SUPPLEMENTARY INFORMATION

Identification of non-substrate-like glycosyltransferase inhibitors from library screening: pitfalls & hits

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CONTENT

Table S1: Composition of inhibitor library

Steroids

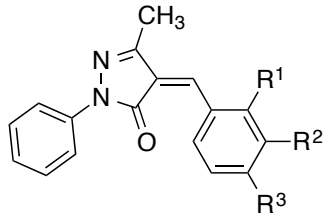
Cmpd	Name	Cmpd	Name
1	Isoflupredone acetate	46	Fluorometholone
2	Norethynodrel	47	Flumethasone
3	Prednisone	48	Medrysone
4	Fulvestrant	49	Alclometasone dipropionate
5	Lynestrenol	50	Norgestrel-(-)-D
6	Danazol	51	Fluocinonide
7	Oxandrolone	52	Clobetasol propionate
8	Triamcinolone	53	Lithocholic acid
9	Dehydrocholic acid	54	Deflazacort
10	Spironolactone	55	Ethynylestradiol 3-methylether
11	Dexamethasone acetate	56	Equilin
12	Canrenoic adic potassium salt	57	Beclomethasone dipropionate
13	Tibolone	58	Digoxigenin
14	Norethindrone	59	Cortisol acetate
15	Ethisterone	60	Adrenosterone
16	Cortisone	61	Ethynodiol diacetate
17	Prednisolone	62	Estrone
18	Methylprednisolone, 6-alpha	63	Dehydroisoandosterone 3-acetate
19	Fludrocortisone acetate	64	Megestrol acetate
20	Chenodiol	65	Deoxycorticosterone
21	Mifepristone	66	Urosiol
22	Betamethasone	67	Canrenone
23	Fusidic acid sodium salt	68	Gestrinone
24	Testosterone propionate	69	Nandrolone
25	Androsterone	70	Proscillaridin A
26	Corticosterone	71	Norgestimate
27	Digitoxigenin	72	Chlormadinone acetate
28	Digoxin	73	Fluticasone propionate
29	Epiandrosterone	74	Alfadolone acetate
30	Estradiol-17 beta	75	Alfaxalone
31	Hydrocortisone base	76	Epitiostanol
32	Stanozolol	77	Rimexolone
33	Progesterone	78	Nomegestrol acetate
34	Budesonide	79	Pancuronium bromide
35	Pregnenolone	80	Prednicarbate
36	Mometasone furoate	81	Cyproterone acetate
37	Exemestane	82	Formestane
38	Diflorasone diacetate	83	Estriol
39	Flunisolide	84	Clocortolone pivalate
40	Flurandrenolide	85	Estradiol Valerate
41	Halcinonide	86	Melengestrol acetate
42	Dydrogesterone	87	Amcinonide

43	Estropipate	88	Ethinylestradiol
44	3-alpha-Hydroxy-5-beta-androstan-17-one	89	Fluocinolone acetonide
45	Finasteride	90	Vecuronium bromide

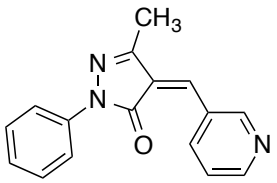
Nucleosides

Cmpd	Name	Cmpd	Name
91	Azaguanine-8	101	Ganciclovir
92	Idoxuridine	102	Abacavir sulfate
93	Acyclovir	103	Stavudine
94	Didanosine	104	Floxuridine
95	Zidovudine, AZT	105	Ribavirin
96	Lamivudine	106	Zalcitabine
97	Valacyclovir hydrochloride	107	Trifluridine
98	(-)-Emtricitabine	108	Famciclovir
99	Penciclovir	109	5-(Indol-6-yl) uridine
100	Vidarabine	110	5-(1-Methyl-indol-4-yl) uridine

Pyrazolones



111-119 & **128-130**



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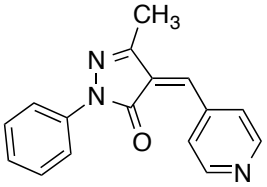

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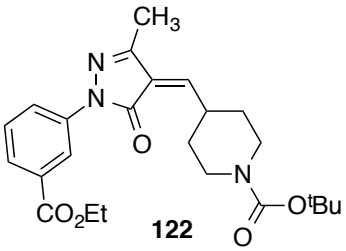

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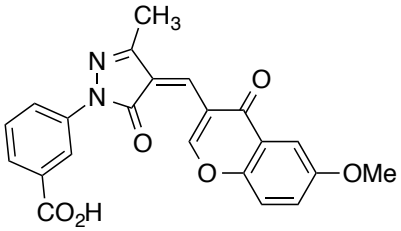

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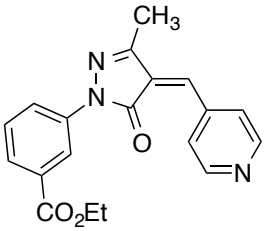

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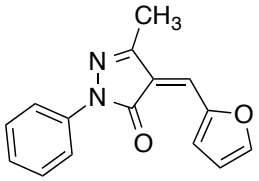

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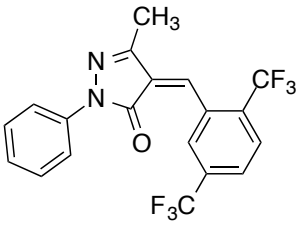

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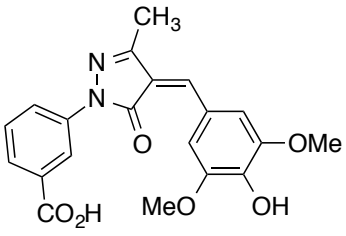
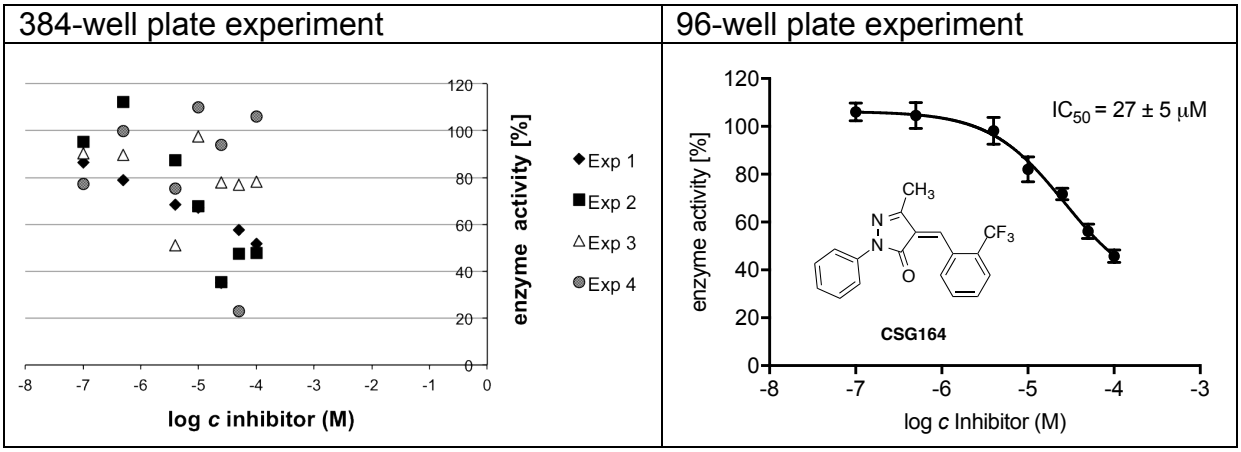


127

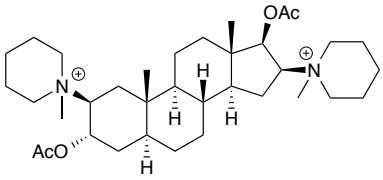
Cmpd	R^1	R^2	R^3
111	H	H	Cl
112	Cl	H	H
113	CH_3	H	H
114	H	H	CH_3
115	H	CH_3	H
116	H	benzyloxy	H
117	H	H	CN
118	H	H	SO_2Me
119	H	OH	H
120	n/a	n/a	n/a
121	n/a	n/a	n/a
122	n/a	n/a	n/a
123	n/a	n/a	n/a
124	n/a	n/a	n/a
125	n/a	n/a	n/a
126	n/a	n/a	n/a
127	n/a	n/a	n/a
128	OH	H	H
129	H	H	OH
130	H	NO_2	H

Fig. S1 Attempted assay miniaturisation with prototype inhibitor **CSG164**[a]



[a]Inhibition assays were carried out under standard conditions. LgtC, $MnCl_2$ (5 mM), chicken egg-white lysozyme (CEL, 1 mg/mL), calf-intestinal phosphatase (CIP, 10 U/mL), Triton X-100 (0.01%), and HEPES buffer (13 mM, pH 7.0) were combined with β-lactose acceptor (2 mM) or buffer (control). Inhibitor (0-100 μM) in DMSO or DMSO only (15 μL, control) was added. The reaction was started by addition of UDP-Gal donor (28 μM) and incubated for 20 mins at 30°C. Malachite Green reagent A was added, and the microplate was shaken carefully. Malachite Green reagent B was added, and the absorbance was recorded at 620 nm. Data were analysed with GraphPad Prism. The 96-well plate experiment was carried out in triplicate.

Fig. S2 LgtC assay results and control experiments for false positive steroid "hits" **79** and **90**



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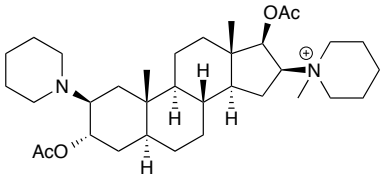
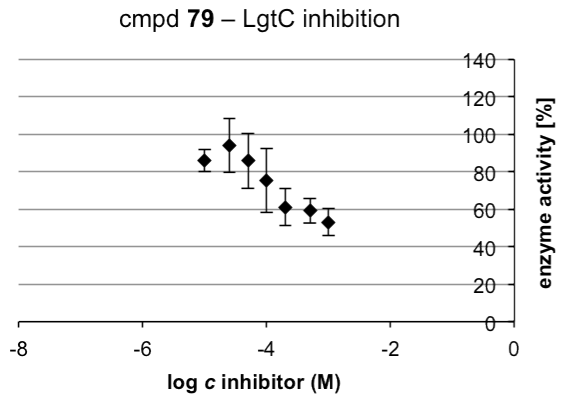
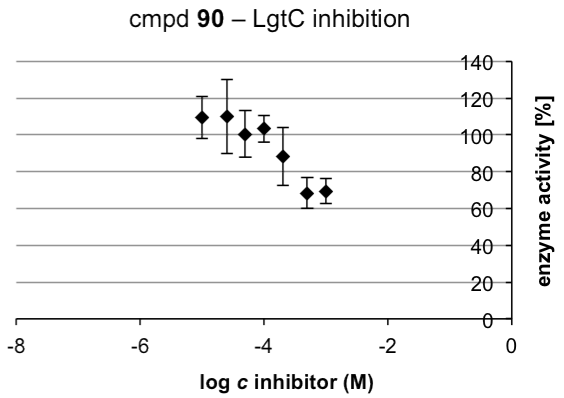


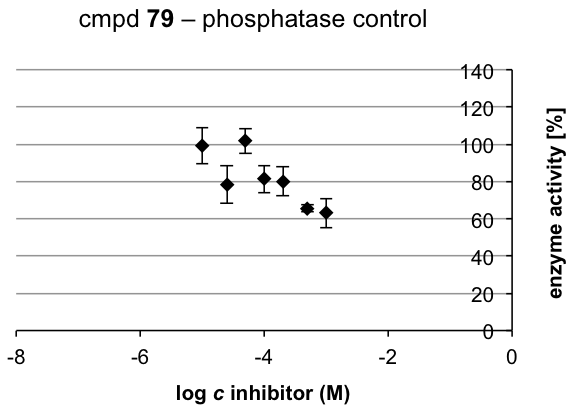
90

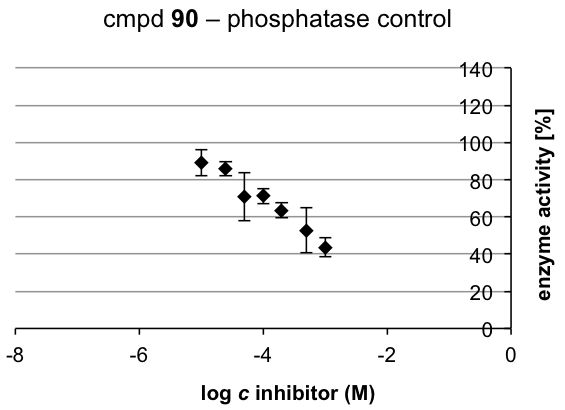
LgtC inhibition assay: IC_{50} experiments



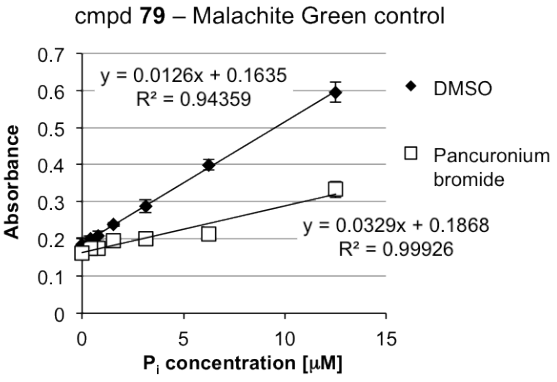


Control experiment: phosphatase reaction





Control experiment: Malachite Green detection



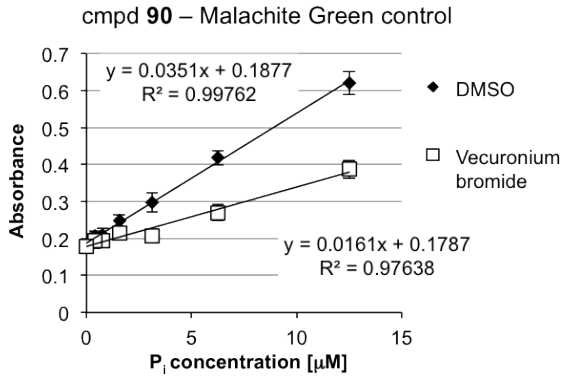
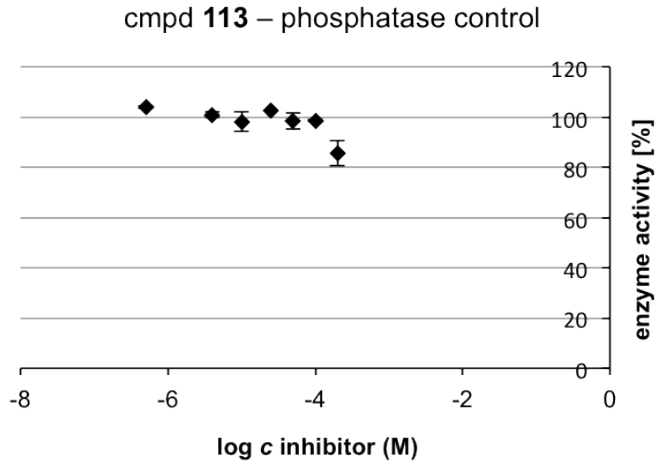


Fig. S3 Control experiments with 5-methyl pyrazol-3-one **113** (YX051)

Control experiment: phosphatase reaction



Control experiment: Malachite Green detection

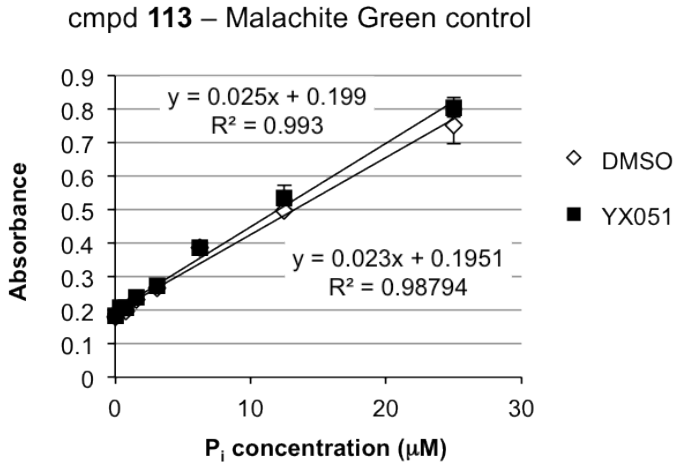
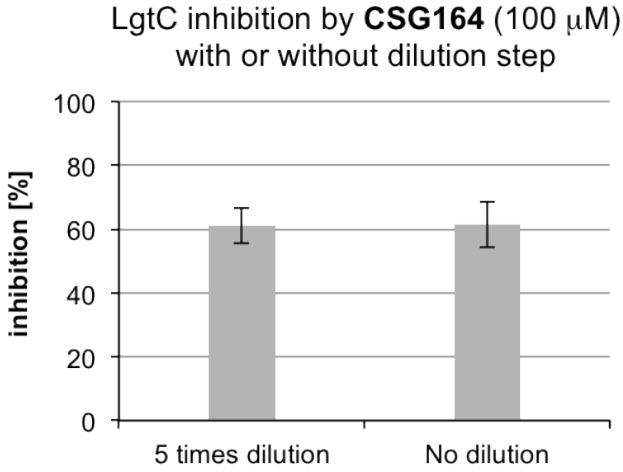
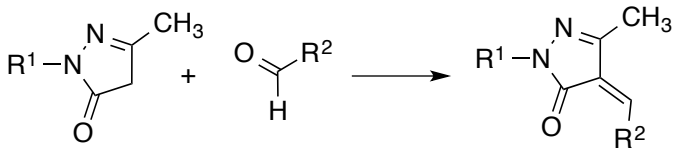


Fig. S4 Validation of the assay dilution step with **CSG164**/LgtC



Synthesis and characterisation of 5-methyl pyrazol-3-ones **111-130**



(Z)-4-(4-Chlorobenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (111). The title compound was obtained as an orange solid (180 mg, 0.61 mmol, 30%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (348 mg, 2.0 mmol) and 4-chlorobenzaldehyde (562 mg, 4.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.28 (s, 3H), 7.10-7.15 (m, 1H), 7.19 (s, 1H), 7.32-7.37 (m, 2H), 7.40 (d, *J* = 8.7 Hz, 2H), 7.84-7.89 (m, 2H), 8.40 (d, *J* = 8.6 Hz, 2H). **^{13}C-NMR** (100 MHz, DMSO) δ (ppm): 13.1, 118.4, 124.7, 127.1, 128.8, 128.9, 131.8, 135.3, 137.9, 138.0, 146.7, 151.8, 161.4. **ESI-MS**: m/z 297.1 (100%) $[M+H]^+$, 329.1 (18%) $[M+MeOH+H]^+$; **HR-MS**: m/z 297.0792 $[M+H]^+$, 297.0789 calcd. for $[C_{17}H_{14}ClN_2O]^+$.

(Z)-4-(2-Chlorobenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (112). The title compound was obtained as an orange solid (98 mg, 0.33 mmol, 33%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (174 mg, 1.0 mmol) and 2-chlorobenzaldehyde (281 mg, 2.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.41 (s, 3H), 7.16-7.26 (m, 1H), 7.55-7.36 (m, 5H), 7.88 (s, 1H), 7.95 (dd, *J* = 8.8, 1.1 Hz, 2H), 8.91 (dd, *J* = 7.8, 1.8 Hz, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.3, 119.0, 125.0, 126.7, 128.8, 128.9, 129.7, 130.1, 133.4, 133.5, 136.4, 138.2, 141.9, 150.8, 161.5. **ESI-MS**: m/z 297.1 (100%) $[M+H]^+$, 329.1 (50%) $[M+MeOH+H]^+$, 351.1 (30%) $[M+MeOH+Na]^+$; **HR-MS**: m/z 297.0793 $[M+H]^+$, calcd for 297.0789 calcd. for $[C_{17}H_{14}ClN_2O]^+$.

(Z)-5-Methyl-4-(2-methylbenzylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (113). 5-Methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (174 mg, 1.0 mmol) and 2-methylbenzaldehyde (240 mg, 2.0 mmol) were dissolved in glacial acetic acid. The reaction mixture was stirred at 110 ^{o}C until TLC showed complete consumption of the starting material. The target compound was obtained as an orange solid in 45% yield (120 mg) after column chromatography. **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.39 (s, 3H), 2.51 (s, 3H), 7.20 (t, *J* = 7.4 Hz, 1H), 7.32 (m, 1H), 7.35 (d, *J* = 7.4 Hz, 1H), 7.38-7.47 (m, 3H), 7.74 (s, 1H), 7.96 (d, *J* = 7.7 Hz, 2H), 8.65 (d, *J* = 7.6 Hz, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.3, 20.2, 118.9, 124.8, 126.0, 127.7, 128.8, 130.5, 130.9, 131.9, 132.6, 138.3, 139.4, 144.3, 150.7, 161.7. **ESI-MS**: m/z 277.1 (100%) $[M+H]^+$; **HR-MS**: m/z 277.1339 $[M+H]^+$, 277.1335 calcd. for $[C_{18}H_{17}N_2O]^+$.

(Z)-5-Methyl-4-(4-methylbenzylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (114). The title compound was obtained as an orange solid (67 mg, 0.24 mmol, 46%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (92 mg, 0.53 mmol) and 4-methylbenzaldehyde (127 mg, 2.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.37 (s, 3H), 2.47 (s, 3H), 7.21 (t, *J* = 7.4 Hz, 1H), 7.34 (d, *J* = 8.2 Hz, 2H), 7.39 (s, 1H), 7.44 (t, *J* = 8.0 Hz, 2H), 7.99 (d, *J* = 7.7 Hz, 2H), 8.46 (d, *J* = 8.2 Hz, 2H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.4, 22.0, 119.1, 124.8, 126.7, 128.8, 129.6, 130.5, 134.0, 138.4, 144.46, 147.1, 150.9, 162.0. **ESI-MS**: m/z 277.1 (100%) $[M+H]^+$; **HR-MS**: m/z 277.1340 $[M+H]^+$, 277.1335 calcd. for $[C_{18}H_{17}N_2O]^+$.

(Z)-5-Methyl-4-(3-methylbenzylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (115). The title compound was obtained as an orange solid (115 mg, 0.42 mmol, 42%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (174 mg, 1.0 mmol) and 3-methylbenzaldehyde (240 mg, 2.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.38 (s, 3H), 2.47 (s, 3H), 7.21 (t, *J* = 7.4 Hz, 1H), 7.37-7.47 (m, 5H), 7.98 (m, 2H), 8.31 (s, 1H), 8.38 (d, *J* = 7.5 Hz, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.4, 21.4, 119.2, 124.9, 127.5, 128.7, 128.8, 130.9, 132.9, 134.0, 134.3, 138.4, 138.5, 147.3, 150.9, 161.9. **ESI-MS**: m/z 277.1 (100%) $[M+H]^+$; **HR-MS**: m/z 277.1340 $[M+H]^+$, 277.1335 calcd. for $[C_{18}H_{17}N_2O]^+$.

(Z)-4-(3-(Benzyloxy)benzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (116). The title compound was obtained as an orange solid (77 mg, 0.21 mmol, 37%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (100 mg, 0.57 mmol) and 3-(benzyloxy)benzaldehyde (220 mg, 1.04 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.34 (s, 3H), 5.19 (s, 2H), 7.21 (t, *J* = 7.4 Hz, 1H), 7.29 (dd, *J* = 8.1, 2.3 Hz, 1H), 7.33-7.38 (m, 1H), 7.39-7.45 (m, 4H), 7.47 (d, *J* = 3.4 Hz, 1H), 7.50 (d, *J* = 7.9 Hz, 3H), 7.80 (s, 1H), 7.91 (d, *J* = 7.7 Hz, 2H), 8.05 (d, *J* = 7.7 Hz, 1H), 8.58 (s, 1H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ (ppm): 13.1, 69.4, 118.4, 118.7, 120.1, 124.6, 126.8, 127.0, 128.0, 128.0, 128.5, 128.8, 129.7, 134.2, 136.6, 138.1, 148.2, 151.8, 158.2, 161.4. **ESI-MS**: m/z 369.2 (100%) $[M+H]^+$, 391.3 (35%) $[M+Na]^+$; **HR-MS**: m/z 369.1599 $[M+H]^+$, 369.1598 calcd. for $[C_{24}H_{21}N_2O_2]^+$.

(Z)-4-((3-Methyl-5-oxo-1-phenyl-1,5-dihydro-4H-pyrazol-4-ylidene)methyl)benzonitrile (117). The title compound was obtained as an orange solid (26 mg, 0.09 mmol, 18%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (87 mg, 0.5 mmol) and 4-formylbenzonitrile (131 mg, 1.0 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.34 (s, 3H), 7.18-7.24 (m, 1H), 7.40-7.46 (m, 2H), 7.85-7.90 (m, 3H), 7.99-8.04 (m, 2H), 8.61-8.65 (m, 2H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ (ppm): 13.1, 114.1, 118.4, 124.9, 128.9, 129.1, 132.3, 133.4, 136.7, 137.9, 145.5, 151.7, 161.1. **ESI-MS**: m/z 288.1 (100%) $[M+H]^+$; **HR-MS**: m/z 288.1134 $[M+H]^+$, 288.1131 calcd. for $[C_{17}H_{15}N_2O_2]^+$.

(Z)-5-Methyl-4-(4-(methylsulfonyl)benzylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (118). The title compound was obtained as an orange solid (29 mg, 0.09 mmol, 17%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (87 mg, 0.5 mmol) and 4-(methylsulfonyl)benzaldehyde (184 mg, 1.0 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.36 (s, 3H), 3.31 (s, 3H), 7.21 (m, 1H), 7.45 (m, 2H), 7.88 (m, 2H), 7.95 (s, 1H), 8.08 (d, *J* = 8.6 Hz, 2H), 8.68 (d, *J* = 8.6 Hz, 2H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ (ppm): 13.1, 43.2, 118.4, 124.9, 126.9, 128.9, 129.1, 133.6, 137.0, 137.9, 143.3, 145.7, 151.8, 161.1. **ESI-MS**: m/z 341.1 (100%) $[M+H]^+$; **HR-MS**: m/z 341.1102 $[M+H]^+$, 341.1106 calcd. for $[C_{18}H_{17}N_2O_3S]^+$.

(Z)-4-(3-Hydroxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (119). The title compound was obtained as an orange solid (22 mg, 0.08 mmol, 15%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (87 mg, 0.5 mmol) and 3-hydroxybenzaldehyde (122 mg, 1.0 mM). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.34 (s, 3H), 7.07 (m, 1H), 7.19 (t, *J* = 7.4 Hz, 1H), 7.33-7.43 (m, 4H), 7.60 (d, *J* = 7.7 Hz, 1H), 7.90 (d, *J* = 7.6 Hz, 2H), 8.52 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.4, 119.2, 119.7, 121.1, 125.4, 127.2, 127.8, 129.0, 129.1, 130.0, 134.2, 138.2, 147.5, 151.3, 156.2, 161.9. **ESI-MS**: m/z 279.1 (100%) $[M+H]^+$; **HR-MS**: m/z 279.1102 $[M+H]^+$, 279.1105 calcd. for $[C_{17}H_{15}N_2O_2]^+$.

(Z)-5-Methyl-2-phenyl-4-(pyridin-3-ylmethylene)-2,4-dihydro-3H-pyrazol-3-one (120). The title compound was obtained as an orange solid (106 mg, 0.41 mmol, 27%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (261 mg, 1.5 mmol) and nicotinaldehyde (321 mg, 3.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.39 (s, 3H), 7.22 (t, *J* = 7.4 Hz, 1H), 7.36 (s, 1H), 7.40-7.50 (m, 3H), 7.95 (dd, *J* = 8.6, 1.0 Hz, 2H), 8.73 (dd, *J* = 4.8, 1.6 Hz, 1H), 9.03 (d, *J* = 2.1 Hz, 1H), 9.49 (m, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.3, 119.1, 123.6, 125.2, 128.9, 129.0, 130.0, 138.1, 139.5, 142.4, 150.4, 152.8, 154.3, 161.6. **ESI-MS**: m/z 264.1 (55%) $[M+H]^+$, 296.1 (100%) $[M+MeOH+H]^+$; **HR-MS**: m/z 264.1134 $[M+H]^+$, 264.1131 calcd. for $[C_{16}H_{14}N_3O]^+$.

(Z)-5-Methyl-2-phenyl-4-(pyridin-4-ylmethylene)-2,4-dihydro-3H-pyrazol-3-one (121). The title compound was obtained as an orange solid (47 mg, 0.18 mmol, 18%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (174 mg, 1.0 mmol) and isonicotinaldehyde (214 mg, 2.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.30 (s, 3H), 7.14 (t, *J* = 7.4 Hz, 1H), 7.19 (s, 1H), 7.35 (t, *J* = 7.9 Hz, 2H), 7.84 (d, *J* = 7.8 Hz, 2H), 8.12 (d, *J* = 4.8 Hz, 2H), 8.73 (d, *J* = 6.1 Hz, 2H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.2, 119.1, 123.3, 125.3, 125.5, 128.9, 131.9, 137.9, 139.3, 142.6, 150.2, 161.0. **ESI-MS**: m/z 264.1 (100%) $[M+H]^+$; **HR-MS**: m/z 264.1129 $[M+H]^+$, 264.1131 calcd. for $[C_{16}H_{14}N_3O]^+$.

^{t}Butyl (Z)-4-((1-(3-(ethoxycarbonyl)phenyl)-3-methyl-5-oxo-1,5-dihydro-4H-pyrazol-4-ylidene)methyl)piperidine-1-carboxylate (122). The title compound was obtained as an orange solid (202 mg, 0.46 mmol, 46%) from ethyl 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoate (174 mg, 1.0 mmol) and ^{t}butyl 4-formylpiperidine-1-carboxylate (426 mg, 2.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.44 (t, *J* = 7.1 Hz, 3H), 1.45-1.50 (m, 11H), 1.79-1.82 (br, 2H), 2.27(s, 3H), 2.91(br, 2H), 4.04 (m, 1H), 4.14 (br, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 6.69 (d, *J* = 9.9 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 9.0 Hz, 1H), 8.18 (d, *J* = 9.3 Hz, 1H), 8.56 (s, 1H). **ESI-MS**: m/z 442.2 (100%) $[M+H]^+$; **HR-MS**: m/z 442.2270 $[M+H]^+$, 442.2264 calcd. for $[C_{24}H_{32}N_3O_5]^+$.

(Z)-3-(4-((6-Methoxy-4-oxo-4H-chromen-3-yl)methylene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (123). The title compound was obtained as an orange solid (57 mg, 0.08 mmol, 14%) from 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (130.9 mg, 0.6 mmol) and 6-methoxy-4-oxo-4H-chromene-3-carbaldehyde (183.8 mg, 0.9 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.35 (s, 3H), 3.88 (s, 3H), 7.46-7.49 (m, 2H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.74-7.79 (m, 2H), 7.87 (s, 1H), 8.12-8.15 (m, 1H), 8.49 (s, 1H), 10.42 (s, 1H), 13.12 (br, 1H). **ESI-MS**: m/z 405.1 (100%) $[M+H]^+$; **HR-MS**: m/z 405.1082 $[M+H]^+$, 405.1081 calcd. for $[C_{22}H_{17}N_2O_6]^+$.

Ethyl (Z)-3-(3-methyl-5-oxo-4-(pyridin-4-ylmethylene)-4,5-dihydro-1H-pyrazol-1-yl)benzoate (124). The title compound was obtained as an orange solid (60 mg, 0.18 mmol, 18%) from ethyl 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoate (246 mg, 1.0 mmol) and isonicotinaldehyde (214 mg, 2.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 1.44 (t, *J* = 7.1 Hz, 3H), 2.41 (s, 3H), 4.43 (q, *J* = 7.1 Hz, 2H), 7.34 (s, 1H), 7.53-7.47 (m, 1H), 7.93-7.87 (m, 1H), 8.24-8.14 (m, 3H), 8.57 (s, 1H), 8.84 (d, *J* = 6.1 Hz, 2H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.3, 14.4, 61.2, 119.9, 123.0, 125.3, 126.2, 129.0, 131.3, 131.5, 138.1, 138.8, 143.3, 150.3, 150.6, 150.7, 161.2. **ESI-MS**: m/z 336.1 (100%) $[M+H]^+$; **HR-MS**: m/z 336.1346 $[M+H]^+$, 336.1343 calcd. for $[C_{19}H_{18}N_3O_3]^+$.

(Z)-4-(Furan-2-ylmethylene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (125). The title compound was obtained as a red solid (472 mg, 1.87 mmol, 92%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (348 mg, 2.0 mmol) and furan-2-carbaldehyde (384 mg, 4.0 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H), 6.94 (m, 1H), 7.19 (t, *J* = 7.4 Hz, 1H), 7.41-7.47 (m, 2H), 7.71 (s, 1H), 7.90-7.93 (m, 2H), 8.27 (d, *J* = 1.6 Hz, 1H), 8.64 (d, *J* = 3.7 Hz, 1H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ (ppm): 12.8, 115.0, 117.8, 118.2, 121.4, 124.5, 128.9, 130.5, 138.3, 150.2, 150.5, 161.6. **ESI-MS**: m/z 253.1 (100%) $[M+H]^+$; **HR-MS**: m/z 253.0812 $[M+H]^+$, 253.0818 calcd. for $[C_{15}H_{13}N_2O_2]^+$.

(Z/E)-4-(2,5-Bis(trifluoromethyl)benzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (126). The title compound (Z:E = 3:1) was obtained as an orange solid (95 mg, 0.24 mmol, 40%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (104 mg, 0.6 mmol) and 2,5-bis(trifluoromethyl)-benzaldehyde (290 mg, 1.2 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 1.79 (s, 3H), 2.35 (s, 3H), 7.19 (t, *J* = 8.4 Hz, 1H), 7.24 (t, *J* = 7.4 Hz, 1H), 7.38-7.45 (m, 2H), 7.45-7.51 (m, 2H), 7.78 (d, *J* = 7.6 Hz, 2H), 7.87 (d, *J* = 7.6 Hz, 2H), 7.99 (d, *J* = 2.3 Hz, 1H), 8.05-8.19 (m, 5H), 8.30 (s, 1H), 8.50 (s, 1H). **ESI-MS**: m/z 399.1 (100%) $[M+H]^+$; **HR-MS**: m/z 399.0867 $[M+H]^+$, 399.0855 calcd. for $[C_{19}H_{13}F_6ON_2]^+$.

(Z)-3-(4-(4-Hydroxy-3,5-dimethoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (127). The title compound was obtained as an orange solid (40 mg, 0.10 mmol, 15%) from 3-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid (153 mg, 0.7 mmol) and syringaldehyde (191 mg, 1.05 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.34 (s, 3H), 3.89 (s, 6H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.75-7.77 (m, 2H), 8.24 (d, *J* = 8.0 Hz, 1H), 8.28 (s, 2H), 8.50 (s, 1H), 10.05 (br, 1H), 13.11 (br, 1H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ (ppm): 13.2, 56.1, 112.8, 118.9, 122.3, 122.5, 123.9, 125.0, 129.2, 131.4, 138.6, 142.5, 147.5, 149.8, 152.2, 162.2, 167.0. **ESI-MS**: m/z 383.1 (100%) $[M+H]^+$, 405.1 (15%) $[M+Na]^+$; **HR-MS**: m/z 383.1239 $[M+H]^+$, 383.1238 calcd. for $[C_{20}H_{19}O_6N_2]^+$.

(Z)-4-(2-Hydroxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (128). The title compound was obtained as an orange solid (14 mg, 0.05 mmol, 10%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (87 mg, 0.5 mmol) and 2-hydroxybenzaldehyde (122 mg, 1.0 mmol). **^{1}H-NMR** (400 MHz, $CDCl_3$) δ (ppm): 2.44 (s, 3H), 7.05 (m, 1H), 7.19 (m, 1H), 7.28 (t, *J* = 7.4 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 2H), 7.53 – 7.57 (m, 2H), 7.61 (s, 1H), 8.00 (d, *J* = 7.7 Hz, 2H), 10.58 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.9, 119.7, 120.9, 122.4, 123.3, 124.6, 126.00, 129.0, 136.0, 137.1, 137.7, 146.1, 152.0, 161.1, 164.1. **ESI-MS**: m/z 279.1 (100%) $[M+H]^+$; **HR-MS**: m/z 279.1102 $[M+H]^+$, 279.1105 calcd. for $[C_{17}H_{15}N_2O_2]^+$.

(Z)-4-(4-Hydroxybenzylidene)-5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (129). The title compound was obtained as an orange solid (20 mg, 0.07 mmol, 13%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (87 mg, 0.5 mmol) and 4-hydroxybenzaldehyde (122 mg, 1.0 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H), 6.95 (d, *J* = 8.2 Hz, 2H), 7.19 (t, *J* = 7.4 Hz, 1H), 7.43 (t, *J* = 8.0 Hz, 2H), 7.71 (s, 1H), 7.93 (d, *J* = 7.7 Hz, 2H), 8.65 (m, 2H), 10.85 (s, 1H). **^{13}C-NMR** (100 MHz, DMSO-d_6) δ (ppm): 13.1, 115.8, 118.2, 122.6, 124.3, 123.9, 128.8, 137.40,138.4, 148.6, 151.8, 161.9, 163.0. **ESI-MS**: m/z 279.1 (100%) $[M+H]^+$; **HR-MS**: m/z 279.1102 $[M+H]^+$, 279.1105 calcd. for $[C_{17}H_{15}N_2O_2]^+$.

(Z)-5-Methyl-4-(3-nitrobenzylidene)-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (130). The title compound was obtained as an orange solid (18 mg, 0.06 mmol, 11%) from 5-methyl-2-phenyl-2,4-dihydro-3H-pyrazol-3-one (87 mg, 0.5 mmol) and 3-nitrobenzaldehyde (151 mg, 1.0 mmol). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ (ppm): 2.38 (s, 3H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 2H), 7.85-7.91 (m, 3H), 8.04 (s, 1H), 8.45 (m, 1H,), 8.86 (d, *J* = 8.0 Hz, 1H), 9.62 (s, 1H). **^{13}C-NMR** (100 MHz, $CDCl_3$) δ (ppm): 13.1, 118.5, 124.9, 126.9, 127.3, 128.8, 128.9, 130.1, 134.0, 137.8, 139.3, 145.4, 147.8, 151.8, 161.2. **ESI-MS**: m/z 308.1 (80%) $[M+H]^+$, 340.1 (100%) $[M+MeOH+H]^+$; **HR-MS**: m/z 308.1032 $[M+H]^+$, 308.1030 calcd. for $[C_{17}H_{13}N_3O_3]^+$.

^{1}H- and ^{13}C-NMR spectra of 5-methyl pyrazol-3-one **113**