

Benzothiazole and pyrrolone flavivirus inhibitors targeting the viral helicase

Noreena L. Sweeney,[†] Alicia M. Hanson,[†] Sourav Mukherjee,[†] Jean Ndjomou,[†] Brian J. Geiss,[‡] J. Jordan Steel,[‡] Kevin J. Frankowski,[§] Kelin Li,[§] Frank J. Schoenen,[§] and David N. Frick^{†*}

[†]Department of Chemistry & Biochemistry, University of Wisconsin- Milwaukee, Milwaukee, Wisconsin 53211, USA

[‡]Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado, 80523 USA.

[§]University of Kansas Specialized Chemistry Center, University of Kansas, 2034 Becker Drive, Lawrence, Kansas 66047, USA

Supplemental Materials and Methods

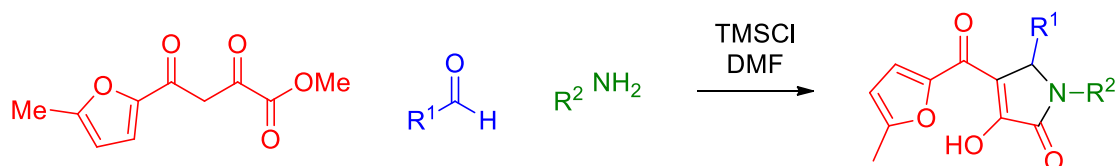
CHEMICAL SYNTHESIS

General synthetic methods.

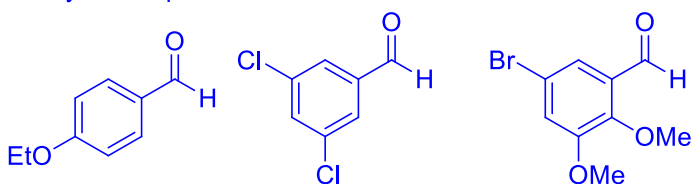
All reagents and materials were purchased from commercial vendors (Sigma, Alfa Aesar, TCI America, Fisher Scientific) and used as received. Ethyl ether, toluene, THF, MeCN and CH₂Cl₂ were degassed with nitrogen and passed through two columns of basic alumina on an Innovative Technology solvent purification system. ¹H and ¹³C NMR spectra were recorded on a Bruker AM 400 spectrometer (operating at 400 and 101 MHz respectively) in CDCl₃ with 0.03% TMS as an internal standard, unless otherwise specified. Chemical shifts are reported in parts per million (ppm) downfield from TMS. ¹³C multiplicities were determined with the aid of an APT pulse sequence, differentiating the signals for methyl and methyne carbons as “d” from methylene and quarternary carbons as “u”. The infrared (IR) spectra were acquired as thin films using a universal ATR sampling accessory on a PerkinElmer Spectrum 100 FT-IR spectrometer and the absorption frequencies are reported in cm⁻¹. Melting points were determined on a Stanford Research Systems Optimelt automated melting point system interfaced through a PC and are uncorrected.

HPLC/MS analysis was carried out with gradient elution (5% CH₃CN to 100% CH₃CN) on an Agilent 1200 RRLLC with a photodiode array UV detector and an Agilent 6224 TOF mass spectrometer (also used to produce high resolution mass spectra). HPLC purification was carried out by mass-directed fractionation (MDF) with gradient elution (a narrow CH₃CN gradient was chosen based on the retention time of the target from LCMS analysis of the crude sample) on an Agilent 1200 instrument with photodiode array detector, an Agilent 6120 quadrupole mass spectrometer, and a HTPAL LEAP autosampler. Fractions were triggered using an MS and UV threshold determined by HPLC/MS analysis of the crude sample. One of two column/mobile phase conditions were chosen for both analysis and purification to promote the targets neutral

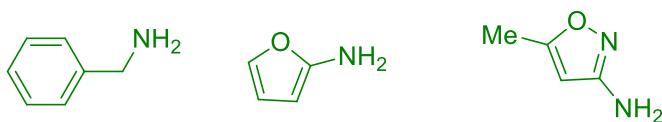
state (0.02% formic acid with Waters Atlantis T3 5um, 19 × 150 mm; or pH 9.8 NH₄OH with Waters XBridge C18 5 um, 19 × 150 mm).



aldehyde component



amine component



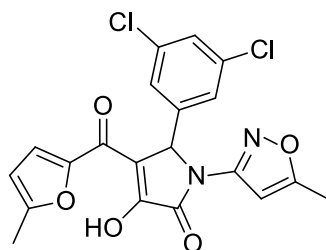
1. Substrate synthesis.

Methyl 4-(5-methylfuran-2-yl)-2,4-dioxobutanoate. To a solution of 2-acetyl-5-methylfuran (480 mg, 3.87 mmol) in THF (20 mL) at -78 °C was added a THF solution of LiHMDS (3.9 mL, 1.0 M, 3.87 mmol). The reaction was stirred at -78 °C for 40 min and a solution of dimethyl oxalate (460 mg, 3.87 mmol) in THF (5 mL) was added and the reaction stirred for 2h, warming to approximately 0 °C. The reaction was quenched by the addition of aqueous, saturated ammonium chloride (20 mL). Brine (20 mL) was added and the mixture was extracted with EtOAc (2 × 25 mL). The organic layers were combined, dried with Na₂SO₄ and evaporated under vacuum. The residue was purified by silica gel chromatography to afford the product as a light yellow solid (315 mg, 1.50 mmol, 39% yield). Mp = 92–94 °C; R_f = 0.09 (1:1 Hexanes: EtOAc); ¹H NMR (CDCl₃) δ 2.44 (s, 3H), 3.93 (s, 3H), 6.26 (d, *J* = 2.8 Hz, 1H), 6.88 (s, 1H), 7.28 (d, *J* = 3.6 Hz, 1H); ¹³C NMR (CDCl₃, APT pulse sequence) δ d 14.2, 53.1, 99.3, 110.2, 120.8; u 149.7, 159.7, 162.7, 164.6, 180.6; IR (neat) 1729, 1630, 1512 cm⁻¹; HRMS (ESI) *m/z* calcd for C₁₀H₁₁O₅ ([M+H]⁺), 211.0606, found 211.0587.

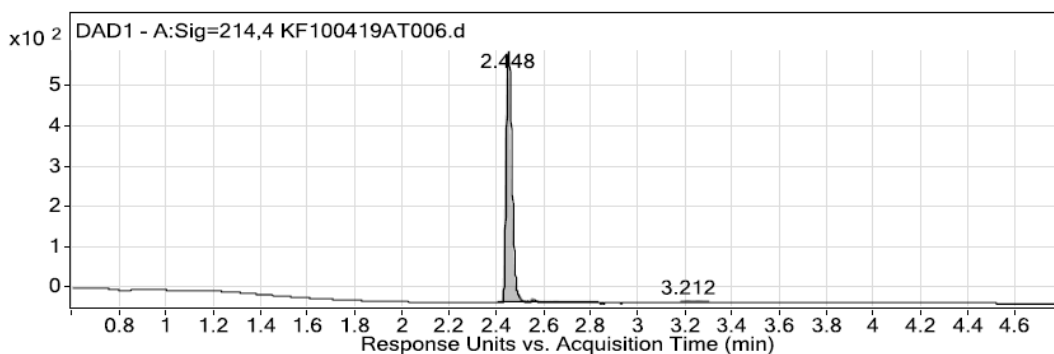
2. Analogue synthesis

General procedure for the synthesis 3-hydroxy-pyrrol-5-one analogs.

A method analogous to that reported by Ryabukhin and coworkers was utilized for the synthesis of pyrrolone analogues.¹ Thus, a DMF solution of methyl 4-(5-methylfuran-2-yl)-2,4-dioxobutanoate (0.5 mL, 1 M, 0.5 mmol) was charged in a reaction vial. To this solution were added DMF solutions of the aldehyde component (0.5 mL, 1 M, 0.5 mmol) and amine component (0.5 mL, 1 M, 0.5 mmol). Trimethylsilyl chloride (0.4 mL, 3.0 mmol, 6.0 equiv) was added and the reaction stirred at rt for 16 h. The reaction was quenched with water (5 mL) precipitating the crude product, which was collected by filtration, washed with water (2 × 2 mL) and purified by reverse-phase, mass-directed, preparative HPLC.

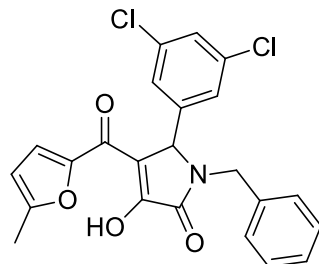


5-(3,5-Dichlorophenyl)-3-hydroxy-4-(5-methylfuran-2-carbonyl)-1-(5-methylisoxazol-3-yl)-1H-pyrrol-2(5H)-one 25 (45382104). 3,5-Dichlorobenzaldehyde and 3-amino-5-methylisoxazole were reacted according to the general procedure to afford the product as a yellow solid (129 mg, 0.30 mmol, 59% yield). Mp = 156–162 °C; ¹H NMR (CDCl₃) δ 2.27 (s, 3H), 2.38 (s, 3H), 6.04 (s, 1H), 6.21 (s, 1H), 6.63 (s, 1H), 7.11–7.14 (m, 2H), 7.30 (s, 2H); IR (neat) 1719, 1608, 1505, 1428 cm⁻¹; HRMS (ESI) *m/z* calcd for C₂₀H₁₅Cl₂N₂O₅ ([M+H]⁺), 433.0353, found 433.0357; HPLC purity = 97.6%.

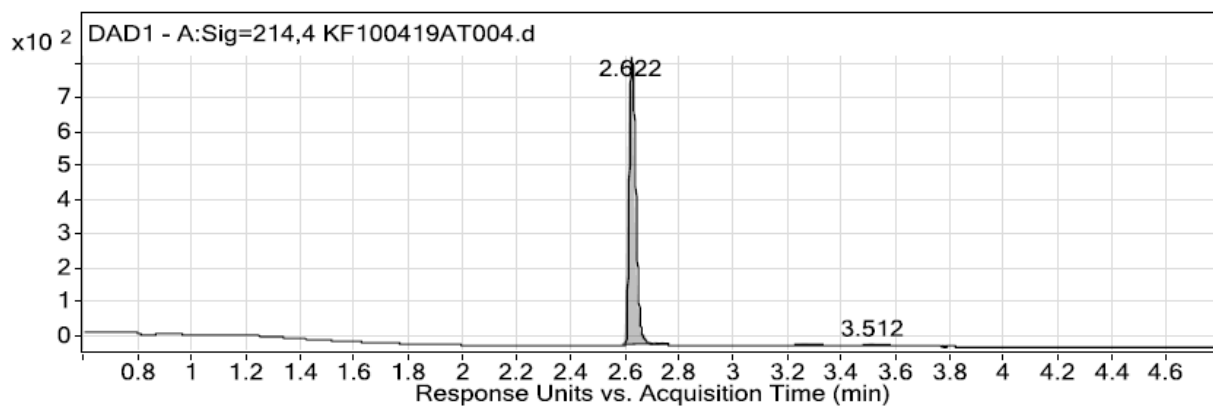


User Chromatogram Peak List

Peak #	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	2.448	619.39	100	1052.92	100	97.63	0.027
2	2.554	9.21	1.49	15.67	1.49	1.45	0.025
3	2.653	1.5	0.24	4.4	0.42	0.41	0.039
4	3.212	2.48	0.4	3.22	0.31	0.3	0.021
5	3.256	1.52	0.25	2.23	0.21	0.21	0.023

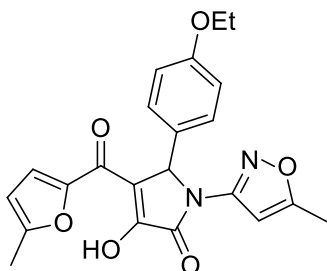


1-Benzyl-5-(3,5-dichlorophenyl)-3-hydroxy-4-(5-methylfuran-2-carbonyl)-1H-pyrrol-2(5H)-one 26 (CID45382103). 3,5-Dichlorobenzaldehyde and benzylamine were reacted according to the general procedure to afford the product as a yellow solid (20 mg, 0.04 mmol, 9% yield). Mp = 106–145 °C; ¹H NMR (CDCl₃) δ 2.67 (s, 3H), 3.62 (d, *J* = 15.2 Hz, 1H), 5.19 (d, *J* = 15.6 Hz, 1H), 5.34 (s, 1H), 6.13 (s, 1H), 7.03 (s, 2H), 7.17 (d, *J* = 6.8 Hz, 2H), 7.24–7.28 (m, 2H), 7.32–7.38 (m, 3H); IR (neat) 1688, 1665, 1613, 1514, 1434, 1406 cm⁻¹; HRMS (ESI) *m/z* calcd for C₂₃H₁₈Cl₂NO₄ ([M+H]⁺), 442.0607, found 442.0604; HPLC purity = 98.7%.

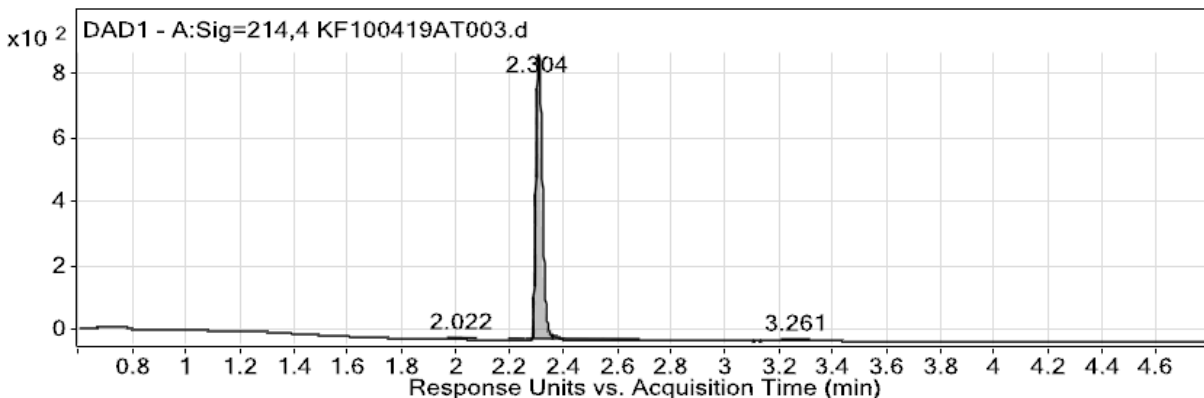


User Chromatogram Peak List

Peak #	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	2.622	845.08	100	1447.68	100	98.65	0.027
2	2.713	4.57	0.54	6.48	0.45	0.44	0.021
3	2.74	4.06	0.48	5.23	0.36	0.36	0.02
4	3.263	1.92	0.23	3.6	0.25	0.25	0.029
5	3.512	3.01	0.36	4.47	0.31	0.3	0.022

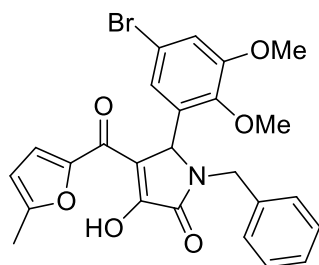


5-(4-Ethoxyphenyl)-3-hydroxy-4-(5-methylfuran-2-carbonyl)-1-(5-methylisoxazol-3-yl)-1H-pyrrol-2(5H)-one 27 (CID4594423). 4-Ethoxybenzaldehyde and 3-amino-5-methylisoxazole were reacted according to the general procedure to afford the product as an orange solid (52 mg, 0.13 mmol, 25% yield). Mp = 113–122 °C; ^1H NMR (DMSO-*d*₆) δ 1.27 (t, J = 7.2 Hz, 3H), 2.27 (s, 3H), 2.32 (s, 3H), 3.93 (q, J = 7.2 Hz, 2H), 5.66 (s, 1H), 6.12 (dd, J = 0.8, 3.2 Hz, 1H), 6.71 (d, J = 8.8 Hz, 2H), 6.77 (d, J = 0.8 Hz, 1H), 7.15 (d, J = 8.8 Hz, 2H), 8.47 (d, J = 3.2 Hz, 1H); ^{13}C NMR (DMSO-*d*₆, APT pulse sequence) δ d 12.1, 13.5, 14.7, 59.3, 95.7, 107.5, 113.1, 117.6, 128.6; u 62.8, 133.0, 152.7, 153.8, 156.4, 157.2, 169.1, 169.4, 171.3; IR (neat) 1710, 1608, 1508, 1425 cm^{-1} ; HRMS (ESI) m/z calcd for $\text{C}_{22}\text{H}_{21}\text{N}_2\text{O}_6$ ($[\text{M}+\text{H}]^+$), 409.1394, found 409.1395; HPLC purity = 97.3%.

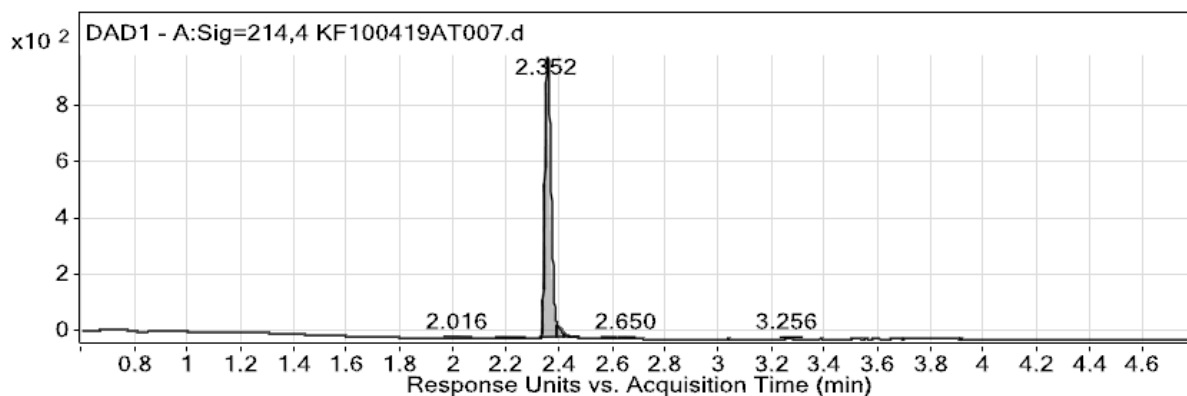


User Chromatogram Peak List

Peak #	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	2.022	3.24	0.36	6.09	0.41	0.4	0.027
2	2.231	1.44	0.16	2.39	0.16	0.16	0.026
3	2.304	888.49	100	1480.17	100	97.29	0.026
4	2.371	11.31	1.27	30.29	2.05	1.99	0.036
5	3.261	1.39	0.16	2.51	0.17	0.17	0.028

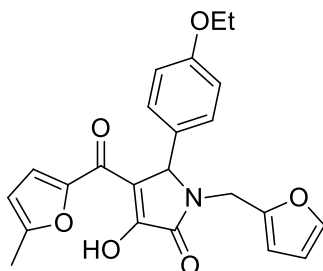


1-Benzyl-5-(5-bromo-2,3-dimethoxyphenyl)-3-hydroxy-4-(5-methylfuran-2-carbonyl)-1H-pyrrol-2(5H)-one 28 (CID45382102). 5-Bromo-2,3-dimethoxybenzaldehyde and benzylamine were reacted according to the general procedure to afford the product as a light orange solid (116 mg, 0.23 mmol, 45% yield). Mp = 160–165 °C; ¹H NMR (DMSO-*d*₆) δ 2.27 (s, 3H), 3.58 (d, *J* = 15.2 Hz, 1H), 3.83 (s, 6H), 4.69 (d, *J* = 14.4 Hz, 1H), 5.56 (s, 1H), 6.19 (dd, *J* = 0.8, 3.2 Hz, 1H), 6.55 (s, 1H), 7.03 (s, 1H), 7.15 (d, *J* = 8.8 Hz, 2H), 7.22–7.32 (complex, 3H), 7.38 (br s, 1H), 8.48 (s, 1H); IR (neat) 1698, 1604, 1511, 1479, 1406 cm⁻¹; HRMS (ESI) *m/z* calcd for C₂₅H₂₃BrNO₆ ([M+H]⁺), 512.0703, found 512.0700; HPLC purity = 95.1%.

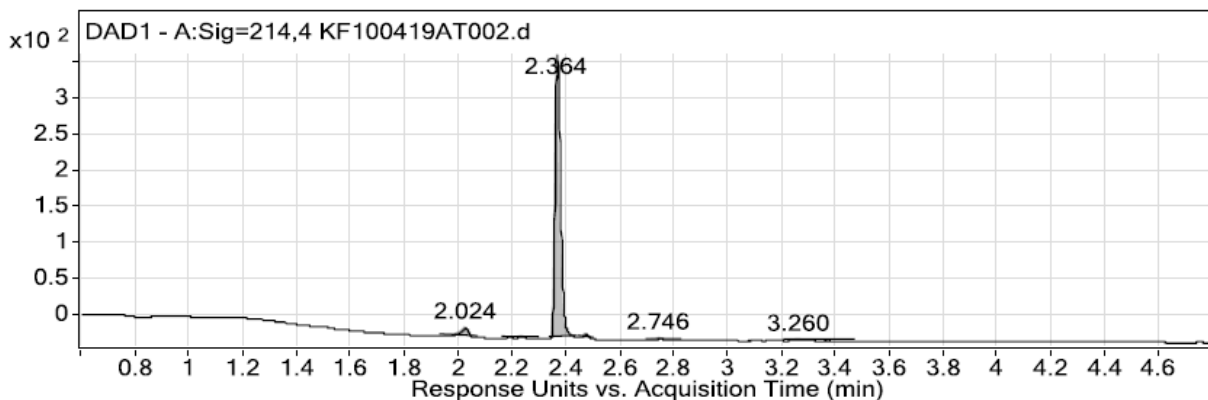


User Chromatogram Peak List

Peak #	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	2.016	3.29	0.33	5.92	0.37	0.35	0.027
2	2.19	2.97	0.3	4.62	0.29	0.27	0.023
3	2.352	998.87	100	1617.79	100	95.1	0.025
4	2.401	35.3	3.53	46.56	2.88	2.74	0.018
5	2.425	10.82	1.08	12.8	0.79	0.75	0.02
6	2.457	4.43	0.44	5.5	0.34	0.32	0.018
7	2.592	1.55	0.16	2.08	0.13	0.12	0.021
8	2.65	2.09	0.21	3.65	0.23	0.21	0.027
9	3.256	1.25	0.13	2.24	0.14	0.13	0.027

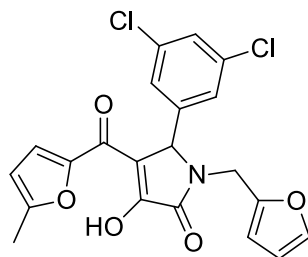


5-(4-Ethoxyphenyl)-1-(furan-2-ylmethyl)-3-hydroxy-4-(5-methylfuran-2-carbonyl)-1H-pyrrol-2(5H)-one 29 (CID4473963). 4-Ethoxybenzaldehyde and 2-(aminomethyl)furan were reacted according to the general procedure to afford the product as an orange solid (13 mg, 0.03 mmol, 6% yield). Mp = 121–138 °C; $^1\text{H NMR}$ (CDCl_3) δ 1.36 (t, $J = 6.9$ Hz, 3H), 2.22 (s, 3H), 3.62 (d, $J = 15.6$ Hz, 1H), 3.93 (q, $J = 6.4$ Hz, 2H), 4.96 (d, $J = 15.6$ Hz, 1H), 5.48 (s, 1H), 5.97 (m, 1H), 6.20 (s, 1H), 6.29 (s, 1H), 6.76 (d, $J = 7.6$ Hz, 2H), 7.07 (m, 1H), 7.13 (d, $J = 8.0$ Hz, 2H), 7.36 (s, 1H); IR (neat) 1713, 1630, 1613, 1512 cm^{-1} ; HRMS (ESI) m/z calcd for $\text{C}_{23}\text{H}_{22}\text{NO}_6$ ($[\text{M}+\text{H}]^+$), 408.1442, found 408.1441; HPLC purity = 92.3%.

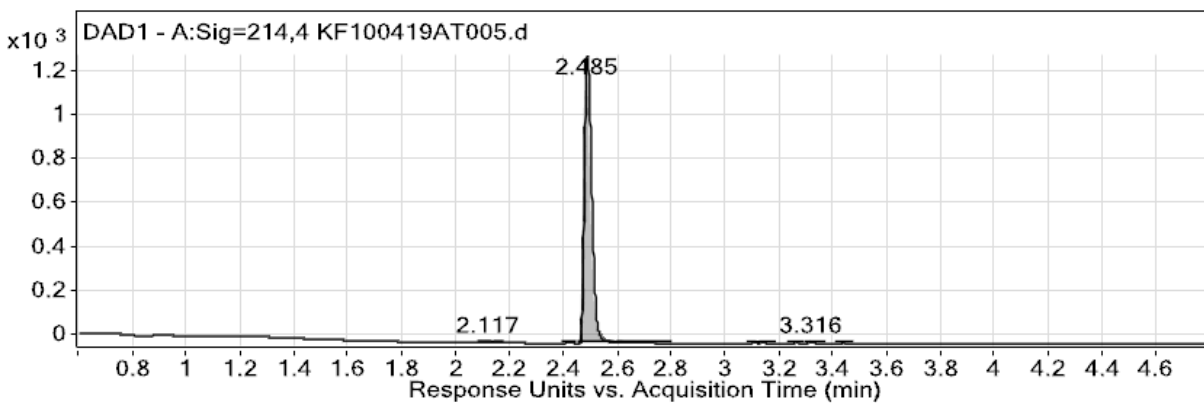


User Chromatogram Peak List

Peak #	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	2.024	11.89	3.07	27	4.92	4.54	0.031
2	2.186	1.04	0.27	1.43	0.26	0.24	0.022
3	2.234	1.96	0.51	3.57	0.65	0.6	0.027
4	2.364	386.71	100	548.49	100	92.32	0.022
5	2.472	4.98	1.29	6.82	1.24	1.15	0.021
6	2.746	2.46	0.64	3.6	0.66	0.61	0.023
7	3.26	1.1	0.28	3.19	0.58	0.54	0.04



5-(3,5-Dichlorophenyl)-1-(furan-2-ylmethyl)-3-hydroxy-4-(5-methylfuran-2-carbonyl)-1H-pyrrol-2(5H)-one 30 (CID45382099). 3,5-Dichlorobenzaldehyde and 2-(aminomethyl)furan were reacted according to the general procedure to afford the product as an orange solid (45 mg, 0.11 mmol, 21% yield). Mp = 124–146 °C; ¹H NMR (CDCl₃) δ 2.01 (s, 3H), 3.75 (d, *J* = 15.6 Hz, 1H), 4.97 (d, *J* = 15.6 Hz, 1H), 5.51 (s, 1H), 6.07 (s, 1H), 6.20 (d, *J* = 3.2 Hz, 1H), 6.31 (dd, *J* = 2.0, 3.2 Hz, 1H), 7.13 (s, 2H), 7.18–7.22 (m, 1H), 7.24 (s, 1H), 7.37 (d, *J* = 1.6 Hz, 1H); IR (neat) 1712, 1632, 1590, 1572, 1513, 1434 cm⁻¹; HRMS (ESI) *m/z* calcd for C₂₁H₁₆Cl₂NO₅ ([M+H]⁺), 432.0406, found 432.0400; HPLC purity = 98.2%.



User Chromatogram Peak List

Peak #	RT	Height	Height %	Area	Area %	Area Sum %	Width
1	2.117	2.17	0.17	3.82	0.15	0.15	0.026
2	2.151	2.01	0.15	2.92	0.12	0.12	0.022
3	2.412	1.05	0.08	1.59	0.06	0.06	0.023
4	2.485	1299.69	100	2475.39	100	98.24	0.03
5	2.611	6.26	0.48	19	0.77	0.75	0.04
6	3.105	2.36	0.18	3.03	0.12	0.12	0.02
7	3.135	2.04	0.16	3.33	0.13	0.13	0.024
8	3.262	1.6	0.12	2.4	0.1	0.1	0.023
9	3.316	4.82	0.37	6.38	0.26	0.25	0.02
10	3.435	1.36	0.1	1.82	0.07	0.07	0.021

¹ Ryabukhin, S. V.; Panov, D. M.; Plaskon, A. S.; Grygorenko, O. O. Approach to the library of 3-hydroxy-1,5-dihydro-2H-pyrrol-2-ones through a three-component condensation. *ACS Comb. Sci.* **2012**, *14*, 631–635.