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Aryl methylene ketones and fluorinated methylene ketones as reversible inhibitors for severe acute respiratory syndrome (SARS) 3C-like proteinase

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Abstract

The severe acute respiratory syndrome (SARS) virus depends on a chymotrypsin-like cysteine proteinase ($3CL^{pro}$) to process the translated polyproteins to functional viral proteins. This enzyme is a target for the design of potential anti-SARS drugs. A series of ketones and corresponding mono- and di-fluoro ketones having two or three aromatic rings were synthesized as possible reversible inhibitors of SARS $3CL^{pro}$. The design was based on previously established potent inhibition of the enzyme by oxa analogues (esters), which also act as substrates. Structure–activity relationships and modeling studies indicate that three aromatic rings, including a 5-bromopyridin-3-yl moiety, are key features for good inhibition of SARS $3CL^{pro}$. Compound **11d**, 2-(5-bromopyridin-3-yl)-1-(5-(4-chlorophenyl)furan-2-yl)ethanone and its α-monofluorinated analogue **12d**, gave the best reversible inhibition with IC_{50} values of 13 μM and 28 μM, respectively. In contrast to inhibitors having two aromatic rings, α-fluorination of compounds with three rings unexpectedly decreased the inhibitory activity.

Keywords: SARS coronavirus; 3C-like proteinase; Ketones; Fluorinated ketones; Reversible inhibitors

1. Introduction

Severe acute respiratory syndrome (SARS) is a life-threatening form of atypical pneumonia that rapidly spread in Asia, North America and Europe in early 2003 [1,2]. SARS is characterized by high fever, malaise, rigor, headache and non-productive cough or dyspnea and may progress to generalized interstitial infiltrates in the lung, requiring intubation and mechanical ventilation [3]. The causative agent of SARS is a coronavirus (SARS-CoV) [1,2,4]. Its 3C-like proteinase ($3CL^{pro}$), a chymotrypsin-like cysteine proteinase which is essential for viral replication, has been recognized as a key target for anti-SARS drug design [4]. The active site of SARS-CoV $3CL^{pro}$ has a catalytic dyad with Cys145 as the nucleophile and His41 as the general base [4,5]. This proteinase cleaves the initially translated polyproteins pp1a and pp1ab at 11 conserved interdomain sites, in which the P1 position has a well-conserved Gln residue, and the P2 position has a hydrophobic amino acid residue [6].

Currently, there are no effective anti-viral agents for the treatment of SARS. However, rational design and library-based screening have identified some small molecules that are potent inhibitors of $3CL^{pro}$, such as the HIV proteinase inhibitor TL-3 [7], bifunctional aryl boronic acids [8], thiophenylcarboxylate [9], keto-glutamine analogues [10a], AG7088 analogues [11,12a], anilides [12b], benzotriazole

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esters [12c] and pyridinyl esters [10b]. In our previous studies, we demonstrated that a series of 5-halopyridin-3-yl aromatic esters act as very potent covalent inhibitors of $3CL^{pro}$ with IC_{50} values in the low nanomolar range (Fig. 1) [10b]. These compounds initially bind competitively and strongly to the active site, but are then hydrolyzed by the enzyme as substrates and released. Despite their potent inhibition of $3CL^{pro}$ and relatively long half life in buffer at neutral pH, they are likely to be problematic as drug candidates because of the propensity of esters to be rapidly hydrolyzed by lipases, esterases and other enzymes in mammalian cells. These compounds and many of the other reported inhibitors of SARS-CoV $3CL^{pro}$ can potentially also react non-specifically with other thiols or nucleophiles in mammalian cells, thereby leading to toxicity.

In order to develop stable and non-covalent inhibitors based on these pyridinyl esters, the ketone **5** was initially investigated as a potential SARS-CoV $3CL^{pro}$ inhibitor (Fig. 2). The carbonyl of this relatively unreactive compound could form a hemithioacetal with the enzyme's active site thiol, but this should be a reversible process.

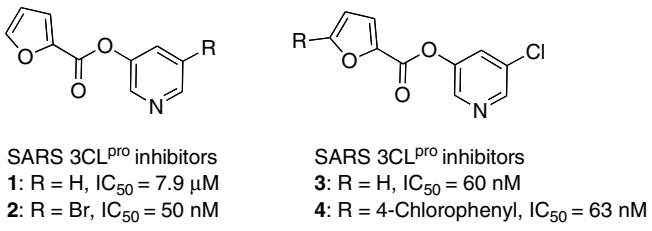


Fig. 1. Pyridinyl esters **1**–**4** as potent SARS $3CL^{pro}$ inhibitors.

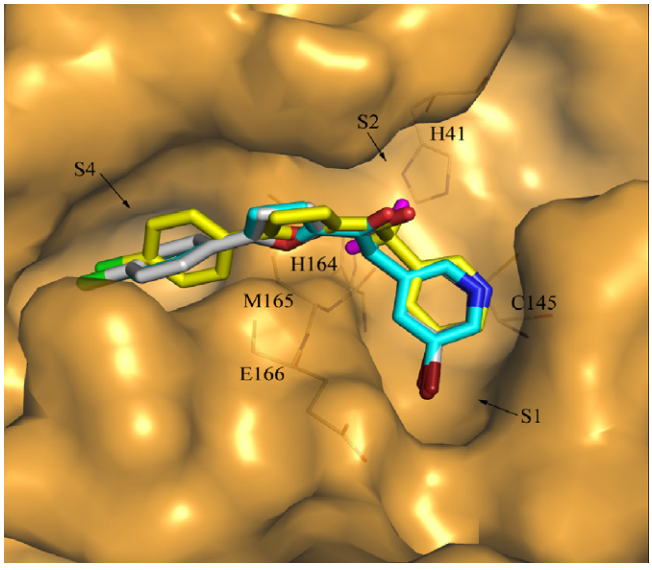


Fig. 2. The modeled binding conformations of **11d** (white carbon sticks), **12d** (cyan carbon sticks) and **13d** (yellow carbon sticks) in the active site of SARS-Cov $3CL^{pro}$ (oxygen atoms are red; nitrogen atoms are blue; chlorine atoms are green; bromine atoms are maroon; and fluorine atoms are purple).

Disappointingly, compared to its oxa analogue, the pyridinyl ester **1** (91% inhibition at 100 μM concentration, $IC_{50} = 7.9$ μM, Fig. 1), the ketone analogue **5** displays no inhibition at 100 μM concentration [10b]. However, it seemed that corresponding fluorinated ketones might be better inhibitors, as they possess the combined features of a more electrophilic carbonyl group and a chemical structure that fits the enzyme active site. The fluorinated ketones should be more stable in vivo than the corresponding pyridinyl esters, and would also avoid the hydrolysis problem that leads to the reactivation of the enzyme [10b]. Furthermore, the difluoromethylene moiety has been proposed as a mimic of an oxygen atom in biological systems [13]. In the present work, we examine a group of methylene ketones and corresponding mono- and di-fluorinated methylene ketones as potential SARS-CoV $3CL^{pro}$ inhibitors.

2. Materials and methods

2.1. General

All reactions involving air or moisture sensitive reactants were done under argon. All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. Solvents were dried for anhydrous reactions. All reactions and fractions from column chromatography were monitored by thin layer chromatography (TLC) using glass plates with a UV fluorescent indicator (normal SiO_2, Merck 60 F_{254}). Flash chromatography was performed using Merck type 60, 230–400 mesh silica gel. Nuclear magnetic resonance (NMR) spectra were obtained on Inova Varian 300, 400 and 500 MHz spectrometers. All the protons were assigned based on the structure numbering system indicated in the paper. The enzyme assay, mass spectrometry of enzyme–inhibitor complexes and molecular docking were performed as described in the literature [10a,10b]. Briefly, the assay was performed at 22 °C in a solution containing 20 mM Bis–TRIS at pH 7.0, 2 mM DTT, 10 μM fluorogenic substrate (Abz-SVTLQSG-Tyr (NO_2)R, 93% purity), 0.2 μM non-tagged $3CL^{pro}$ and 1% DMSO without any preincubation. The increase in fluorescence (λ_{ex} 340 nm, λ_{em} 420 nm) was monitored using a spectrofluorimeter. Initial rates were calculated using the first 5 min of the progress curves.

2.2. 2-Fluoro-2-(pyridin-3-yl)-1-(thiophen-2-yl)ethanone (6) and 2,2-difluoro-2-(pyridin-3-yl)-1-(thiophen-2-yl)-ethanone (7)

To a solution of **5** [10b] (0.14 g, 0.70 mmol) in dry THF (25 mL) at −78 °C was added LiHMDS (0.84 mL, 1.0 M solution in THF, 0.84 mmol) dropwise over 15 min. After 1.5 h of stirring at − 78 °C, a solution of *N*-fluorobenzenesulfonimide (NFSi) (0.24 g, 0.77 mmol) in THF (5 mL) was added slowly. The mixture was stirred at −78 °C for 6 h and then quenched with 1 M aqueous HCl (1 mL). Satu-

rated $NaHCO_3$ was added to adjust the pH to 9 and the resulting solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and concentrated in vacuo. Purification of the product by flash chromatography on silica gel (EtOAc) afforded **6** (98 mg, 63%) and **7** (9.7 mg, 6 %) as solids.

Data for **6**. IR ($CHCl_3$ cast): 3093, 1673, 1592, 1577, 1515, 1479, 1428, 1412 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) 8.81–8.77 (m, 1H, Py*H*), 8.66–8.62 (m, 1H, Py*H*), 7.99–7.95 (m, 1H, Py*H*), 7.79 (d, 1H, $J = 7.6$ Hz, Py*H*), 7.72 (d, 1H, $J = 4.9$ Hz, H_5), 7.31 (dd, 1H, $J = 7.3$, 5.0 Hz, H_3), 7.14 (dd, 1H, $J = 4.8$, 4.0 Hz, H_4), 6.28 (d, 1H, $J_{H-F} = 47.9$ Hz, COC*H*F); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 187.0 (d, $J_{C-F} = 23.8$ Hz), 150.7 (d, $J_{C-F} = 2.0$ Hz), 148.1 (d, $J_{C-F} = 7.3$ Hz), 139.5 (d, $J_{C-F} = 3.0$ Hz), 135.9 (d, $J_{C-F} = 1.6$ Hz), 134.6 (d, $J_{C-F} = 7.3$ Hz), 134.4 (d, $J_{C-F} = 5.8$ Hz), 130.5 (d, $J_{C-F} = 20.6$ Hz), 128.6, 123.7, 93.0 (d, $J_{C-F} = 189.4$ Hz); HRMS (EI) calcd for $C_{11}H_8FNOS$ (M^+), 221.0311; found: 221.0311.

Data for **7**. IR ($CHCl_3$ cast): 3105, 1677, 1650, 1632, 1593, 1514, 1502, 1480, 1424, 1410 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) 8.92–8.88 (m, 1H, Py*H*), 8.77–8.73 (m, 1H, Py*H*), 8.07–8.04 (m, 1H, Py*H*), 7.91 (d, 1H, $J = 8.0$ Hz, Py*H*), 7.80 (d, 1H, $J = 5.0$ Hz, H_5), 7.38 (dd, 1H, $J = 7.9$, 4.9 Hz, H_3), 7.19 (dd, 1H, $J = 4.9$ Hz, H_4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.8 (t, $J_{C-H} = 33.0$ Hz), 152.1, 147.2 (t, $J_{C-H} = 6.7$ Hz), 137.7, 137.0, 136.2 (t, $J_{C-H} = 5.2$ Hz), 133.9 (t, $J_{C-H} = 5.7$ Hz), 128.9, 128.8 (t, $J_{C-H} = 25.8$ Hz), 123.3, 115.9 (t, $J_{C-H} = 255.0$ Hz); HRMS (EI) calcd for $C_{11}H_7F_2NOS$ (M^+), 239.0216; found: 239.0216.

2.3. Methyl 2-(5-chloropyridin-3-yl)acetate (*9a*)

To a solution of **19** (0.32 g, 2.10 mmol) in H_2O (5 mL) was added conc. HCl (5 mL), and the mixture was heated to reflux at 100 °C overnight. The solvent was removed in vacuo to afford (5-chloronicotin-3-yl)acetic acid, which was used for next reaction without further purification. A solution of this acid in MeOH (5 mL) was treated with a solution of MeOH (3 mL) and $SOCl_2$ (1 mL). The resulting mixture was heated to reflux overnight, and then the solvent was removed in vacuo. The residue was diluted with saturated $NaHCO_3$, and the solution was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (15 mL), dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (EtOAc) to yield **9a** as a liquid (0.28 g, 72% over two steps). IR ($CHCl_3$ cast): 3043, 3003, 2954, 1740, 1584, 1560, 1436, 1424 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.46 (d, 1H, $J = 2.3$ Hz, H_6), 8.36 (d, 1H, $J = 1.8$ Hz, H_2), 7.63 (dd, 1H, $J = 2.3$, 1.9 Hz, H_4), 3.70 (s, 3H, CO_2CH_3), 3.61 (s, 2H, CH_2CO_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4, 148.1, 147.5, 136.6, 131.9, 130.8, 52.4, 37.6; HRMS (EI) calcd for $C_8H_8ClNO_2$ (M^+), 185.0244; found: 185.0241.

2.4. Methyl 2-(3-chlorophenyl)acetate(*9b*)

This was obtained from 3-chlorophenylacetic acid (1.71 g, 10 mmol) following the procedure described for the preparation of **9a**. Purification by flash chromatography on silica gel (10:90 EtOAc/hexanes) afforded **9b** as a liquid (1.63 g, 88%). Literature compound [14]. IR ($CHCl_3$ cast): 3000, 2953, 2843, 1741, 1599, 1576, 1477, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.31–7.14 (m, 4H, Ph*H*), 3.71 (s, 2H, CH_2CO_2), 3.61 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_9H_9ClO_2$ (M^+), 184.0291; found: 184.0290.

2.5. Methyl 2-(5-bromopyridin-3-yl)acetate (*9c*)

This was obtained from (5-bromopyridin-3-yl)acetic acid (**8c**) (1.04 g, 4.80 mmol) following the procedure for **9a**. Purification by flash chromatography on silica gel (EtOAc) afforded **9c** as a liquid (1.00 g, 91%). Literature compound [15]. IR ($CHCl_3$ cast): 3040, 2953, 1740, 1583, 1558, 1436, 1425 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.56 (d, 1H, $J = 1.8$ Hz, H_6), 8.40 (s, 1H, H_2), 7.78 (dd, 1H, $J = 1.8$, 1.8 Hz, H_4), 3.69 (s, 2H, CH_2CO_2), 3.59 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_8H_8BrNO_2$ (M^+), 230.9769; found: 230.9786.

2.6. Methyl 2-(5-chloropyridin-3-yl)-3-(furan-2-yl)-3-oxopropanoate (*10a*)

To a solution of **9a** (175 mg, 0.94 mmol) in THF (5 mL) at −78 °C was added LiHMDS (1.0 mL of 1.0 M solution in THF, 1.0 mmol) dropwise over 15 min. The solution was stirred for 1 h at −78 °C. To this solution was added dropwise over 15 min a solution of 2-furoic acid (50 mg, 0.45 mmol) and carbonyl diimidazole (CDI) (80 mg, 0.49 mmol) in anhydrous THF (5 mL), which had been previously stirred for 1 h at 20 °C. The mixture was stirred for 4 h at −78 °C, and quenched with 1.0 M aqueous HCl (10 mL). The pH was adjusted to ~8 by adding saturated aqueous $NaHCO_3$, and the solution was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography (50:50 EtOAc/hexanes) to yield **10a** as a liquid (90 mg, 72%). IR ($CHCl_3$ cast): 3134, 3043, 2955, 1747, 1677, 1567, 1464, 1427 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.53 (d, 1H, $J = 2.4$ Hz, $H_{2'}$ or $H_{6'}$), 8.50 (d, 1H, $J = 2.0$ Hz, $H_{2'}$ or $H_{6'}$), 7.92 (dd, 1H, $J = 2.4$, 2.0 Hz, $H_{4'}$), 7.63 (dd, 1H, $J = 1.7$, 0.7 Hz, H_5), 7.34 (dd, 1H, $J = 3.7$, 0.7 Hz, H_3), 6.59 (dd, 1H, $J = 3.7$, 1.7 Hz, H_4); 5.51 (s, 1H, COC*H*CO_2), 3.79 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_{13}H_{10}ClNO_4$ (M^+), 279.0298; found: 279.0298.

2.7. Methyl 2-(3-chlorophenyl)-3-(furan-2-yl)-3-oxopropanoate (*10b*)

This was obtained from **9b** (550 mg, 3.0 mmol) following the procedure for **10a**. Purification by flash chromatography

on silica gel (10:90 EtOAc/hexanes) afforded **10b** as a liquid(300 mg, 75%). IR ($CHCl_3$ cast): 3135, 3006, 2954, 1748, 1676, 1597, 1568, 1464, 1434 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.57 (dd, 1H, $J = 1.7$, 0.7 Hz, H_5), 7.42 (dd, 1H, $J = 1.8$, 1.8 Hz, Ph*H*), 7.38–7.01 (m, 4H, H_3 and 3× Ph*H*), 6.53 (dd, 1H, $J = 3.6$, 1.7 Hz, H_4), 5.44 (s, 1H, $COCHCO_2$), 3.77 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_{14}H_{11}ClO_4$ (M^+), 278.0346; found: 278.0345.

2.8. Methyl 2-(5-bromopyridin-3-yl)-3-(furan-2-yl)-3-oxopropanoate (*10c*)

This was obtained from **9c** (0.91 g, 3.95 mmol) following the procedure for **10a**. Purification by flash chromatography on silica gel (50:50 EtOAc/hexanes) afforded **10c** as a liquid (0.59 g, 96%). Literature compound [10b]. IR ($CHCl_3$ cast): 3134, 2954, 1746, 1676, 1567, 1464, 1426, 1393 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.61 (d, 1H, $J = 2.0$ Hz, $H_{2'}$ or $H_{6'}$), 8.52 (d, 1H, $J = 1.6$ Hz, $H_{2'}$ or $H_{6'}$), 8.05 (dd, 1H, $J = 2.0$, 1.6 Hz, $H_{4'}$), 7.62 (d, 1H, $J = 1.6$ Hz, H_5), 7.23 (d, 1H, $J = 3.7$ Hz, H_3), 6.57 (dd, 1H, $J = 3.7$, 1.6 Hz, H_4), 5.49 (s, 1H, $COCHCO_2$), 3.76 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_{13}H_{10}BrNO_4$ (M^+), 322.9793; found: 322.9793.

2.9. Methyl 2-(5-bromopyridin-3-yl)-3-(5-(4-chlorophenyl)furan-2-yl)-3-oxopropanoate (*10d*)

This was obtained from **9c** (1.81 g, 7.86 mmol) following the procedure for **10a**. Purification by flash chromatography on silica gel (33:67 EtOAc/$CHCl_3$) afforded **10d** as an oil (1.35 g, 83%). IR ($CHCl_3$ cast): 3129, 3035, 2953, 1748, 1670, 1603, 1581, 1561, 1516, 1471, 1442, 1426, 1412 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.61 (d, 1H, $J = 2.1$ Hz, $H_{2'}$ or $H_{6'}$), 8.55 (d, 1H, $J = 1.7$ Hz, $H_{2'}$ or $H_{6'}$), 8.06 (dd, 1H, $J = 2.1$, 1.7 Hz, $H_{4'}$), 7.64 (d, 2H, $J = 8.5$ Hz, H_7), 7.40 (d, 2H, $J = 8.5$ Hz, H_8), 7.38 (d, 1H, $J = 3.8$ Hz, H_4), 6.78 (d, 1H, $J = 3.8$ Hz, H_3), 5.46 (s, 1H, $COCHCO_2$), 3.78 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_{19}H_{13}BrClNO_4$ (M^+), 434.9696; found: 434.9693.

2.10. 2-(5-Chloropyridin-3-yl)-1-(furan-2-yl)ethanone (*11a*)

A solution of **10a** (70 mg, 0.25 mmol) in 50% H_2SO_4 (5 mL) was heated to reflux at 100 °C for 8 h. NaOH (6.25 M, 10 mL) and saturated $NaHCO_3$ (9 mL) were then added to neutralize the solution to pH 7. The solution was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (EtOAc) to yield **11a** as a solid (47 mg, 85%). IR ($CHCl_3$ cast): 3132, 3042, 2910, 1675, 1569, 1467, 1443, 1425 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.48 (d, 1H, $J = 2.2$ Hz, $H_{2'}$ or $H_{6'}$), 8.42 (d, 1H, $J = 1.7$ Hz, $H_{2'}$ or $H_{6'}$), 7.66 (dd, 1H, $J = 2.2$, 1.7 Hz, $H_{4'}$), 7.63 (dd, 1H, $J = 1.8$, 0.8 Hz, H_5), 7.29 (dd, 1H, $J = 3.7$, 0.8 Hz, H_3), 6.59 (dd, 1H, $J = 3.7$, 1.8 Hz, H_4); 4.14 (s, 2H, $COCH_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 184.7, 152.0, 148.4, 147.5, 146.9, 136.9, 131.9, 130.8, 118.1, 112.8, 41.6; HRMS (EI) calcd for $C_{11}H_8ClNO_2$ (M^+), 221.0244; found: 221.0243.

2.11. 2-(3-Chlorophenyl)-1-(furan-2-yl)ethanone (*11b*)

This was obtained from **10b** (160 mg, 0.58 mmol) following the procedure for **11a**. Purification by flash chromatography on silica gel (25:75 EtOAc/hexanes) afforded **11b** as an oil (75 mg, 59%), which solidified upon cooling to 4 °C. Literature compound [16]. IR ($CHCl_3$ cast): 3135, 1732, 1673, 1598, 1570, 1466, 1432 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.59 (dd, 1H, $J = 1.7$, 0.7 Hz, H_5), 7.28–7.18 (m, 5H, H_3 and Ph*H*), 6.53 (dd, 1H, $J = 3.6$, 1.7 Hz, H_4), 4.10 (s, 2H, $COCH_2$); HRMS (EI) calcd for $C_{12}H_9ClO_2$ (M^+), 220.0291; found: 220.0291.

2.12. 2-(5-Bromopyridin-3-yl)-1-(furan-2-yl)ethanone (*11c*)

This was obtained from **10c** (0.52 g, 1.59 mmol) following the procedure for **11a**. Purification by flash chromatography on silica gel (EtOAc) afforded **11c** as a solid (0.30 g, 68%) that sublimes under vacuum. Literature compound [10b]. IR ($CHCl_3$ cast): 3130, 3039, 2926, 1703, 1677, 1650, 1631, 1568, 1555, 1466, 1439, 1424, 1392, 1334 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.57 (s, 1H, $H_{2'}$ or $H_{6'}$), 8.45 (s, 1H, $H_{2'}$ or $H_{6'}$), 7.81 (s, 1H, $H_{4'}$), 7.63 (m, 1H, H_5), 7.28 (d, 1H, $J = 1.6$ Hz, H_3), 6.57 (m, 1H, H_4), 4.10 (s, 2H, $COCH_2$); HRMS (EI) calcd for $C_{11}H_8BrNO_2$ (M^+), 264.9738; found: 264.9739.

2.13. 2-(5-Bromopyridin-3-yl)-1-(5-(4-chlorophenyl)furan-2- yl)ethanone(*11d*)

This was obtained from **10d** (1.22 g, 2.81 mmol) following the procedure for **11a**. Purification by flash chromatography on silica gel (EtOAc) afforded **11d** as a solid (0.90 g, 85%). IR ($CHCl_3$ cast): 3127, 3030, 2914, 1675, 1665, 1518, 1469, 1436, 1410, 1402 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) 8.60 (d, 1H, $J = 2.2$ Hz, $H_{2'}$ or $H_{6'}$), 8.51 (d, 1H, $J = 1.9$ Hz, $H_{2'}$ or $H_{6'}$), 7.86 (dd, 1H, $J = 2.2$, 1.9 Hz, $H_{4'}$), 7.72 (d, 2H, $J = 8.7$ Hz, H_7), 7.44 (d, 2H, $J = 8.5$ Hz, H_8), 7.36 (d, 1H, $J = 3.7$ Hz, H_3), 6.81 (d, 1H, $J = 3.7$ Hz, H_4), 4.18 (s, 2H, $COCH_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 184.1, 157.2, 151.1, 149.6, 148.7, 139.7, 135.5, 131.4, 129.3, 127.6, 126.2, 120.7, 120.4, 108.2, 41.7; HRMS (EI) calcd for $C_{17}H_{11}BrClNO_2$ (M^+), 374.9662; found: 376.9642.

2.14. 2-(5-Chloropyridin-3-yl)-2-fluoro-1-(furan-2-yl)-ethanone (*12a*)

To a solution of **11a** (20 mg, 0.09 mmol) in dry THF (5 mL) was added LiHMDS (0.1 mL, 1.0 M solution in THF, 0.1 mmol) over 5 min. The mixture was stirred for 1 h at −78 °C. A solution of NFSi (32 mg, 0.1 mmol) in dry THF (3 mL) was added dropwise to the mixture over

10 min. This was stirred for another 2 h at −78 °C. Saturated $NaHCO_3$ (5 mL) was added to adjust the pH to 9, and the solution was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (50:50 EtOAc/hexanes) to yield **12a** as a solid (16 mg, 74%). IR ($CHCl_3$ cast): 3136, 3059, 1690, 1584, 1569, 1464, 1425 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) 8.67 (dd, 1H, $J = 1.9$, 1.7 Hz, $H_{2'}$ or $H_{6'}$), 8.60 (d, 1H, $J = 1.9$ Hz, $H_{2'}$ or $H_{6'}$), 7.89–7.87 (m, 1H, $H_{4'}$), 7.69 (dd, 1H, $J = 1.7$, 0.7 Hz, H_5), 7.48 (ddd, 1H, $J = 3.7$, 2.1, 0.7 Hz, H_3), 6.62 (dd, 1H, $J = 3.7$, 1.7 Hz, H_4), 6.37 (d, 1H, $J = 47.2$ Hz, COC*H*F); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 182.2 (d, $J_{C-H} = 23.5$ Hz), 150.2 (d, $J_{C-H} = 1.7$ Hz), 150.1 (d, $J_{C-H} = 1.7$ Hz), 149.0, 146.2 (d, $J_{C-H} = 7.0$ Hz), 135.3 (d, $J_{C-H} = 6.2$ Hz), 133.4, 132.3 (d, $J_{C-H} = 21.0$ Hz), 122.0 (d, $J_{C-H} = 7.4$ Hz), 113.6 (d, $J_{C-H} = 1.2$ Hz), 91.0 (d, $J_{C-H} = 189.2$ Hz); ^{19}F NMR ($CDCl_3$, 376 MHz) −186.6 (d, $J_{H-F} = 47.2$ Hz); HRMS (EI) calcd for $C_{11}H_7ClFNO_2$ (M^+), 239.0149; found: 239.0145.

2.15. 2-(3-Chlorophenyl)-2-fluoro-1-(furan-2-yl)ethanone (*12b*)

This was obtained from **11b** (40 mg, 0.181 mmol) following the procedure for **12a**. Purification by flash chromatography on silica gel (25:75 EtOAc/hexanes) afforded **12b** as an oil (38 mg, 88%), which solidified upon cooling to 4 °C. IR ($CHCl_3$ cast): 3140, 1689, 1597, 1569, 1464, 1433 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.63 (dd, 1H, $J = 1.7$, 0.7 Hz, H_5), 7.53–7.51 (m, 1H, Ph*H*), 7.40 (ddd, 1H, $J = 3.7$, 2.0, 0.7 Hz, H_3), 7.42–7.31 (m, 3H, Ph*H*), 6.56 (dd, 1H, $J = 3.7$, 1.7 Hz, H_4), 6.24 (d, 1H, $J = 47.6$ Hz, COC*H*F); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 183.2 (d, $J_{C-H} = 24.3$ Hz), 149.9, 148.1, 136.3 (d, $J_{C-H} = 20.7$ Hz), 135.2, 130.4, 130.0 (d, $J_{C-H} = 2.1$ Hz), 127.2 (d, $J_{C-H} = 6.7$ Hz), 125.2 (d, $J_{C-H} = 6.2$ Hz), 121.1 (d, $J_{C-H} = 7.2$ Hz), 113.0, 93.0 (d, $J_{C-H} = 187.9$ Hz); ^{19}F NMR ($CDCl_3$, 376 MHz) −183.4 (d, $J_{H-F} = 47.5$ Hz); HRMS (EI) calcd for $C_{12}H_8ClFO_2$ (M^+), 238.0197; found: 238.0120.

2.16. 2-(5-Bromopyridin-3-yl)-2,2-difluoro-1-(furan-2- yl)-ethanone(*12c*)

This was obtained from **11c** (53 mg, 0.20 mmol) following the procedure for **12a**. Purification by flash chromatography on silica gel (EtOAc) afforded **12c** as an oil (42 mg, 74%), which solidified upon cooling to 4 °C. IR ($CHCl_3$ cast): 3136, 2960, 2924, 2850, 1732, 1690, 1569, 1463, 1425, 1394, 1261 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) 8.86 (m, 2H, $H_{2'}$ and $H_{6'}$), 7.95 (dd, 1H, $J = 2.2$, 2.0 Hz, $H_{4'}$), 7.65 (d, 1H, $J = 1.6$ Hz, H_5), 7.44 (dd, 1H, $J = 3.7$, 2.0 Hz, H_3), 6.58 (dd, 1H, $J = 3.8$, 1.7 Hz, H_4), 6.32 (d, 1H, $J = 47.2$ Hz, COC*H*F); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.8 (d, $J_{C-H} = 23.2$ Hz), 152.3 (d, $J_{C-H} = 2.1$ Hz), 149.8, 148.5, 146.7 (d, $J_{C-H} = 7.2$ Hz), 137.2 (d, $J_{C-H} = 6.2$ Hz), 131.8 (d, $J_{C-H} = 21.2$ Hz), 121.5 (d, $J_{C-H} = 7.2$ Hz), 121.2, 113.3, 90.8 (d, $J_{C-H} = 188.9$ Hz); ^{19}F NMR ($CDCl_3$, 376 MHz) −186.1 (d, $J_{H-F} = 46.9$ Hz); HRMS (EI) calcd for $C_{11}H_7BrFNO_2$ (M^+), 282.9644; found: 282.9647.

2.17. 2-(5-Bromopyridin-3-yl)-1-(5-(4-chlorophenyl)furan-2-yl)-2- fluoroethanone(*12d*)

This was obtained from **11d** (57 mg, 0.15 mmol) following the procedure for **12a**. Purification by flash chromatography on silica gel (25:75 EtOAc/hexanes) afforded **12d** as an oil (30 mg, 50%), which solidified upon cooling to 4 °C. IR ($CHCl_3$ cast): 3309, 3146, 3087, 3067, 2925, 1656, 1626, 1588, 1513, 1467, 1446, 1425, 1411 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) 8.70 (m, 1H, $H_{2'}$ or $H_{6'}$), 8.68 (d, 1H, $J = 1.6$ Hz, $H_{2'}$ or $H_{6'}$), 7.99 (dd, 1H, $J = 1.8$, 1.6 Hz, $H_{4'}$), 7.68 (d, 2H, $J = 8.7$ Hz, H_7), 7.53 (dd, 1H, $J = 3.8$, 2.3 Hz, H_3), 7.41 (d, 2H, $J = 8.7$ Hz, H_8), 6.80 (d, 1H, $J = 3.9$ Hz, H_4), 6.31 (d, 1H, $J = 47.3$ Hz, COC*H*F); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.2 (d, $J_{C-H} = 23.2$ Hz), 158.9, 152.3 (d, $J_{C-H} = 2.1$ Hz), 148.8, 146.5 (d, $J_{C-H} = 7.2$ Hz), 137.1 (d, $J_{C-H} = 6.2$ Hz), 136.4, 132.1 (d, $J_{C-H} = 21.2$ Hz), 129.7, 127.4, 126.8, 124.0 (d, $J_{C-H} = 8.3$ Hz), 121.3, 108.6, 91.3 (d, $J_{C-H} = 190.0$ Hz); ^{19}F NMR ($CDCl_3$, 376 MHz) −185.9 (d, $J_{H-F} = 47.5$ Hz); HRMS (EI) calcd for $C_{17}H_{10}BrClFNO_2$ (M^+), 394.9538; found: 394.9531.

2.18. 2-(5-Chloropyridin-3-yl)-2,2-difluoro-1-(furan-2-yl)-ethanone (*13a*)

To a solution of **11a** (10 mg, 0.045 mmol) in dry THF (5 mL) was added LiHMDS (0.1 mL, 1.0 M solution in THF, 0.1 mmol) over a period of 5 min. The mixture was stirred for 1 h at −78 °C, and a solution of NFSi (32 mg, 0.1 mmol) in dry THF (3 mL) was added dropwise over 10 min. The mixture was stirred for another 2 h at −78 °C. Saturated $NaHCO_3$ (5 mL) was added to adjust the pH to 9, and the solution was extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (10 mL), dried over $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (50:50 EtOAc/hexanes) to yield **13a** as a solid (7.1 mg, 61%). IR ($CHCl_3$ cast): 3142, 3068, 2933, 1687, 1563, 1460, 1424 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) 8.72 (d, 1H, $J = 1.9$ Hz, $H_{2'}$ or $H_{6'}$), 8.67 (d, 1H, $J = 2.2$ Hz, $H_{2'}$ or $H_{6'}$), 7.90 (dd, 1H, $J = 2.2$, 1.9 Hz, $H_{4'}$), 7.74 (dd, 1H, $J = 1.7$, 0.6 Hz, H_5), 7.58 (ddt, 1H, $J = 3.7$, 1.9, 0.7 Hz, H_3), 6.63 (dd, 1H, $J = 3.8$, 1.7 Hz, H_4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.8 (t, $J_{C-H} = 33.0$ Hz), 151.5, 149.8, 148.1, 145.3 (t, $J_{C-H} = 6.2$ Hz), 140.0 (t, $J_{C-H} = 6.2$ Hz), 132.5, 130.0 (t, $J_{C-H} = 25.8$ Hz), 124.0 (t, $J_{C-H} = 5.2$ Hz), 114.9 (t, $J_{C-H} = 255.5$ Hz), 113.3; ^{19}F NMR ($CDCl_3$, 376 MHz) −102.1; HRMS (EI) calcd for $C_{11}H_6ClF_2NO_2$ (M^+), 257.0055; found: 257.0055.

2.19. 2-(3-Chlorophenyl)-2,2-difluoro-1-(furan-2-yl)-ethanone (**13b**)

This was obtained from **11b** (36 mg, 0.163 mmol) following the procedure for **13a**. Purification by flash chromatography on silica gel (25:75 EtOAc/hexanes) afforded **13b** as an oil(24 mg, 57%), which solidified upon cooling to 4 °C. IR ($CHCl_3$ cast): 1687, 1578, 1560, 1477, 1461, 1427, 1394 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.71 (dd, 1H, $J = 1.7$, 0.7 Hz, H_5), 7.61 (dd, 1H, $J = 1.9$, 1.7 Hz, Ph*H*), 7.53–7.45 (m, 3H, H_3 and 2× Ph*H*), 7.37 (dd, 1H, $J = 7.9$, 7.5 Hz, Ph*H*), 6.59 (dd, 1H, $J = 3.8$, 1.7 Hz, H_4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.1 (t, $J_{C-H} = 33.0$ Hz), 149.4, 148.3, 135.2, 134.7 (t, $J_{C-H} = 25.4$ Hz), 131.5, 130.4, 126.3 (t, $J_{C-H} = 6.2$ Hz), 124.3 (t, $J_{C-H} = 6.2$ Hz), 123.5 (t, $J_{C-H} = 5.0$ Hz), 115.6 (t, $J_{C-H} = 254.5$ Hz), 113.1; ^{19}F NMR ($CDCl_3$, 376 MHz) −102.0; HRMS (EI) calcd for $C_{12}H_7ClF_2O_2$ (M^+), 256.0103; found: 256.0106.

2.20. 2-(5-Bromopyridin-3-yl)-2-fluoro-1-(furan-2-yl)-ethanone(**13c**)

This was obtained from **11c** (114 mg, 0.39 mmol) following the procedure for **13a**. Purification by flash chromatography on silica gel (50:50 EtOAc/hexanes) afforded **13c** as a solid (60 mg, 51%). IR ($CHCl_3$ cast): 3139, 3126, 3033, 1691, 1653, 1559, 1467, 1437, 1424, 1395, 1308, 1261 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) 8.82–8.78 (m, 2H, $H_{2'}$ and $H_{6'}$), 8.08 (dd, 1H, $J = 2.1$, 2.1 Hz, $H_{4'}$), 7.78 (dd, 1H, $J = 1.6$, 0.7 Hz, H_5), 7.59–7.56 (m, 1H, H_3), 6.66 (dd, 1H, $J = 3.7$, 1.6 Hz, H_4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 176.9 (t, $J_{C-H} = 32.6$ Hz), 154.2, 150.5, 148.7, 146.2 (t, $J_{C-H} = 6.6$ Hz), 137.3 (t, $J_{C-H} = 6.2$ Hz), 130.9 (t, $J_{C-H} = 26.0$ Hz), 124.5 (t, $J_{C-H} = 5.0$ Hz), 121.4, 115.3 (t, $J_{C-H} = 255.7$ Hz), 113.8; ^{19}F NMR ($CDCl_3$, 376 MHz) −102.1; HRMS (EI) calcd for $C_{11}H_6BrF_2NO_2$ (M^+), 300.9550; found: 300.9555.

2.21. 2-(5-Bromopyridin-3-yl)-1-(5-(4-chlorophenyl)furan-2-yl)-2,2- difluoroethanone(**13d**)

This was obtained from **11d** (57 mg, 0.15 mmol) following the procedure for **13a**. Purification by flash chromatography on silica gel (50:50 EtOAc/hexanes) afforded **13d** as a solid (53 mg, 85%). IR ($CHCl_3$ cast): 3369, 3175, 3036, 1691, 1582, 1522, 1472, 1424, 1412 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) 8.78 (m, 2H, $H_{2'}$ and $H_{6'}$), 8.08 (dd, 1H, $J = 2.2$, 1.9 Hz, $H_{4'}$), 7.71 (d, 2H, $J = 8.8$ Hz, H_7), 7.61 (dt, 1H, $J = 3.8$, 1.8 Hz, H_3), 7.41 (d, 2H, $J = 8.8$ Hz, H_8), 6.83 (d, 1H, $J = 3.9$ Hz, H_4); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 175.5 (t, $J_{C-H} = 32.5$ Hz), 160.1, 153.6, 147.3, 145.6 (t, $J_{C-H} = 6.7$ Hz), 136.8, 136.7, 130.6 (t, $J_{C-H} = 26.0$ Hz), 129.2, 127.2, 127.0, 126.2 (t, $J_{C-H} = 5.2$ Hz), 121.0, 115.0 (t, $J_{C-H} = 256.0$ Hz), 108.7; ^{19}F NMR ($CDCl_3$, 376 MHz) −101.9; HRMS (EI) calcd for $C_{17}H_9BrClF_2NO_2$ (M^+), 412.9453; found: 412.9452.

2.22. Methyl 5-aminonicotinate(**15**)

Acetyl chloride (30 mL) was slowly added to dry MeOH (30 mL) at 0 °C to generate HCl and MeOAc. This solution was stirred for 10 min at 0 °C, and then was added to a solution of 5-aminonicotinic acid (9.89 g, 71.6 mmol) in MeOH (120 mL) at 0 °C. The mixture was heated to reflux for 18 h and then the solvent was removed in vacuo. The residue was treated with saturated $NaHCO_3$ until pH of the solution was ~7, and the resulting solution was extracted with EtOAc (3 × 80 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (EtOAc) to yield **15** as a solid (8.58 g, 79%). Literature compound [17]. IR ($CHCl_3$ cast): 3316, 3135, 2993, 2962, 1726, 1646, 1581, 1473, 1446, 1435 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz) δ 8.62 (d, 1H, $J = 1.8$ Hz, Py*H*), 8.27 (d, 1H, $J = 2.8$ Hz, Py*H*), 7.58 (dd, 1H, $J = 2.8$, 1.8 Hz, Py*H*), 4.05–3.70 (br, 2H, NH_2), 3.93 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_7H_8N_2O_2$ (M^+), 152.0586; found: 152.0585.

2.23. Methyl 5-chloronicotinate (**16**)

A solution of $NaNO_2$ (3.26 g, 47.2 mmol) in H_2O (21 mL) was added over 30 min to a solution of **15** (5.91 g, 38.9 mmol) in conc. HCl (42 mL) and H_2O (21 mL) at 0 °C. The mixture was stirred for another 30 min at 0 °C. To this was added HCl solution (10% w/w, 50 mL), and then a solution of $CuCl_2$ (8.82 g) and CuCl (42 mg) in HCl solution (10% w/w, 30 mL). The mixture was stirred for 4 h at 0 °C and then allowed to warm to 20 °C. NaOH solution and saturated $NaHCO_3$ were added to neutralize the solution to pH ~ 7. The aqueous layer (250 mL) was extracted with EtOAc (3 × 80 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (50:50 EtOAc/hexanes) to yield **16** as a solid (4.74 g, 71%). Literature compound [18]. IR ($CHCl_3$ cast): 3056, 1725, 1579, 1444, 1425 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 9.06 (s, 1H, Py*H*), 8.71 (d, 1H, $J = 2.0$ Hz, Py*H*), 8.25 (d, 1H, $J = 1.7$ Hz, Py*H*), 4.00 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_7H_6ClNO_2$ (M^+), 171.0087; found: 171.0088.

2.24. 5-Chloronicotinic acid HCl salt(**17**)

To a solution of **16** (0.51 g, 2.96 mmol) in MeOH (5 mL) and H_2O (5 mL) was added KOH solution (0.24 g, 10% w/w) to bring the pH between 10 and 11. The mixture was stirred for 24 h at 20 °C. A precipitate appeared upon acidifying the mixture to pH 1 with 1 N HCl (5 mL). This solid was collected and then washed several times with H_2O. The filtrate was concentrated in vacuo and then dissolved in dry MeOH. The insoluble impurities were removed by gravity filtration, and the filtrate was concentrated in vacuo to afford **17** as a solid (0.48 g, 84%). Literature compound

[19]. IR (microscope): 3351 (broad), 3055, 1853, 1632, 1585, 1540, 1431 cm^{-1}. ^1H NMR (CD_3OD, 500 MHz) δ 9.05 (s, 1H, Py*H*), 8.78 (s, 1H, Py*H*), 8.31 (d, 1H, $J = 1.4$ Hz, Py*H*); HRMS (EI) calcd for $C_6H_4ClNO_2$ (M^+), 156.9931; found: 156.9930.

2.25. 3-Chloro-5-(chloromethyl)pyridine (*18*)

To a solution of **17** (0.49 g, 2.54 mmol) in THF (20 mL) at 0 °C was added dry Et_3N (0.78 mL, 5.60 mmol), followed by ethyl chloroformate (0.29 mL, 3.05 mmol). The mixture was stirred for 1.5 h at 0 °C, and then the precipitate, $Et_3N \cdot HCl$, was removed by gravity filtration. To the filtrate at −78 °C was added $LiAlH_4$ (3.05 mL of 1.0 M solution in THF, 3.05 mmol) over a period of 15 min. The mixture was stirred at −78 °C for another 4 h, and then quenched with 5% NaOH (8 mL). The solvent was removed in vacuo and the residue was diluted with H_2O (20 mL). Saturated NH_4Cl was added to adjust pH of the solution to 8. The resulting mixture was stirred for 1 h and then EtOAc (30 mL) was added. The solution was filtered through celite, and then the two layers were separated. The aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and the solvent was removed to yield the product (5-chloropyridin-3-yl)methanol as an oil (0.38 g), which was used for next reaction without further purification. To a solution of the above alcohol (0.38 g, 2.65 mmol) in CH_2Cl_2 (15 mL) at 20 °C was added $SOCl_2$ (0.98 mL, 16.0 mmol). The mixture was stirred for 42 h. The solvent was removed in vacuo, and the residue was treated with saturated $NaHCO_3$ (25 mL) to give pH 8. The solution was then extracted with EtOAc (3 × 40 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (50:50 EtOAc/hexanes) to yield **18** as a solid (0.32 g, 76% over three steps). IR ($CHCl_3$ cast): 3046, 2964, 1584, 1563, 1556, 1461, 1442, 1423 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 8.53 (d, 1H, $J = 2.3$ Hz, H_2 or H_6), 8.52 (d, 1H, $J = 1.9$ Hz, H_2 or H_6), 7.96 (dd, 1H, $J = 2.3$, 1.9 Hz, H_4), 4.70 (s, 2H, CH_2Cl); ^{13}C NMR (CD_3OD, 100 MHz) δ 148.8, 148.2, 137.7, 137.2, 133.3, 42.5; HRMS (EI) calcd for $C_6H_5Cl_2N$ (M^+), 160.9799; found: 160.9803.

2.26. (5-Chloropyridin-3-yl)acetonitrile (*19*)

A solution of **18** (0.10 g, 0.62 mmol) and KCN (0.10 g, 1.54 mmol) in dry DMF (3 mL) was stirred for 48 h at 20 °C. The solvent was removed in vacuo, and the residue was treated with K_2CO_3 solution (15 mL, 10% w/w). The solution was then extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (EtOAc) to yield **19** as a solid (53 mg, 56%). IR ($CHCl_3$ cast): 3048, 3031, 2928, 2251, 2231, 1583, 1566, 1447, 1413 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.57 (d, 1H, $J = 2.3$ Hz, H_2 or H_6), 8.47 (d, 1H, $J = 2.0$ Hz, H_2 or H_6), 7.72 (dd, 1H, $J = 2.3$, 2.0 Hz, H_4), 3.78 (s, 2H, CH_2CN); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.5, 146.7, 135.2, 132.4, 127.2, 116.1, 20.7; HRMS (EI) calcd for $C_7H_5ClN_2$ (M^+), 152.0141; found: 152.0138.

2.27. Ethyl 3-diazo-2-oxopropanoate(*21*)

This was prepared by modification of the literature procedure [20]. To a solution of ethyl chloro-oxoacetate (1.6 mL, 14 mmol) in THF (20 mL) was added dropwise $TMSCHN_2$ (21 mL, 2 M solution in hexane, 42 mmol). After 3 h of stirring at 20 °C, the solvent was removed in vacuo and the product was purified by column chromatography on silica gel (25:75 EtOAc/hexanes) to yield **21** as a solid (1.35 g, 68%). IR ($CHCl_3$ cast): 3458, 3241, 3080, 2994, 2971, 2943, 2909, 2869, 2432, 2159, 2109, 1734, 1697, 1641, 1530, 1476, 1459, 1442 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.15 (s, 1H, COC*H*N_2), 4.27 (q, 2H, $J = 7.1$ Hz, OCH_2CH$_3$), 1.74 (t, 3H, $J = 7.1$ Hz, OCH$_2$CH_3); HRMS (ES) calcd for $C_5H_6N_2O_3Na$ ($[M + Na]^+$), 165.0271; found: 165.0273.

2.28. Ethyl 2-(4-chlorophenyl)oxazole-5-carboxylate(*22*)

To a stirred suspension of bis-copper acetylacetonate (5.2 mg) in benzene (5 mL) and 4-chlorobenzonitrile (1.8 g, 13.1 mmol) at reflux temperature was added ethyl diazopyruvate (**21**) (1.0 g, 6.06 mmol) in benzene (14 mL) during a period of 3 h. The mixture was heated until the complete consumption of the starting material was confirmed by TLC (ca 3 h). The solvent was removed in vacuo, and the residue was diluted with saturated $NaHCO_3$ solution (30 mL). The solution was then extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (20 mL), dried over $MgSO_4$ and then concentrated in vacuo. The residue was purified by column chromatography on silica gel (25:75 EtOAc/hexanes) to afford **22** as a solid (240 mg, 14%). Literature compound [21]. IR ($CHCl_3$ cast): 3089, 2983, 1735, 1606, 1587, 1574, 1534, 1475, 1408 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, 2H, $J = 8.6$ Hz, H_7), 7.83 (s, 1H, H_4), 7.47 (d, 2H, $J = 8.6$ Hz, H_8), 4.42 (q, 2H, $J = 7.1$ Hz, OCH_2CH$_3$), 1.41 (t, 3H, $J = 7.1$ Hz, OCH$_2$CH_3); HRMS (EI) calcd for $C_{12}H_{10}ClNO_3$ (M^+), 253.0349; found: 253.0347.

2.29. 2-(4-Chlorophenyl)oxazole-5-carboxylic acid(*23*)

To a solution of **22** (180 mg, 0.72 mmol) in THF/H_2O (8 mL/8 mL) at 0 °C was added LiOH (39 mg, 0.93 mmol). The resulting solution was stirred for 2 h until complete consumption of the starting material was confirmed by TLC. The reaction was quenched with 1 N HCl to give pH 3, and solvent was removed in vacuo. The residue was diluted with H_2O (20 mL) and then extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (10 mL) and dried over $MgSO_4$. The solvent was removed in vacuo to afford **23** as a solid (150 mg, 94%). IR ($CHCl_3$

cast): 2917, 2849, 2633, 2528, 1739, 1603, 1569, 1530, 1475, 1411 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 8.07 (d, 2H, $J = 8.5$ Hz, H_7), 7.86 (s, 1H, H_4), 7.54 (d, 2H, $J = 8.5$ Hz, H_8); ^{13}C NMR (CD_3OD, 100 MHz) δ 164.1, 161.3, 145.8, 138.9, 135.2, 130.5, 129.6, 126.5; HRMS (EI) calcd for $C_{10}H_6ClNO_3$ (M^+), 223.0036; found: 223.0040.

2.30. Methyl 2-(5-bromopyridin-3-yl)-3-(2-(4-chlorophenyl)oxazol-5-yl)-3-oxopropanoate (24)

This was obtained from **23** (112 mg, 0.5 mmol) following the procedure described for **10a**. Purification by flash chromatography on silica gel (25:75 EtOAc/hexanes) afforded **24** as a solid (50 mg, 23%). (Mixture of enol isomer A and keto isomer B, 3:2 ratio). IR ($CHCl_3$ cast): 2954, 1744, 1683, 1650, 1603, 1580, 1556, 1526, 1473, 1443, 1408 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) (isomer A) δ 8.75 (d, 1H, $J = 2.2$ Hz, $H_{6'}$), 8.44 (d, 1H, $J = 1.9$ Hz, $H_{2'}$), 7.80 (dd, 1H, $J = 2.2$, 1.9 Hz, $H_{4'}$), 7.49 (d, 2H, $J = 8.8$ Hz, H_7), 7.43 (s, 1H, H_4), 7.37 (d, 1H, $J = 8.8$ Hz, H_8), 3.81 (s, 3H, CO_2CH_3); ^1H NMR ($CDCl_3$, 300 MHz) (isomer B) δ 8.68 (d, 1H, $J = 2.2$ Hz, $H_{6'}$), 8.57 (d, 1H, $J = 1.9$ Hz, $H_{2'}$), 8.08 (dd, 1H, $J = 2.2$, 1.9 Hz, $H_{4'}$), 8.06 (d, 2H, $J = 8.8$ Hz, H_7), 7.99 (s, 1H, H_4), 7.52 (d, 2H, $J = 8.8$ Hz, H_8), 5.35 (s, 1H, $COCHCO_2$), 3.82 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_{18}H_{12}BrClN_2O_4$ (M^+), 435.9640; found: 435.9642.

2.31. 2-(5-Bromopyridin-3-yl)-1-(2-(4-chlorophenyl)oxazol-5- yl)ethanone(25)

This was obtained from **24** (26 mg, 0.060 mmol) following the procedure for **11a**. Purification by flash chromatography on silica gel (EtOAc) afforded **25** as a solid (13 mg, 58%). IR ($CHCl_3$ cast): 3039, 2926, 1680, 1603, 1580, 1556, 1526, 1472, 1408 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.65-8.61 (m, 1H, $H_{2'}$ or $H_{6'}$), 8.52–8.48 (m, 1H, $H_{2'}$ or $H_{6'}$), 8.10 (d, 2H, $J = 8.7$ Hz, H_7), 7.97 (s, 1H, H_4), 7.85 (dd, 1H, $J = 2.1$, 1.8 Hz, $H_{4'}$), 7.52 (d, 2H, $J = 8.7$ Hz, H_8), 4.16 (s, 2H, $COCH_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.3, 163.8, 149.9, 148.7, 148.5, 139.6, 138.5, 136.0, 130.3, 129.4, 128.6, 124.3, 120.7, 42.3; HRMS (EI) calcd for $C_{16}H_{10}BrClN_2O_2$ (M^+), 377.9594; found: 377.9608.

2.32. Methyl 2-(5-bromopyridin-3-yl)-3-(5-(4-chlorophenyl)isoxazol-3-yl)-3- oxopropanoate (27)

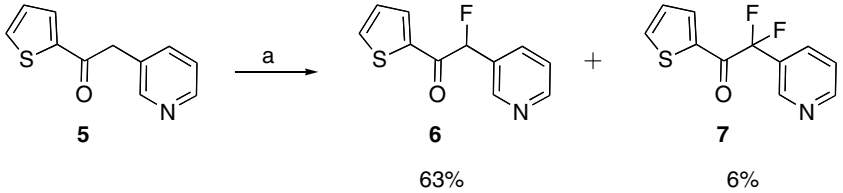
This was obtained from 5-(4-chlorophenyl)isoxazole-3-carboxylic acid (**26**) (630 mg, 2.82 mmol) following the procedure for **10a**. Purification by flash chromatography on silica gel (50:50 EtOAc/hexanes) afforded **27** as a solid (230 mg, 19%). IR ($CHCl_3$ cast): 3217, 3031, 2953, 1739, 1719, 1653, 1607, 1576, 1559, 1490, 1436 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.67 (d, 1H, $J = 2.1$ Hz, $H_{6'}$), 8.59 (d, 1H, $J = 1.9$ Hz, $H_{2'}$), 8.03 (dd, 1H, $J = 2.1$, 1.9 Hz, $H_{4'}$), 7.74 (d, 2H, $J = 8.9$ Hz, H_7), 7.49 (d, 2H, $J = 8.9$ Hz, H_8), 6.92 (s, 1H, H_4), 5.84 (s, 1H. $COCHCO_2$), 3.81 (s, 3H, CO_2CH_3); HRMS (EI) calcd for $C_{18}H_{12}BrClN_2O_4$ (M^+), 435.9648; found: 435.9651.

2.33. 2-(5-Bromopyridin-3-yl)-1-(5-(4-chlorophenyl)isoxazol-3- yl)ethanone (28)

This was obtained from **27** (60 mg, 0.138 mmol) following the procedure for **11a**. Purification by flash chromatography on silica gel (50:50 EtOAc/hexanes) afforded **28** as a solid (30 mg, 58%). IR ($CHCl_3$ cast): 3217, 3090, 3045, 2894, 1709, 1608, 1589, 1560, 1492, 1440 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.59 (s, 1H, $H_{2'}$ or $H_{6'}$), 8.49 (s, 1H, $H_{2'}$ or $H_{6'}$), 7.82 (dd, 1H, $J = 2.0$, 2.0 Hz, $H_{4'}$), 7.72 (d, 2H, $J = 8.8$ Hz, H_7), 7.46 (d, 2H, $J = 8.8$ Hz, H_8), 6.90 (s, 1H, H_4), 4.39 (s, 2H, $COCH_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 190.0, 171.0, 161.8, 149.8, 149.0, 139.9, 137.2, 130.4, 129.6, 127.2, 124.9, 120.7, 89.3, 24.8; HRMS (EI) calcd for $C_{16}H_{10}BrClN_2O_2$ (M^+), 377.9594; found: 377.9604.

3. Results and discussion

Initially, the fluorinated methylene ketones **6** and **7** were prepared from the ketone **5** [10b] (Scheme 1). Deprotonation of ketone **5** with 1.05 equivalent of LiHMDS, followed by fluorination with 1.1 equivalent of *N*-fluorobenzenesulfonimide (NFSi), provides both the monofluoromethylene ketone **6** and the difluoromethylene ketone **7** in 63% and 6% yields, respectively. Compounds **6** and **7** were then tested as inhibitors of SARS $3CL^{pro}$ using a continuous fluorometric assay in which enzymatic cleavage of an internally quenched fluorogenic peptide substrate, (Abz-SVTLQSG-Tyr(NO_2)R) leads to enhanced fluorescence [10a,10b]. The monofluoro ketone **6** shows weak inhibition against $3CL^{pro}$ (10% at 100 μM concentration, Table 1), and no improved inhibition was observed after 2 h preincubation with the $3CL^{pro}$. However, the di-fluoro ketone **7** displays stronger inhibition against $3CL^{pro}$ (38% at 100 μM concentration, Table 1). Similarly, preincubation of **7** with the $3CL^{pro}$ for 2 h shows no improved inhibition.



Scheme 1. Reagents and conditions: (a) LiHMDS (1.05 equivalent), NFSi (1.1 equivalent), THF, −78 °C, 4 h.

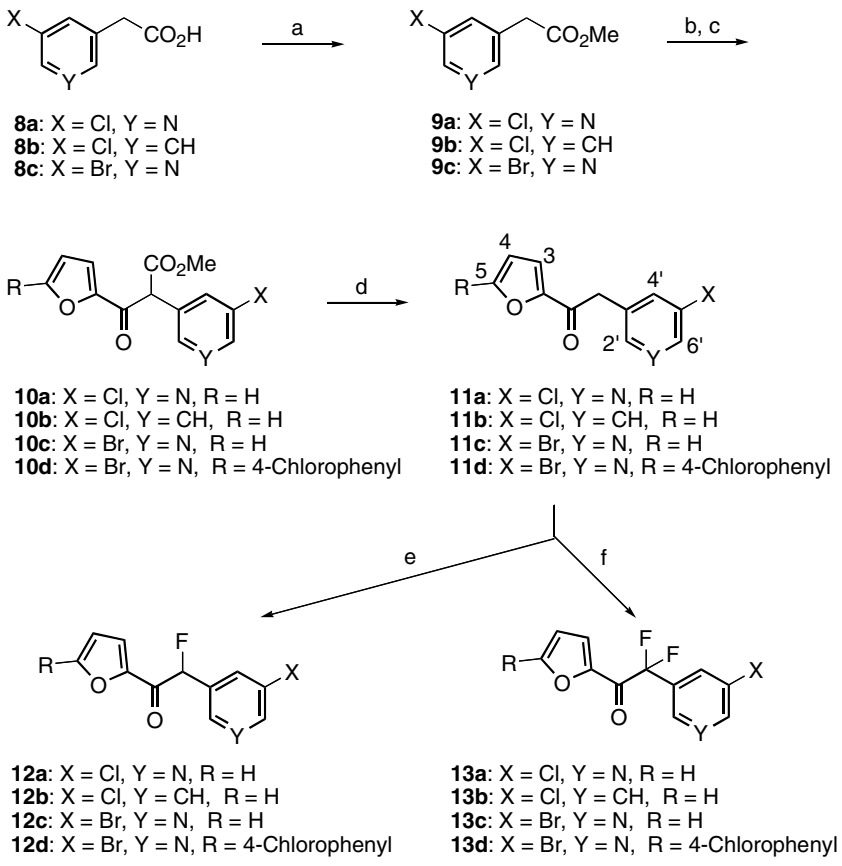
Table 1
Evaluation of aryl methylene ketones and fluorinated methylene ketones as SARS 3CL^{pro} inhibitors

Compounds	Inhibition (100 μM)	IC_{50} (μM)
5	—	
6	10%	
7	38%	
11a	—	
12a	14%	
13a	27%	
11b	—	
12b	15%	
13b	13%	
11c	—	
12c	21%	
13c	—	
11d		13
12d		28
13d		57
25		75
28	35%	

Dash represents <10% inhibition.

These results suggest that the fluorinated ketones **6**, **7** are non-covalent and completely reversible inhibitors for SARS 3CL^{pro}. As proposed, fluorination leads to the substantial improvement in the inhibitory activity against the 3CL^{pro}. The di-fluoro ketone **7** appears to be a reasonable mimic of the corresponding pyridinyl ester **1** (91% inhibition at 100 μM), with only 2- to 3-fold less potent inhibition. As the pyridinyl ester **1** (IC_{50} = 7.9 μM) is only a moderate inhibitor among the pyridinyl esters (**2**–**4**, IC_{50} = 50–63 nM), it seemed that the difluoromethylene ketone mimics of the very potent esters (i.e. **2**–**4**) could inhibit SARS 3CL^{pro} very strongly in a non-covalent and reversible fashion.

Based on this assumption, a series of methylene ketones and fluorinated methylene ketones were synthesized. Synthesis started from halogen-substituted phenyl or pyridinyl acetic acids, most of which are commercially available materials except the 5-chloropyridinyl acetic acid **8a** (Scheme 2). Esterification of carboxylic acids **8a**–**c** yields **9a**–**c** in 88% to quantitative yields. Treatment of **9a**–**c** with LiHMDS to generate the anions, followed by the addition of pre-activated CDI/acid solutions, provides the β-keto esters **10a**–**d** in 72–96% yields. Hydrolytic decarboxylation of the β-keto esters **10a**–**d** gives the corresponding ketones **11a**–**d** in 59–85% yields. Fluorination of the ketones **11a**–**d** with 1.1 equivalent of LiHMDS and NFSi affords the corresponding monofluoromethylene ketones **12a**–**d** in 50–88% yields. Similarly, fluorination of the ketones **11a**–**d** with 2.2 equivalent of LiHMDS and NFSi generates the corresponding difluoromethylene ketones **13a**–**d** in 34–85% yields.



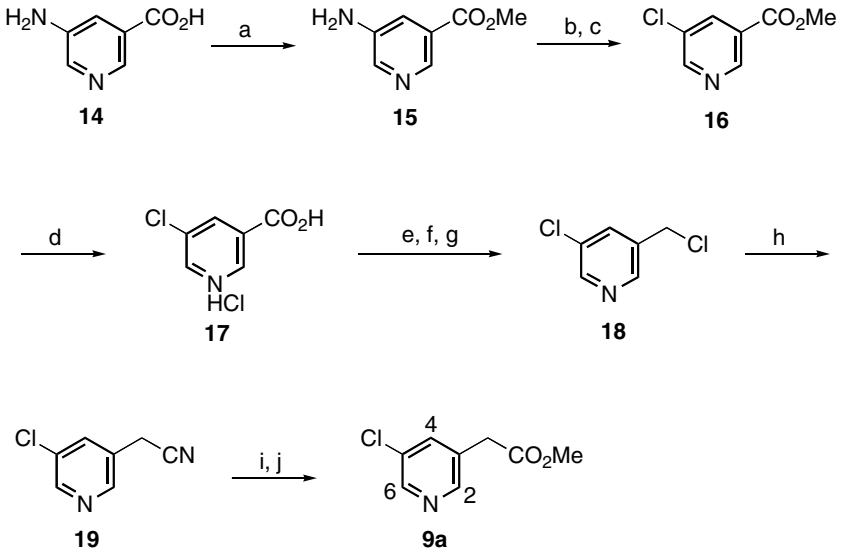
Scheme 2. Reagents and conditions: (a) MeOH, HCl, reflux, 12 h, 88%-quant.; (b) LiHMDS, THF, −78 °C, 1 h; (c) furan-2-carboxylic acid or 5-(4-chlorophenyl)furan-2-carboxylic acid, CDI, THF, 3 h, 72–96%; (d) 50% aqueous H_2SO_4, reflux, 8 h, 59–85%; (e) LiHMDS (1.1 equivalent), NFSi (1.1 equivalent), THF, −78 °C, 4 h, 50–88%; (f) LiHMDS (2.2 equivalent), NFSi (2.2 equivalent), THF, −78 °C, 4 h, 34–85%.

Compound 5-chloronicotinate **9a** is not commercially available and was prepared as described in Scheme 3. Esterification of the commercially available 5-aminonicotinic acid (**14**) affords methyl 5-aminonicotinate (**15**) in 88% yield. Then treating the free amine of **15** with sodium nitrite under acidic conditions, followed by the addition of copper (I) chloride and copper (II) chloride yields the methyl 5-chloronicotinate (**16**) in 79% yield. Hydrolysis of **16** with potassium hydroxide, and then acidic workup generates the acid **17** in 84% yield. Activation of **17** with ethyl chloroformate, followed by lithium aluminum hydride reduction produces the corresponding alcohol, which is readily converted by thionyl chloride to the chloride **18** in 76% yield over 3 steps. Nucleophilic attack of **18** by potassium cyanide provides compound **19** in 56% yield. Hydrolysis of **19**, followed by esterification gives the desired ester **9a** in quantitative yield.

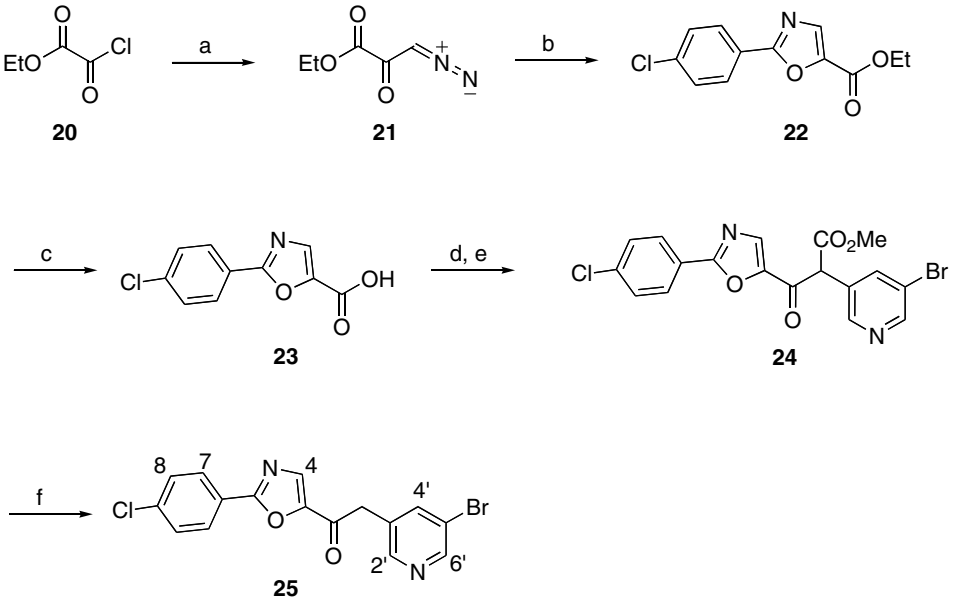
Compounds **11a–d**, **12a–d**, **13a–d** were tested as inhibitors of SARS 3CL^{pro} using the continuous fluorometric assay described above. The testing results, which only examine the initial binding affinity, are listed in Table 1. Surprisingly, most of the methylene ketones (**11a–c**) as well as their fluorinated methylene ketone analogues (**12a–c**, **13a–c**) inhibit 3CL^{pro} poorly. However, the methylene ketone **11d** and its fluorinated methylene ketone analogues **12d**, **13d** are good inhibitors of 3CL^{pro} with IC_{50} values of 13–57 μM. Interestingly, introduction of a fluorine substituent to this group of inhibitors bearing the *p*-chlorophenyl ring (**11d**, **12d**, **13d**) decreases the inhibitory activity ~2-fold, which stands in contrast to results with inhibitors **11a–c**, **12a–c**, **13a–c** that are lacking this ring. This suggests that the three-ringed inhibitors (**11d**, **12d**, **13d**) may have a different binding mode from the two-ringed inhibitors (**11a–c**, **12a–c**, **13a–c**). After 2 h preincubation of 25 μM methylene ketone (**11d**) or fluorinated methylene ketones (**12d**, **13d**) with 3CL^{pro}, no improved inhibitory activity is observed, indicating that these compounds act as non-covalent fully reversible inhibitors of SARS 3CL^{pro}. The inhibition mechanism was also examined on a longer time scale using electrospray ionization-mass spectrometry (ESI-MS) studies. After mixing 10 equivalent of inhibitor **11d**, **12d** or **13d** with 1 equivalent of 3CL^{pro}, and incubating the solution for 24 h, no mass change is observed in the major mass peak of 3CL^{pro}. This further demonstrates that compounds **11a–13d** utilize a non-covalent reversible mechanism of inhibition. These compounds represent a new class of SARS-CoV 3CL^{pro} inhibitors, and to our knowledge, are among the most potent non-covalent and reversible inhibitors of this enzyme.

As furan moieties have some potential to be metabolized rapidly in mammalian cells, two additional ketone analogues **25** and **28** were prepared and examined. Synthesis of **25** started from commercially available ethyl 2-chloro-2-oxoacetate (**20**), as shown in Scheme 4. Nucleophilic reaction of **20** with $TMSCHN_2$ provides the diazo compound **21** in 68% yield. Treating the diazo compound **21** with copper salt generates the carbene intermediate, which readily reacts with 4-chlorobenzonitrile through [3+2] cyclization to form the ester **22** with the desired oxazole moiety in 14% yield. Hydrolysis of **22** with lithium hydroxide produces the carboxylic acid **23** in 94% yield. Activation of the acid **23** with CDI, and addition of this to the pre-generated anion formed by deprotection of **9c** with LiHMDS, yields the β-keto ester **24** in 23% yield. Hydrolytic decarboxylation of the β-keto ester **24** gives the desired ketone **25** in 58% yield. The ketone **28** was also prepared in a similar method as described in Scheme 5.

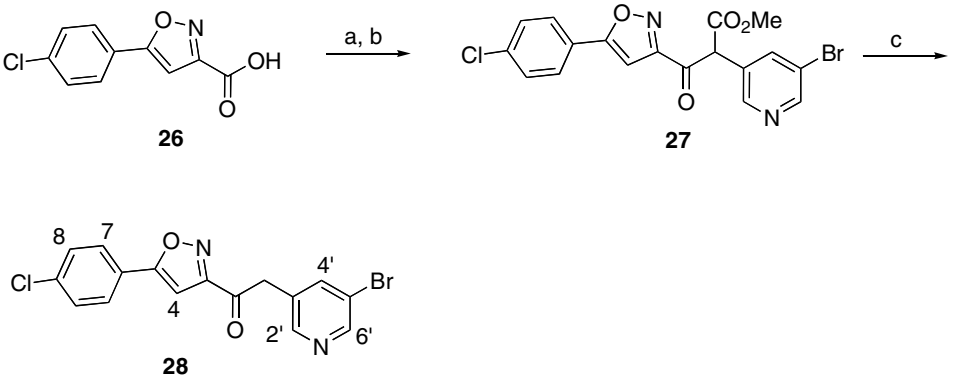
Compounds **25** and **28** were tested against SARS 3CL^{pro}. Compound **25** inhibits the proteinase with an IC_{50} value of 75 μM, and compound **28** displays 35% inhi-



Scheme 3. Reagents and conditions: (a) MeOH, $SOCl_2$, reflux, 8 h, 88%; (b) $NaNO_2$, Conc. HCl, 0 °C, 1 h; (c) 10% HCl, CuCl, $CuCl_2$, 0 °C, 4 h, 79%; (d) KOH, MeOH/H_2O, rt, 24 h, 84%; (e) Et_3N, EtOCOCl, 0 °C, 1.5 h; (f) $LiAlH_4$, −78 °C, 4 h; (g) $SOCl_2$, rt, 42 h, 76%; (h) KCN, DMF, rt, 48 h, 56%; (i) Conc. HCl, H_2O, reflux, 12 h; (j) MeOH, $SOCl_2$, reflux, 8 h, 72%.



Scheme 4. Reagents and conditions: (a) $TMSCHN_2$, THF, rt, 3 h, 68%; (b) 4-chlorobenzonitrile, $Cu(acac)_2$, benzene, reflux, 12 h, 14%; (c) LiOH, THF/H_2O, 0 °C, 2 h; (d) CDI, THF, 1H; (e) **9c**, LiHMDS, THF, −78 °C, 4 h, 23%; (f) 50% aqueous H_2SO_4, reflux, 8 h, 58%.



Scheme 5. Reagents and conditions: (a) CDI, THF, 1 h; (b) **9c**, LiHMDS, THF, −78 °C, 4 h, 19%; (c) 50% aqueous H_2SO_4, reflux, 8 h, 58%.

bition at a concentration of 100 μM (Table 1). It is known that for non-covalent and reversible inhibitors, hydrogen bonds, ionic and van der Waal's interactions play crucial roles in binding affinity to the target enzyme [22]. For inhibitors **11d**–**13d**, the oxygen atoms of their furan rings are suspected to have hydrogen bonds with the $3CL^{pro}$ [10b,23]. In contrast, that position may be occupied by the oxygen atom of the oxazole ring of **25** and the carbon atom of the isoxazole ring of **28**, both of which have lower electron density and may thus give weaker binding to the $3CL^{pro}$.

To gain further insight into the inhibition mechanism, modeling studies (Fig. 2) of $3CL^{pro}$ with inhibitors **11a**–**13d** were conducted [10b]. Since the S2 and S4 pockets in the active site of $3CL^{pro}$ are relatively large, the shorter two-ringed compounds (e.g. **11c**) cannot occupy the maximal volume in these S sites in any docked conformations. However, due to their extended end-to-end length, the three-ringed compounds can occupy more of the binding surface from the S2/S4 to the S1 pocket. Therefore, the three aromatic ring compounds (**11d**, **12d**, **13d**) are more effective in blocking the binding of substrates into the active site, and thus exhibit better inhibition against $3CL^{pro}$ than the two-ringed ones, as revealed in the enzymatic assay. Based on our previous modeling studies [10b,23], the three-ringed esters utilize a non-covalent and reversible mechanism of inhibition in a S4–S1 binding mode, by blocking entry of substrates into the active site of SARS-CoV $3CL^{pro}$. This is supported by a recently reported crystal structure of a three-ringed thioester with the $3CL^{pro}$ [24]. Docking results suggest that these ketone analogues (**11d**, **12d**, **13d**) adopt binding conformations similar to that of the corresponding esters (e.g. **4**). Each of these three compounds is oriented in an extended conformation from S4 to S1 pocket, with the oxygen atom of their furan ring forming a hydrogen bond with the main chain NH of Glu166, an interaction that was also predicted for the three-ringed esters. The pyridinyl moiety preferentially binds inside

the S1 specificity pocket, which limits the possible spatial orientations of the substituents at the α-position of the central ketone group. In the 3CL^{pro}:**12d**and 3CL^{pro}:**13d** complexes, the fluorine substituents of the inhibitor point towards the main chain carbonyl oxygen of His164 (Fig. 2). This pushes **12d** and **13d** slightly out towards the solvent. Thus, the van der Waals interactions between the fluorinated compounds and the active site residues of the enzyme are likely to be weaker than those for **11d**.

4. Conclusion

A series of aryl methylene ketones and fluorinated methylene ketones have been prepared and tested as potential SARS 3CL^{pro} inhibitors. Derivatives **11d**, **12d** and **13d** comprising three aromatic rings, including a 5-bromopyridin-3-yl moiety, show the best inhibition (IC_{50} = 13–57 μM). Interestingly, in this series (as opposed to analogues with two aryl rings), fluorination decreases inhibition despite enhancing electrophilicity of the carbonyl carbon. Enzyme kinetics and ESI-MS studies indicate that these inhibitors utilize a non-covalent, reversible mechanism, providing a basis for development of more potent specific inhibitors.

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