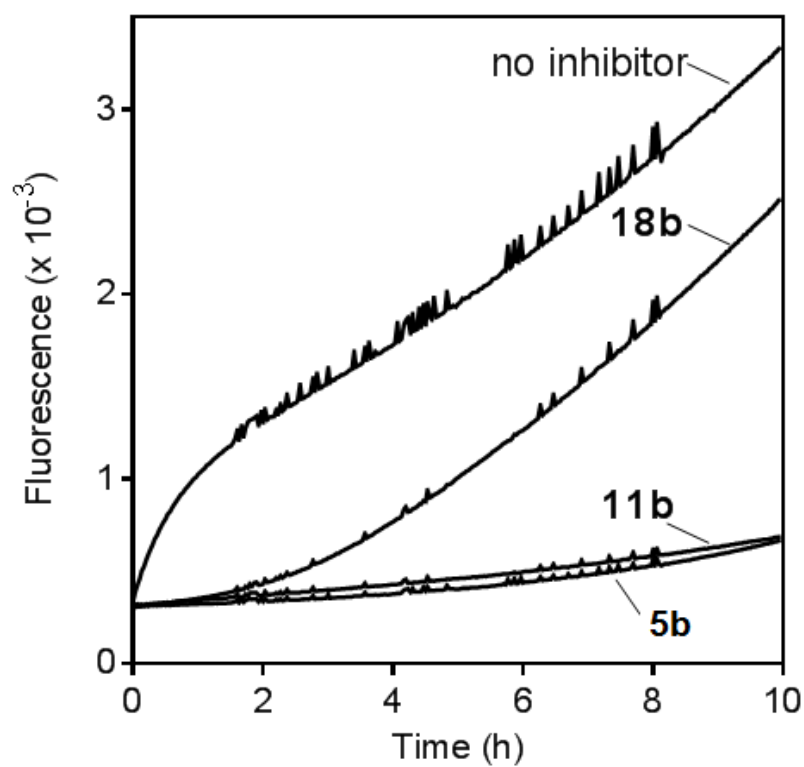


Figure S3. Evaluation of HNE inhibition by selected cinnoline derivatives **11b** and **18b** and comparison with indazole **5b**²⁸. HNE was incubated with the indicated compounds (10 μ M), and kinetic curves monitoring substrate cleavage catalyzed by HNE from 0 to 10 h are shown. Representative curves are from two independent experiments.

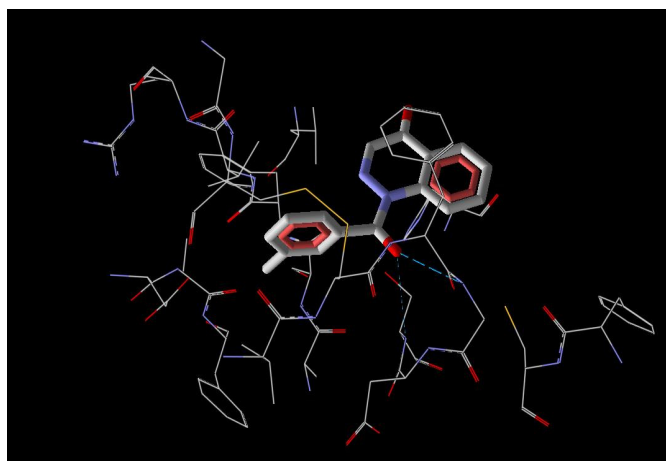


Figure S4. Docking pose of compound **18a**. Gly193 and Ser195 are H-bonded with the benzoyl oxygen. Residues within 6 Å of the pose are visible.

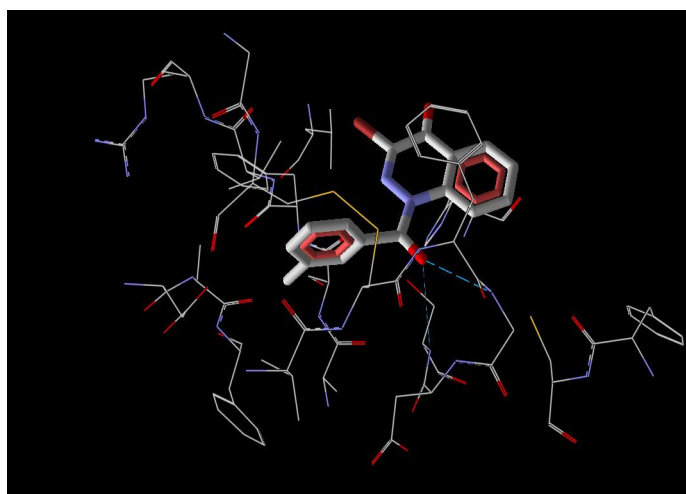


Figure S5. Docking pose of compound **16b**. Gly193 and Ser195 are H-bonded with the benzoyl oxygen. Residues within 6 Å of the pose are visible.



Figure S6. Docking pose of compound **17b**. Gly193, Asp194, and Ser195 are H-bonded with the benzoyl oxygen, while Val216 forms hydrogen bonds with nitrogen atoms in the heterocycle. Residues within 6 Å of the pose are visible.

Table S1. Elemental analyses for all new compounds

Compound	Formula	Calculated			Found		
		C	N	H	C	N	H
2	C ₁₅ H ₁₉ N ₃ O ₃	62.27	14.52	6.62	62.51	14.57	6.64
4	C ₁₅ H ₁₄ N ₂ O ₄	62.93	9.79	4.93	63.15	9.82	4.95
5	C ₁₂ H ₁₂ N ₂ O ₃	62.06	12.06	5.21	62.24	12.09	5.22
6	C ₁₉ H ₁₈ N ₂ O ₃	70.79	8.69	5.63	70.96	8.71	5.64
7	C ₁₉ H ₁₆ N ₂ O ₄	67.85	8.33	4.79	67.98	8.35	4.80
8	C ₁₇ H ₁₂ N ₂ O ₄	66.23	9.09	3.92	65.96	9.05	3.90

10b	C ₁₁ H ₁₀ N ₂ O	70.95	15.04	5.41	70.70	14.98	5.39
10c	C ₁₁ H ₁₂ N ₂ O	70.19	14.88	6.43	69.98	14.83	6.41
11a	C ₂₂ H ₁₆ N ₂ O ₂	77.63	8.23	4.74	77.44	8.20	4.72
11b	C ₁₉ H ₁₆ N ₂ O ₂	74.98	9.20	5.30	74.83	9.18	5.28
11c	C ₁₉ H ₁₈ N ₂ O ₂	74.49	9.14	5.92	74.34	9.12	5.90
11d	C ₁₇ H ₁₄ N ₂ O ₃	69.38	9.52	4.79	69.10	9.48	4.77
12	C ₁₄ H ₁₆ N ₂ O ₃	64.60	10.76	6.20	64.37	10.72	6.17
13	C ₂₂ H ₂₂ N ₂ O ₄	69.83	7.40	5.86	69.62	7.37	5.84
16a	C ₁₆ H ₁₁ ClN ₂ O ₂	64.33	9.38	3.71	64.58	9.41	3.72
16b	C ₁₆ H ₁₁ BrN ₂ O ₂	56.00	8.16	3.23	56.17	8.18	3.24
16c	C ₁₆ H ₁₁ IN ₂ O ₂	49.25	7.18	2.84	49.37	7.20	2.85
17a	C ₁₆ H ₁₁ ClN ₂ O ₂	64.33	9.38	3.71	64.58	9.41	3.72
17b	C ₁₆ H ₁₁ BrN ₂ O ₂	56.00	8.16	3.23	56.17	8.18	3.24
17c	C ₁₆ H ₁₁ IN ₂ O ₂	49.25	7.18	2.84	49.37	7.20	2.85
18a	C ₁₆ H ₁₂ N ₂ O ₂	72.72	10.60	4.58	72.95	10.63	4.59
18b	C ₁₀ H ₈ N ₂ O ₂	63.82	14.89	4.28	64.04	14.94	4.29
18c	C ₁₁ H ₁₀ N ₂ O ₂	65.34	13.85	4.98	65.47	13.87	4.99
18d	C ₁₂ H ₁₂ N ₂ O ₂	66.65	12.96	5.59	66.81	12.99	5.60
18e	C ₁₃ H ₁₄ N ₂ O ₂	67.81	12.17	6.13	68.01	12.20	6.15
18f	C ₁₂ H ₁₀ N ₂ O ₂	67.28	13.08	4.71	67.51	13.12	4.73
18g	C ₁₄ H ₁₄ N ₂ O ₂	69.41	11.56	5.82	69.67	11.60	5.84
19	C ₁₆ H ₁₆ N ₂ O ₂	71.62	10.44	6.01	71.33	10.39	5.98

21a	$C_{16}H_{11}N_3O_4$	62.14	13.59	3.58	61.92	13.54	3.56
21b	$C_{16}H_{11}ClN_2O_2$	64.33	9.38	3.71	64.13	9.35	3.69
21c	$C_{16}H_{11}BrN_2O_2$	56.00	8.16	3.23	55.86	8.13	3.22
21d	$C_{16}H_{11}IN_2O_2$	49.25	7.18	2.84	49.15	7.16	2.85
22	$C_{16}H_{11}N_3O_4$	62.14	13.59	3.58	61.92	13.54	3.56
24	$C_{24}H_{19}N_3O_3$	72.53	10.57	4.82	72.28	10.53	4.80