

Supporting Information

Rational design of 5-(4-(isopropylsulfonyl)phenyl)-3-(3-(4-((methylamino)methyl)phenyl)isoxazol-5-yl)pyrazin-2-amine (VX-970, M6620): Optimization of intra- and inter-molecular polar interactions of a new ataxia telangiectasia mutated and Rad3-related (ATR) kinase inhibitor

Ronald Knegt^{*}, Jean-Damien Charrier, Steven Durrant, Chris Davis, Michael O'Donnell, Pierre Storck, Somhairle MacCormick[†], David Kay, Joanne Pinder, Anisa Virani, Heather Twin, Matthew Griffiths, Philip Reaper, Peter Littlewood, Steve Young[‡], Julian Golec, John Pollard

Vertex Pharmaceuticals (Europe) Ltd, 86-88 Jubilee Avenue, Milton Park, Abingdon, Oxfordshire OX14 4RW, United Kingdom

[†]Present Address: PharmaCytics Operations BV, Novio Tech Campus, Office 3.12, Transistorweg 5v, 6534 AT Nijmegen, The Netherlands

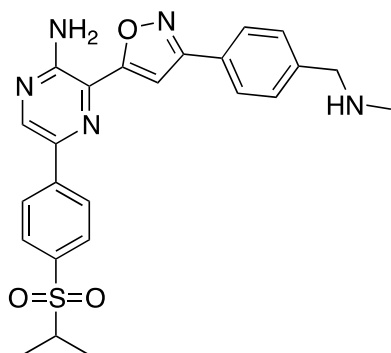
[‡]Present Address: Sygnature Discovery Limited, BioCity, Pennyfoot Street, Nottingham, NG1 1GF, United Kingdom

Content:

1. Analytical data for compounds 2-20	S3
2. HPLC Characterization of purity for compounds 2-20	S13
3. Preparation of key intermediates	S23
4. Enzyme assays	S29
5. Cellular assays	S30

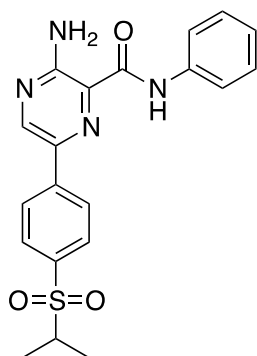
Analytical data for compounds 2-20

5-(4-(Isopropylsulfonyl)phenyl)-3-(3-(4-((methylamino)methyl)phenyl)isoxazol-5-yl)pyrazin-2-amine (2).



Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.92 (s, 1 H), 8.85 (br s, 2H), 8.43 (m, 2H), 8.12 (m, 2H), 7.85 (m, 2H), 7.82 (s, 1H), 7.65 (m, 2H), 7.26 (br s, 2H), 4.21-4.25 (m, 2H), 3.55 (m, J 7.5 Hz, 1H), 2.61-2.65 (m, 3H), 1.22 (d, J 7.5 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.35, 162.38, 152.08, 143.00, 141.66, 138.04, 136.14, 134.81, 131.27, 129.64, 129.10, 127.43, 126.17, 124.86, 102.76, 54.68, 51.10, 32.25, 15.56; MS (ES $^+$) 464.4; HRMS (ES $^+$) calcd for $\text{C}_{24}\text{H}_{25}\text{N}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 464.1751; found: 464.1754.

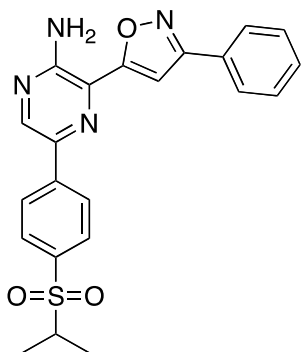
3-Amino-6-(4-(isopropylsulfonyl)phenyl)-*N*-phenylpyrazine-2-carboxamide (3).



Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 10.47 (s, 1H), 9.05 (s, 1H), 8.53 (d, J 8.5 Hz, 2H), 7.93 (d, J 8.5 Hz, 2H), 7.82 (d, J 7.8 Hz, 2H), 7.41 (t, J 7.8 Hz, 2H), 7.18 (m, 1H), 3.48 (m, J 6.8 Hz, 1H), 1.19 (d, J 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 164.72, 155.14, 145.80, 141.17, 138.20, 136.74, 136.16, 129.36, 128.99, 126.56, 124.87, 124.68, 121.62,

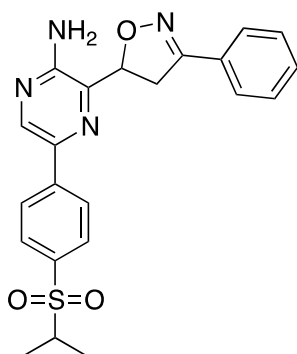
54.56, 15.59; MS (ES+) m/z 397.3 (M + H)⁺; HRMS (ES+) calcd for C₂₀H₂₀N₄O₃S (M + H)⁺ 397.1334; found: 397.1343.

5-(4-(Isopropylsulfonyl)phenyl)-3-(3-phenylisoxazol-5-yl)pyrazin-2-amine (4).



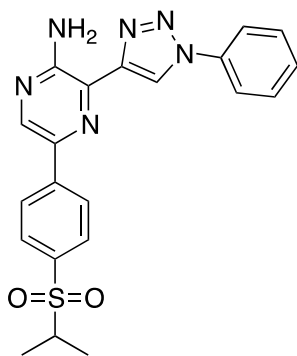
Isolated as a yellow solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.95 (s, 1H), 8.44 (m, 2H), 8.01 - 8.06 (m, 2H), 7.95 (m, 2H), 7.67 (s, 1H), 7.55 - 7.63 (m, 2H), 7.22 - 7.35 (m, 2H), 3.47 (m, *J* 6.9 Hz, 1H), 1.14 (d, *J* 6.9 Hz, 6H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 168.93, 163.52, 153.04, 143.78, 142.32, 138.82, 137.00, 131.76, 131.74, 130.44, 129.54, 128.08, 126.96, 125.72, 103.46, 55.46, 16.47; MS (ES+) m/z 421.3 (M + H)⁺; HRMS (ES+) calcd for C₂₂H₂₀N₄O₃S (M + H)⁺ 421.1334; found: 421.1339.

5-(4-(Isopropylsulfonyl)phenyl)-3-(3-phenyl-4,5-dihydroisoxazol-5-yl)pyrazin-2-amine (5).



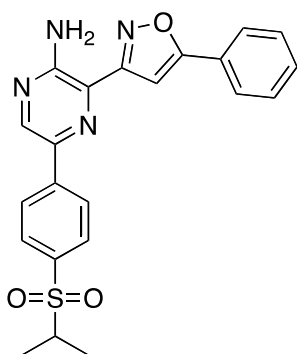
Isolated as a white solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.75 (s, 1H), 8.22 (m, 2H), 7.78 - 7.85 (m, 4H), 7.48-7.53 (m, 3H), 6.95 (br s, 2H), 6.02 (m, 1H), 4.28 (m, 1H), 3.75 (m, 1H), 3.44 (m, J 6.8 Hz, 1H), 1.15 (d, J 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 157.78, 154.40, 142.19, 141.24, 136.48, 136.02, 135.65, 130.70, 129.67, 129.56, 129.37, 127.26, 125.53, 78.88, 54.65, 37.03, 15.66; MS (ES+) m/z 423.4 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{22}\text{H}_{22}\text{N}_4\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 423.1491; found: 423.1487.

5-(4-(Isopropylsulfonyl)phenyl)-3-(1-phenyl-1H-1,2,3-triazol-4-yl)pyrazin-2-amine (6).



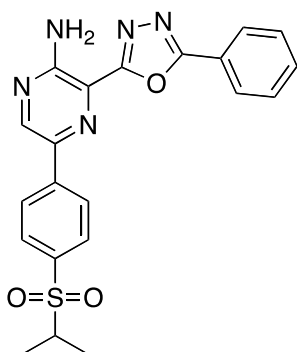
Isolated as a pale yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.66 (s, 1H), 8.85 (s, 1H), 8.46 (m, 2H), 8.12 (m, 2H), 7.91 (m, 2H), 7.74 (br s, 2H), 7.67 (m, 2H), 7.58 (m, 1H), 3.47 (m, J 6.8 Hz, 1H), 1.19 (d, J 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 152.28, 148.04, 142.08, 140.53, 137.22, 136.85, 135.86, 130.40, 129.74, 129.48, 128.22, 125.99, 123.44, 121.08, 54.72, 15.71; MS (ES+) m/z 421.1 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{21}\text{H}_{20}\text{N}_6\text{O}_2\text{S}$ ($\text{M} + \text{H}$) $^+$ 421.1447; found: 421.1445.

5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenylisoxazol-3-yl)pyrazin-2-amine (7).



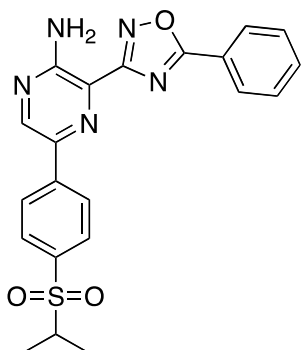
Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.95 (s, 1H), 8.42 (m, 2H), 8.02 - 8.05 (m, 2H), 7.93 (m, 2H), 7.78 (s, 1H), 7.55 - 7.61 (m, 3H), 7.27 (br s, 2H), 3.55 (m, J 6.9 Hz, 1H), 1.22 (d, J 6.9 Hz, 6H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 166.65, 161.23, 150.76, 141.50, 140.03, 136.52, 134.71, 129.45, 128.17, 128.15, 127.25, 125.79, 124.67, 123.43, 101.18, 53.18, 14.18; MS (ES+) m/z 421.1 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 421.1334; found: 421.1354.

5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)pyrazin-2-amine (8).



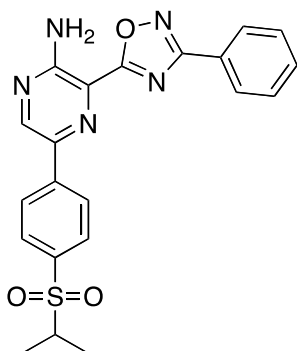
Isolated as an orange solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.06 (s, 1H), 8.40 (m, 2H), 8.19 - 8.17 (m, 2H), 7.98 (m, 4H), 7.72 - 7.66 (m, 3H), 3.47 (m, J 6.8 Hz, 1H), 1.20 (d, J 6.8 Hz, 6H); MS (ES+) m/z 422.0 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{21}\text{H}_{19}\text{N}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 422.1287; found: 422.1283.

5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyl-1,2,4-oxadiazol-3-yl)pyrazin-2-amine (9).



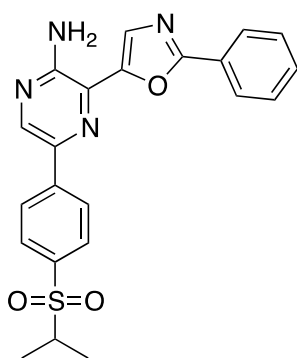
Isolated as a pale orange solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.86 (s, 1H), 8.18 (m, 2H), 8.12 (m, 2H), 7.79 (m, 2H), 7.61 (m, 1H), 7.53 (m, 2H), 7.42 (br s, 2H), 3.29 (m, J 6.8 Hz, 1H), 1.02 (d, J 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 175.45, 167.51, 153.68, 144.06, 141.50, 138.15, 136.14, 134.00, 129.99, 129.64, 128.60, 126.09, 123.50, 123.21, 54.60, 15.59; MS (ES $^+$) m/z 422.2 ($\text{M} + \text{H}$) $^+$; HRMS (ES $^+$) calcd for $\text{C}_{21}\text{H}_{19}\text{N}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 422.1287; found: 422.1298.

5-(4-(Isopropylsulfonyl)phenyl)-3-(3-phenyl-1,2,4-oxadiazol-5-yl)pyrazin-2-amine (10).



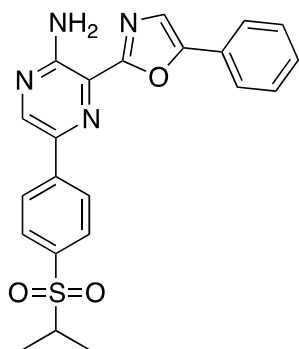
Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.16 (s, 1H), 8.37 (m, 2H), 8.26 (m, 2H), 8.07 (br s, 2H), 7.98 (m, 2H), 7.68 - 7.61 (m, 3H), 3.48 (m, J 6.8 Hz, 1H), 1.20 (d, J 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 172.90, 168.02, 153.71, 146.42, 141.05, 138.42, 136.46, 132.25, 129.70, 129.65, 127.82, 126.22, 126.13, 119.48, 54.58, 15.58; MS (ES+) m/z 422.2 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{21}\text{H}_{19}\text{N}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 422.1287; found: 422.1283.

5-(4-(Isopropylsulfonyl)phenyl)-3-(2-phenyloxazol-5-yl)pyrazin-2-amine (11).



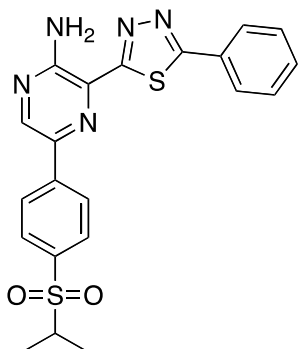
Isolated as an off-white solid; ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.45 (s, 1H), 8.12 - 8.07 (m, 2H), 8.06 - 8.01 (m, 2H), 7.97 - 7.87 (m, 3H), 7.56 - 7.25 (m, 3H), 3.17 (m, J 6.9 Hz, 1H), 1.27 (d, J 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 158.88, 153.04, 151.78, 143.07, 141.75, 137.82, 136.18, 129.76, 129.73, 129.67, 127.47, 126.16, 124.93, 124.16, 122.96, 54.75, 15.74; MS (ES+) m/z 421.0 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 421.1334; found: 421.1335.

5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyloxazol-2-yl)pyrazin-2-amine (12).



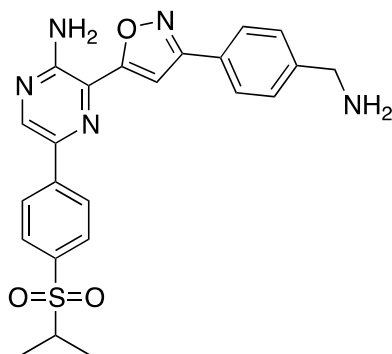
Isolated as an off-white solid; ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 8.96 (s, 1H), 8.40 (d, J 8.5 Hz, 2H), 8.07 (s, 1H), 7.97 (d, J 8.5 Hz, 2H), 7.94 - 7.88 (m, 2H), 7.56 (m, 2H), 7.47 (m, 1H), 3.47 (m, J 6.8 Hz, 1H), 1.21 (d, J 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 161.47, 151.60, 148.70, 141.84, 140.80, 137.91, 136.08, 131.46, 129.66, 129.16, 129.07, 127.02, 126.87, 126.66, 126.07, 54.72, 15.72; MS (ES+) m/z 421.0 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{22}\text{H}_{20}\text{N}_4\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 421.1334; found: 421.1347.

5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)pyrazin-2-amine (13).



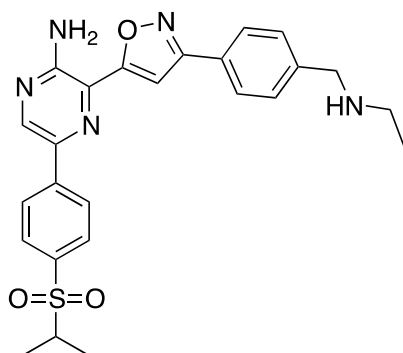
Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 9.03 (s, 1H), 8.32 (m, 2H), 8.12 - 8.18 (m, 3H), 7.98 (m, 2H), 7.61 - 7.65 (m, 3H), 2.72 (m, J 6.8 Hz, 1H), 1.75 (d, J 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 171.60, 169.50, 152.86, 144.74, 141.73, 138.76, 137.06, 132.88, 130.68, 130.43, 130.37, 128.95, 126.74, 126.2, 54.62, 15.63; MS (ES+) m/z 438.0 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{21}\text{H}_{19}\text{N}_5\text{O}_2\text{S}_2$ ($\text{M} + \text{H}$) $^+$ 438.1058; found: 438.1056.

3-(3-(4-(Aminomethyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (14).



Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.97 (s, 1H), 8.21 (br s, 2H), 8.43 (m, 2H), 8.14 (m, 2H), 7.95 (m, 2H), 7.82 (s, 1H), 7.65 (m, 2H), 7.25 (br s, 2H), 4.21-4.25 (m, 2H), 3.55 (m, J 6.8 Hz, 1H), 1.22 (d, J 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.42, 162.25, 152.27, 143.09, 141.52, 138.12, 136.71, 136.31, 130.06, 129.63, 128.86, 127.49, 126.17, 124.85, 102.70, 54.71, 42.51, 15.70; MS (ES+) m/z 450.4 ($\text{M} + \text{H}$) $^+$; HRMS (ES+) calcd for $\text{C}_{23}\text{H}_{23}\text{N}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}$) $^+$ 450.1600; found: 450.1603.

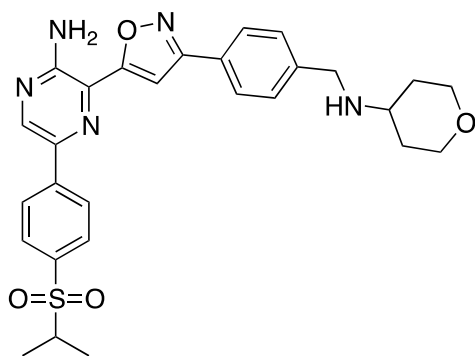
3-(3-(4-((Ethylamino)methyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (15).



Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.97 (s, 1H), 8.82 (br m, 1H), 8.41 (m, 2H), 8.10 (m, 2H), 7.95 (m, 2H), 7.82 (s, 1H), 7.65 (m, 2H), 7.25 (br s, 2H), 4.23 (m, 2H), 3.55 (m, J 6.9 Hz, 1H), 3.05 (m, 2H), 1.25 (t, J 7.5 Hz, 3H), 1.22 (d, J 6.9 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.44, 162.21, 152.27, 143.10, 141.52, 138.14, 136.32, 134.81,

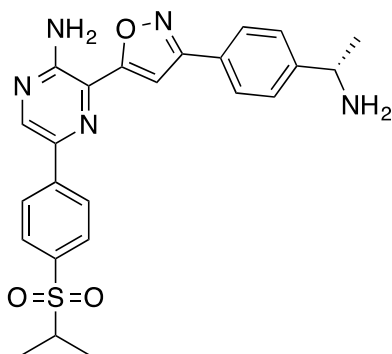
131.04, 129.63, 129.29, 127.58, 126.18, 124.83, 102.71, 54.71, 49.74, 42.50, 15.70, 11.45; MS (ES⁺) *m/z* 478.4 (M + H)⁺; HRMS (ES⁺) calcd for C₂₅H₂₇N₅O₃S (M + H)⁺ 478.1913; found: 478.1915.

5-(4-(Isopropylsulfonyl)phenyl)-3-(3-(4-(((tetrahydro-2H-pyran-4-yl)amino)methyl)phenyl)isoxazol-5-yl)pyrazin-2-amine (16).



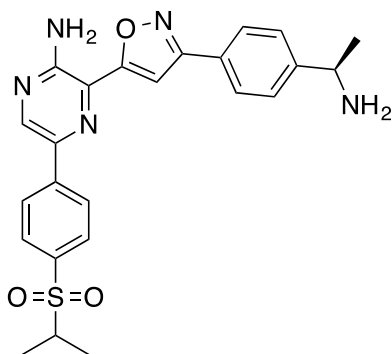
Isolated as a yellow solid; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.93 (s, 1H), 8.37 (m, 2H), 7.93 - 7.98 (m, 4H), 7.74 (s, 1H), 7.52 (m, 2H), 7.18 (br s, 2H), 3.79-3.82 (m, 4H), 3.44 (m, *J* 6.8 Hz, 1H), 3.25 (m, 2H), 2.59 (m, 1H), 2.12 (br s, 1H), 1.78 (m, 2H), 1.29 (m, 2H), 1.19 (d, *J* 6.8 Hz, 6H); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 167.56, 162.13, 151.71, 144.04, 142.38, 141.04, 137.57, 135.74, 129.10, 128.45, 126.52, 126.37, 125.65, 124.50, 102.06, 65.79, 54.22, 52.58, 49.18, 33.16, 15.17; MS (ES⁺) *m/z* 534.2 (M + H)⁺; HRMS (ES⁺) calcd for C₂₈H₃₁N₅O₄S (M + H)⁺ 534.2175; found: 534.2173.

3-[3-[4-[(1S)-1-Aminoethyl]phenyl]isoxazol-5-yl]-5-(4-isopropylsulfonylphenyl)pyrazin-2-amine (17).



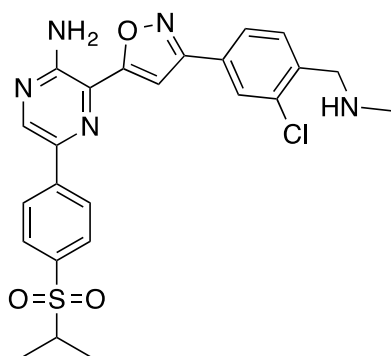
Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.95 (s, 1H), 8.39 (d, J 8.5 Hz, 2H), 7.96 - 7.93 (m, 4H), 7.77 (s, 1H), 7.56 (d, J 8.3 Hz, 2H), 7.20 (s, 2H), 4.07 (q, J 6.6 Hz, 1H), 3.47 (m, J 6.8 Hz, 1H), 1.95 (br s, 2H), 1.29 (d, J 6.6 Hz, 3H), 1.20 (d, J 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.02, 162.66, 152.23, 151.87, 142.91, 141.55, 138.08, 136.25, 129.63, 127.08, 127.05, 126.68, 126.18, 125.01, 102.60, 54.72, 50.97, 26.55, 15.70; MS (ES+) 464.3; HRMS (ES+) calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4\text{O}_3\text{S}$ ($\text{M} - \text{NH}_2$) $^+$ 447.1485; found: 447.1491.

3-[3-[4-[(1R)-1-Aminoethyl]phenyl]isoxazol-5-yl]-5-(4-isopropylsulfonylphenyl)pyrazin-2-amine (18).



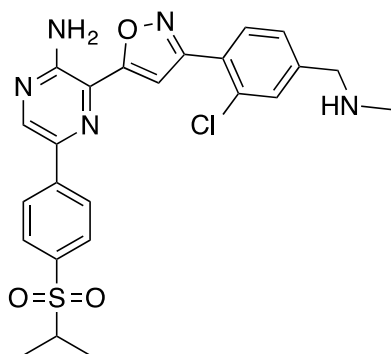
Isolated as a yellow solid (TFA salt); ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.96 (s, 1H), 8.39 (m, 3H), 8.38 (d, J 8.5 Hz, 2H), 8.11 (d, J 8.2 Hz, 2H), 7.94 (d, J 8.5 Hz, 2H), 7.83 (s, 1H), 7.69 (d, J 8.3 Hz, 2H), 7.22 (br s, 2H), 4.54 (m, 1H), 3.47 (q, J 6.8 Hz, 1H), 1.56 (d, J 6.8 Hz, 3H) and 1.20 (d, J 6.8 Hz, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.38, 162.22, 152.26, 143.07, 141.83, 141.52, 138.12, 136.30, 129.63, 128.86, 128.03, 127.64, 126.18, 124.85, 102.71, 54.71, 50.18, 21.02, 15.70; MS (ES+) 464.1; HRMS (ES+) calcd for $\text{C}_{24}\text{H}_{23}\text{N}_4\text{O}_3\text{S}$ ($\text{M} - \text{NH}_2$) $^+$ 447.1485; found: 447.1497.

3-(3-(3-Chloro-4-((methylamino)methyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (19).



Isolated as a yellow solid; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.98 (br s, 2H), 8.38 (m, 2H), 8.25 (m, 1H), 8.12 (m, 1H), 7.94-7.96 (m, 3H), 7.79 (m, 1H), 7.24 (br s, 2H), 4.37 (s, 2H), 3.48 (m, 1H), 2.71 (s, 3H), 1.19 (d, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 168.64, 161.19, 158.24, 152.27, 143.20, 141.49, 138.14, 136.32, 134.83, 132.67, 132.47, 131.18, 129.64, 128.16, 126.17, 124.66, 102.86, 54.71, 48.85, 33.30, 15.69; MS (ES+) 498.2; HRMS (ES+) calcd for $\text{C}_{24}\text{H}_{24}\text{ClN}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}$)⁺ 498.1361; found: 498.1360.

3-(3-(2-Chloro-4-((methylamino)methyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (20).



Isolated as a yellow solid (TFA salt); ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 8.98 (s, 1H), 8.87 (br s, 2H), 8.36 (m, 2H), 7.90-7.96 (m, 3H), 7.85 (m, 1H), 7.63-7.65 (m, 2H), 7.25 (br s, 2H), 4.26 (m, 2H), 3.49 (m, 1H), 2.63 (t, 3H), 1.18 (d, 6H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 167.99, 160.97, 158.32, 152.30, 143.24, 141.44, 138.19, 136.32, 132.46, 132.18, 132.06, 129.66, 129.56, 128.46, 126.19, 124.62, 105.16, 54.70, 50.71, 32.81, 15.69; MS (ES+) 498.2; HRMS (ES+) calcd for $\text{C}_{24}\text{H}_{24}\text{ClN}_5\text{O}_3\text{S}$ ($\text{M} + \text{H}$)⁺ 498.1361; found: 498.1379.

HPLC Characterization of purity for compounds 2-20

Purity of compounds **2-20** was measured by HPLC. Several methods have been used and are reported below. Retention times and purity of compounds **2-20** are summarized in Table 3.

Method 1: analytical reverse phase HPLC-MS was carried out on an agilent 100 HPLC with a waters Quattro MS system equipped with an ACE 5mm C-18 reverse phase column (4.6 mm x 150 mm, 5 μ m). The mobile phases were acetonitrile/methanol (1:1) and water (10 mM ammonium acetate), 5 or 12 min run.

Method 2: analytical reverse phase UPLC-MS was carried out on a waters Acquity UPLC-MS system equipped with a waters BEH 1.7mm C-18 reverse phase column (2.1 mm x 50 mm, 1.7 μ m). The mobile phases were acetonitrile and water/acetonitrile (95:5 with 10 mM ammonium formate, pH9).

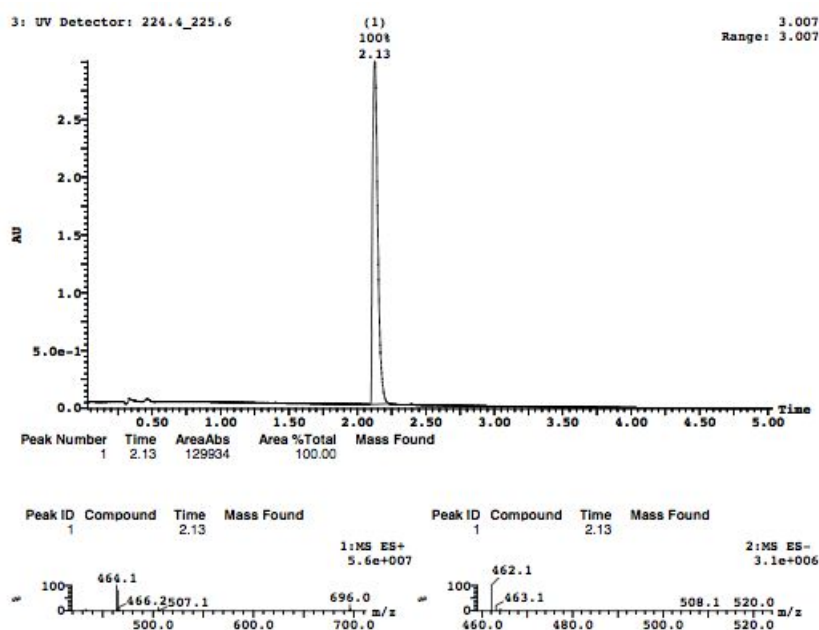
Method 3: analytical reverse phase UPLC-MS was carried out on a waters Acquity UPLC-MS system equipped with a waters BEH 1.7mm C-18 reverse phase column (2.1 mm x 50 mm, 1.7 μ m). The mobile phases were acetonitrile and water/acetonitrile (95:5 with 10 mM ammonium acetate, pH7).

Table 3: Retention times and purity of compounds **2-20**

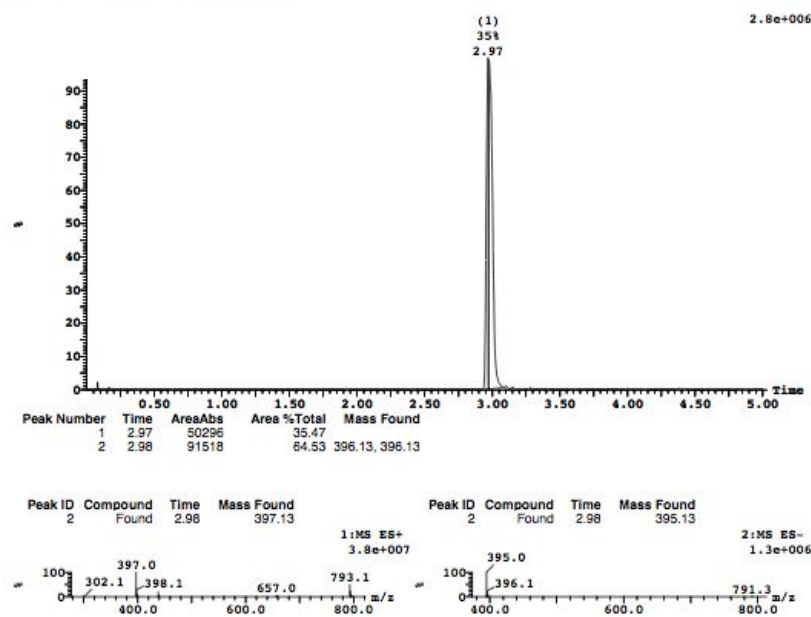
Compound	Retention time (min)	% Purity	Method
2	2.13	100	3
3	2.97	100	3
4	3.00	100	3
5	3.10	100	3

6	3.07	91	3
7	2.77	99	2
8	9.44	98	1
9	2.96	95	3
10	3.16	100	3
11	2.88	100	2
12	3.17	100	2
13	3.11	99	3
14	3.09	100	1
15	2.97	100	1
16	2.31	98	3
17	2.15	100	3
18	2.13	100	3
19	9.15	100	1
20	2.26	100	3

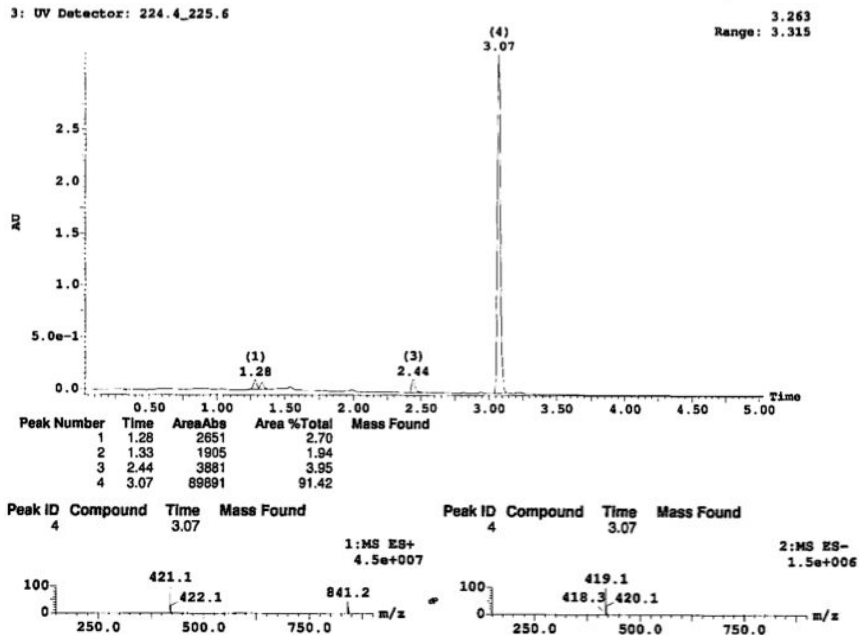
5-(4-(Isopropylsulfonyl)phenyl)-3-(3-(4-((methylamino)methyl)phenyl)isoxazol-5-yl)pyrazin-2-amine (2)



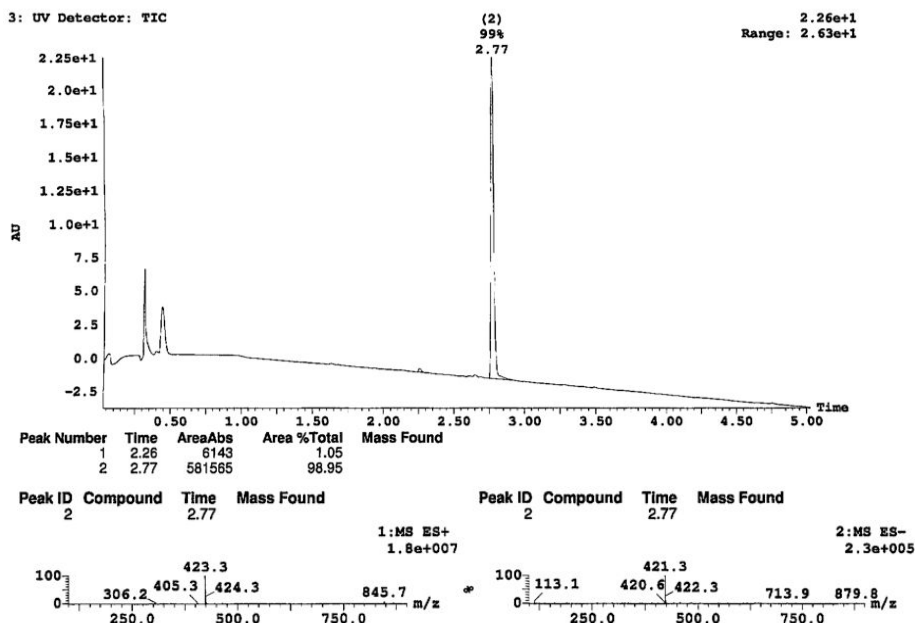
3-Amino-6-(4-(isopropylsulfonyl)phenyl)-N-phenylpyrazine-2-carboxamide (3)



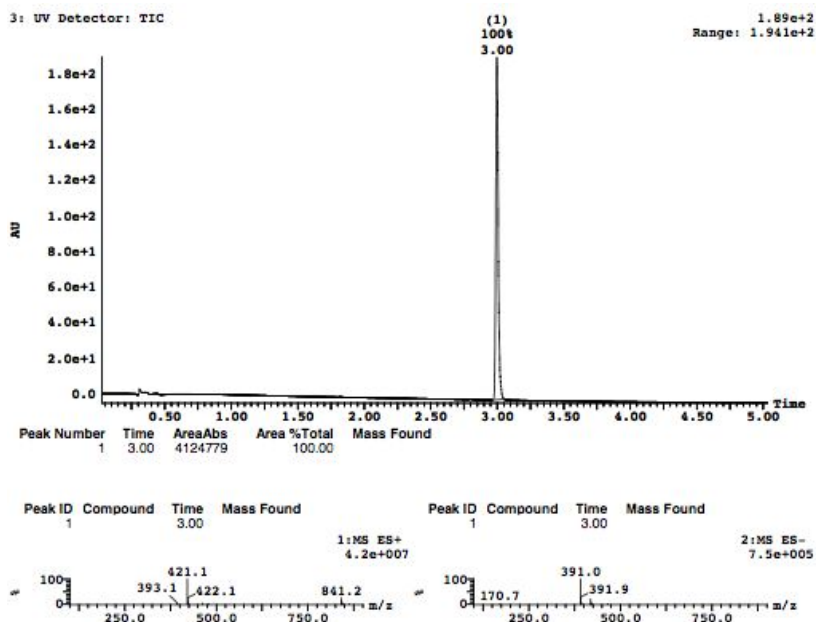
5-(4-(Isopropylsulfonyl)phenyl)-3-(3-phenylisoxazol-5-yl)pyrazin-2-amine (4)



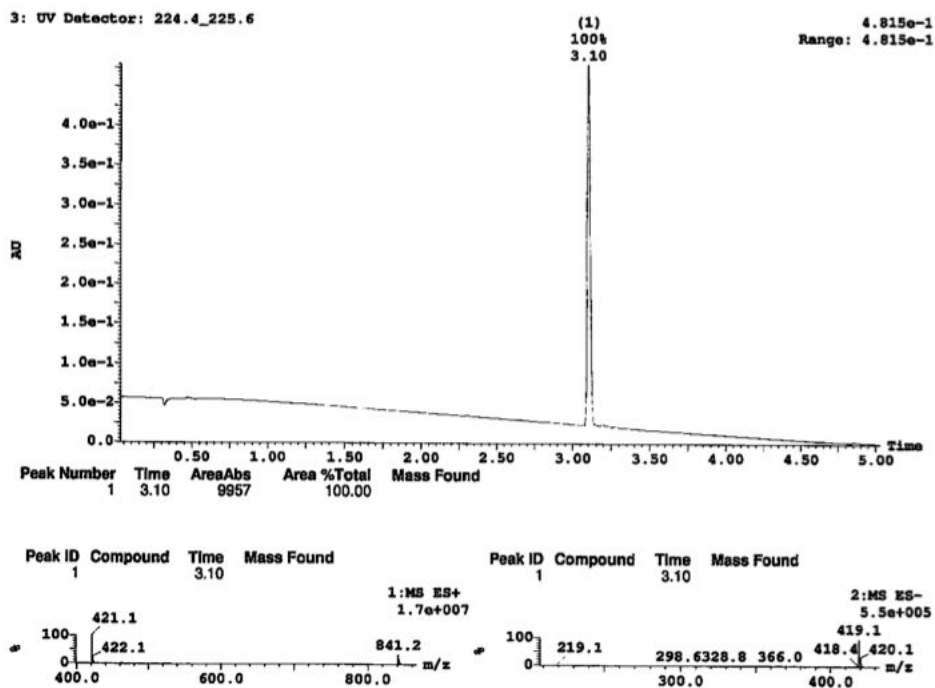
5-(4-(Isopropylsulfonyl)phenyl)-3-(3-phenyl-4,5-dihydroisoxazol-5-yl)pyrazin-2-amine (5)



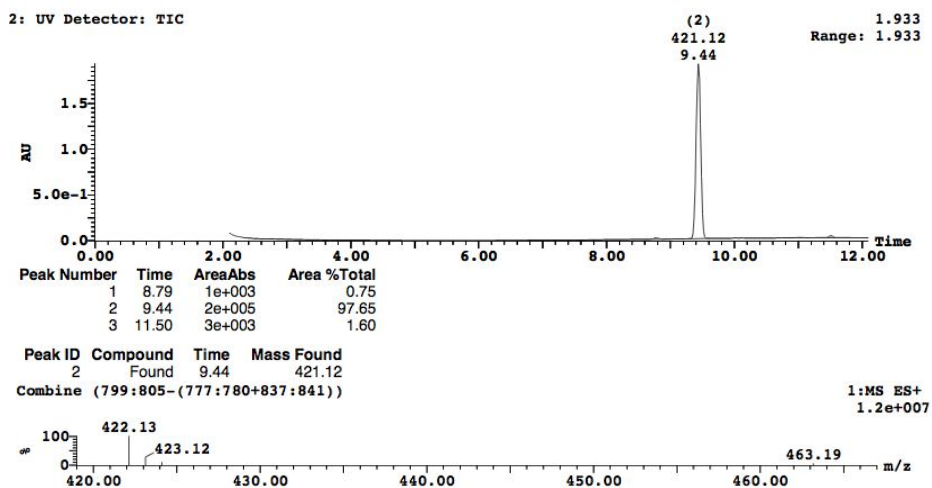
5-(4-(Isopropylsulfonyl)phenyl)-3-(1-phenyl-1*H*-1,2,3-triazol-4-yl)pyrazin-2-amine (6)



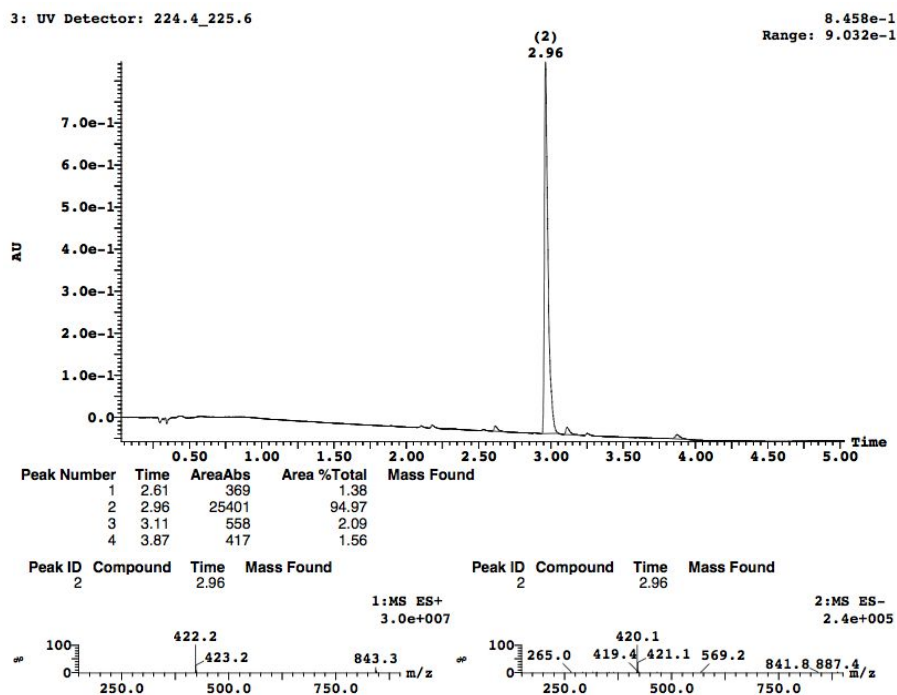
5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenylisoxazol-3-yl)pyrazin-2-amine (7)



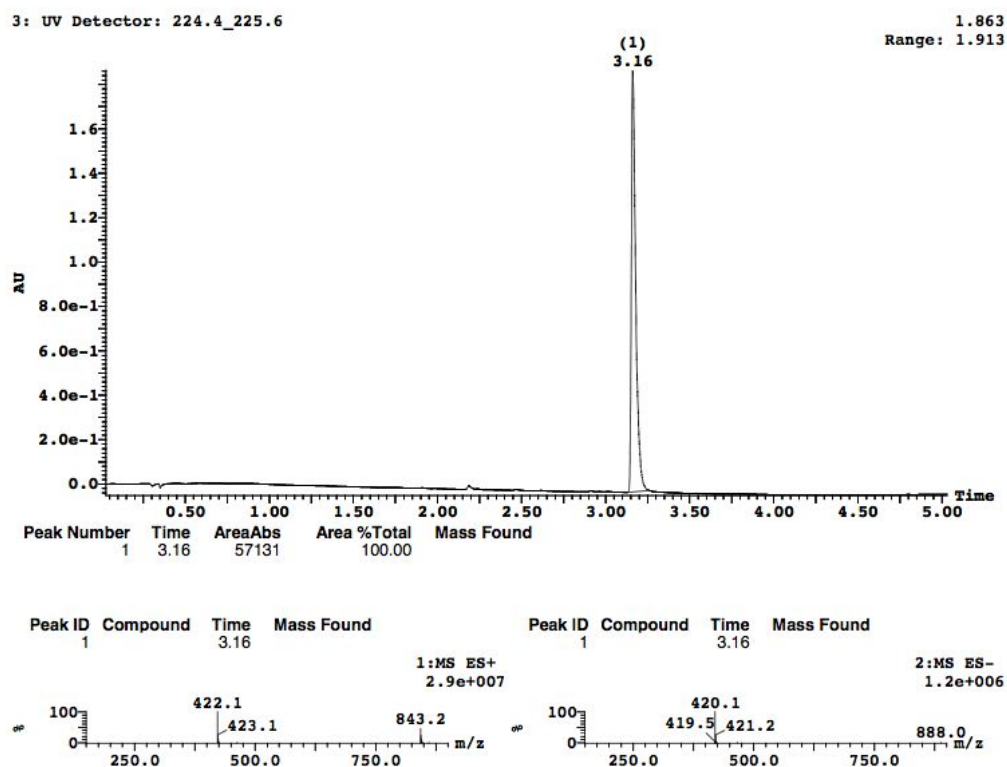
5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyl-1,3,4-oxadiazol-2-yl)pyrazin-2-amine (8)



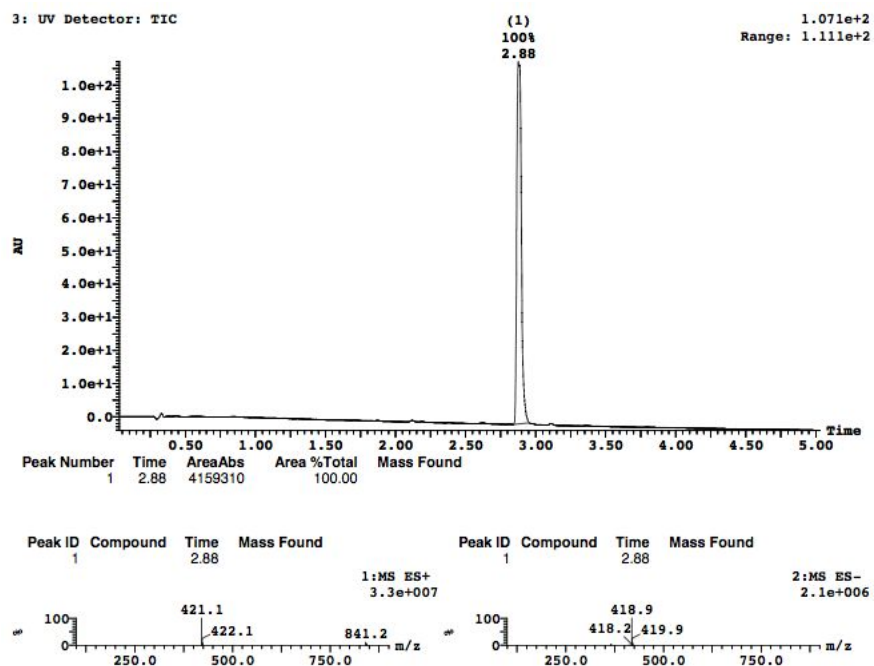
5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyl-1,2,4-oxadiazol-3-yl)pyrazin-2-amine (9)



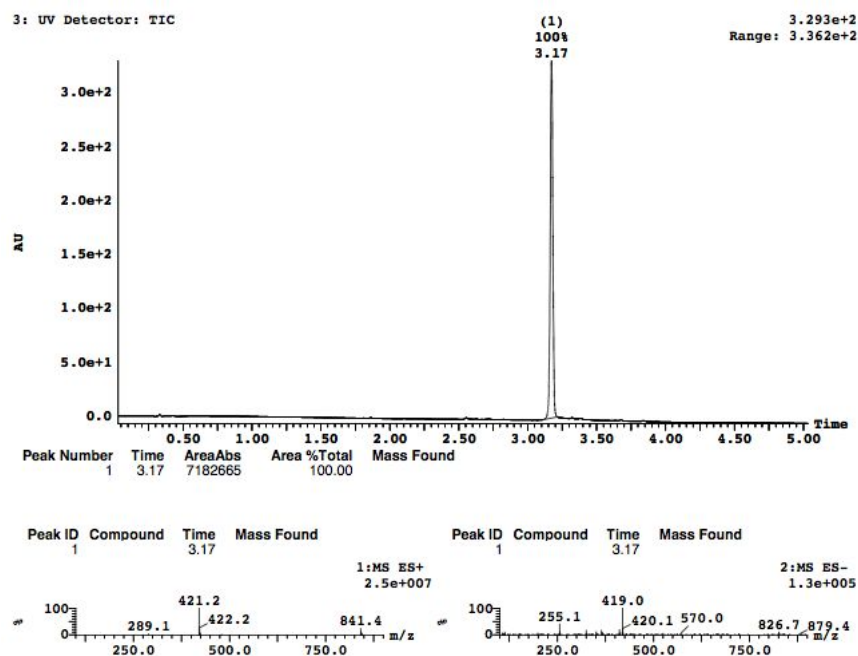
5-(4-(Isopropylsulfonyl)phenyl)-3-(3-phenyl-1,2,4-oxadiazol-5-yl)pyrazin-2-amine (10)



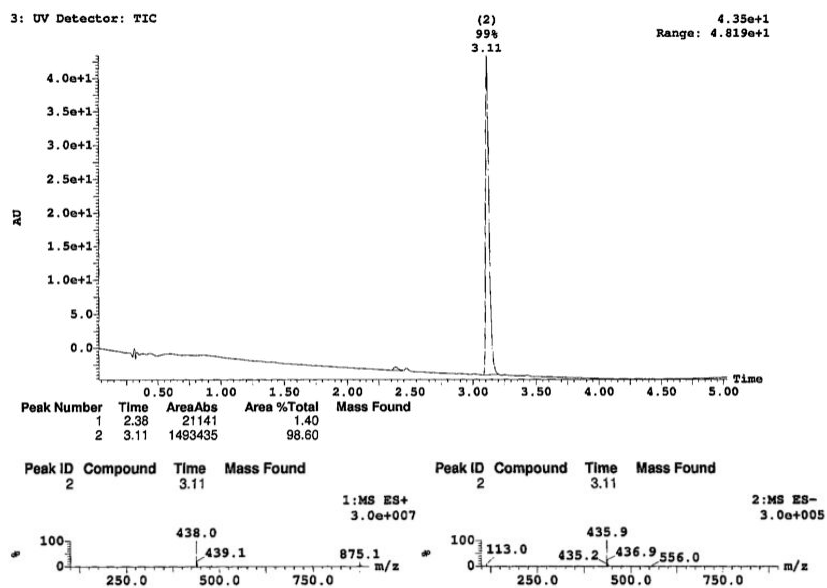
5-(4-(Isopropylsulfonyl)phenyl)-3-(2-phenyloxazol-5-yl)pyrazin-2-amine (11)



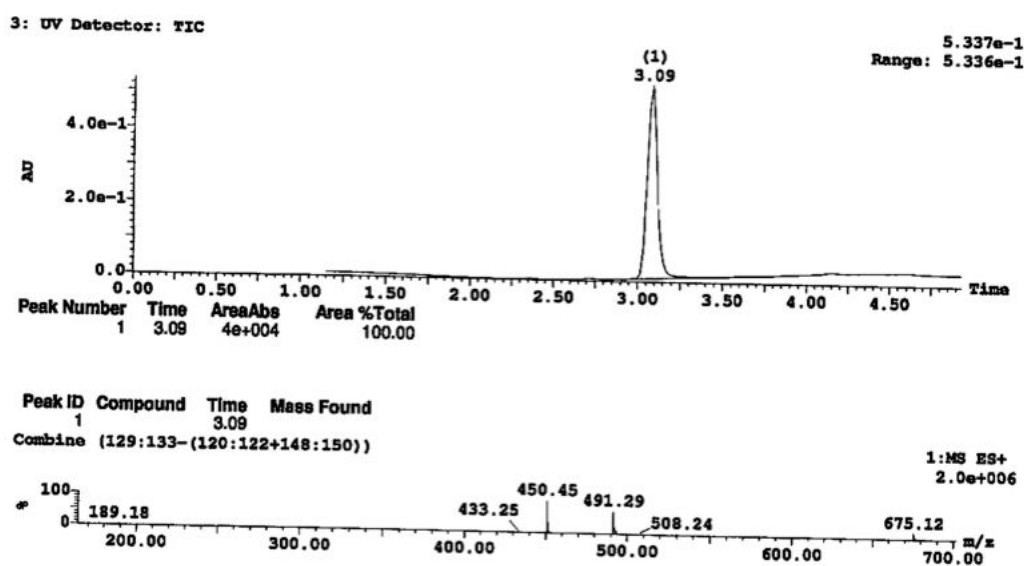
5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyloxazol-2-yl)pyrazin-2-amine (12)



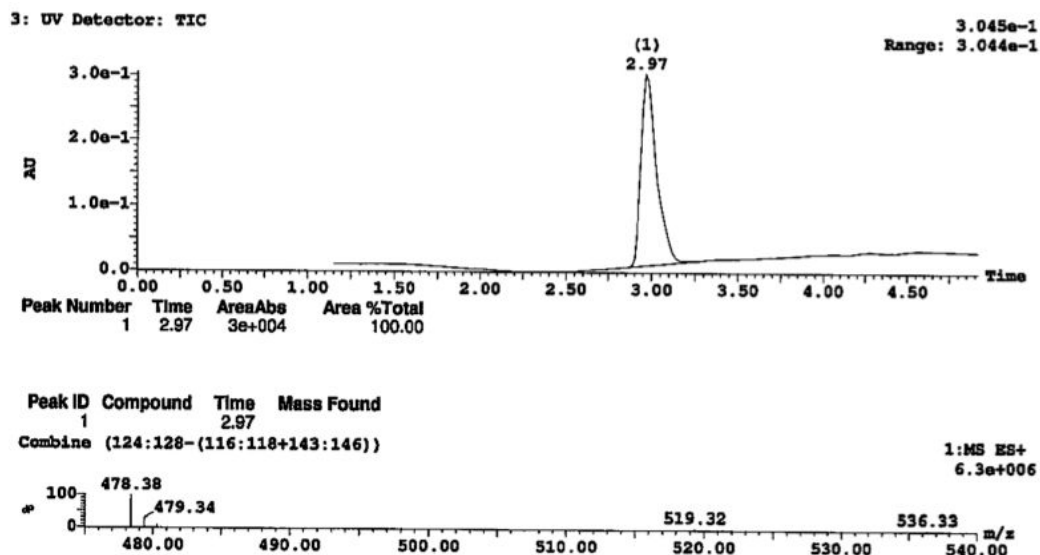
5-(4-(Isopropylsulfonyl)phenyl)-3-(5-phenyl-1,3,4-thiadiazol-2-yl)pyrazin-2-amine (13)



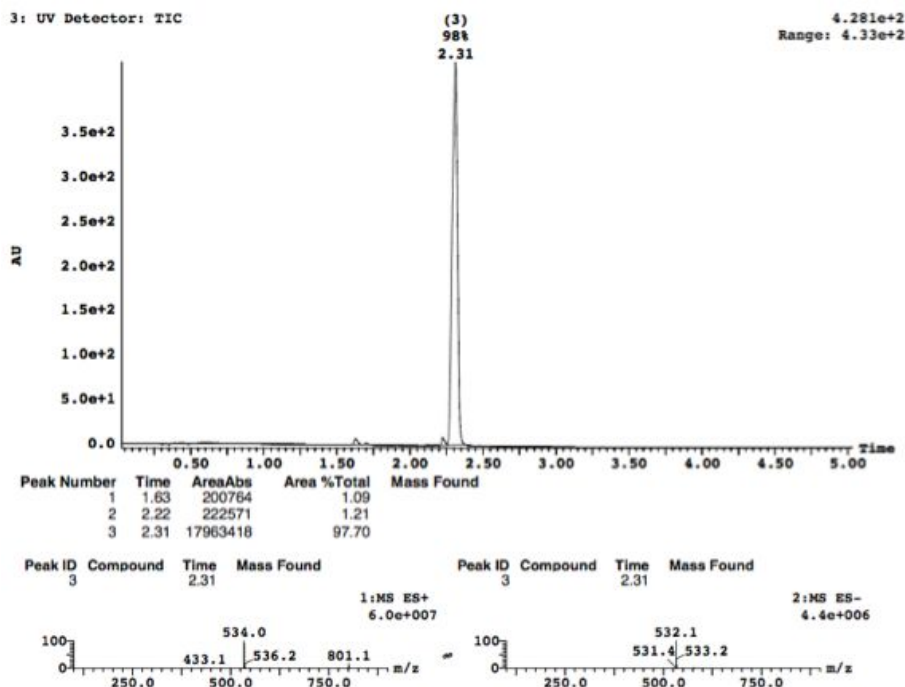
3-(3-(4-(Aminomethyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine
(14)



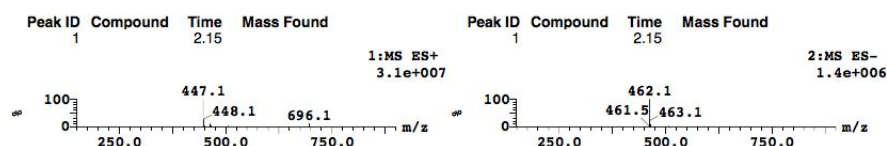
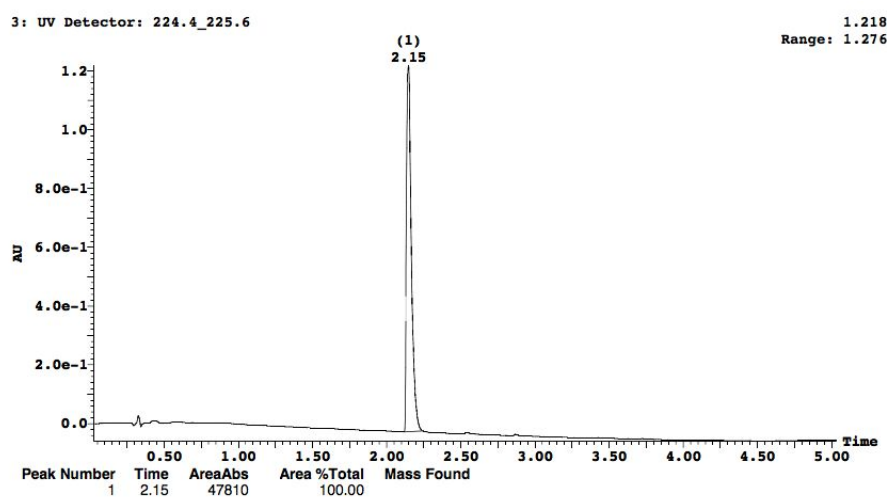
3-(3-(4-((Ethylamino)methyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine
(15)



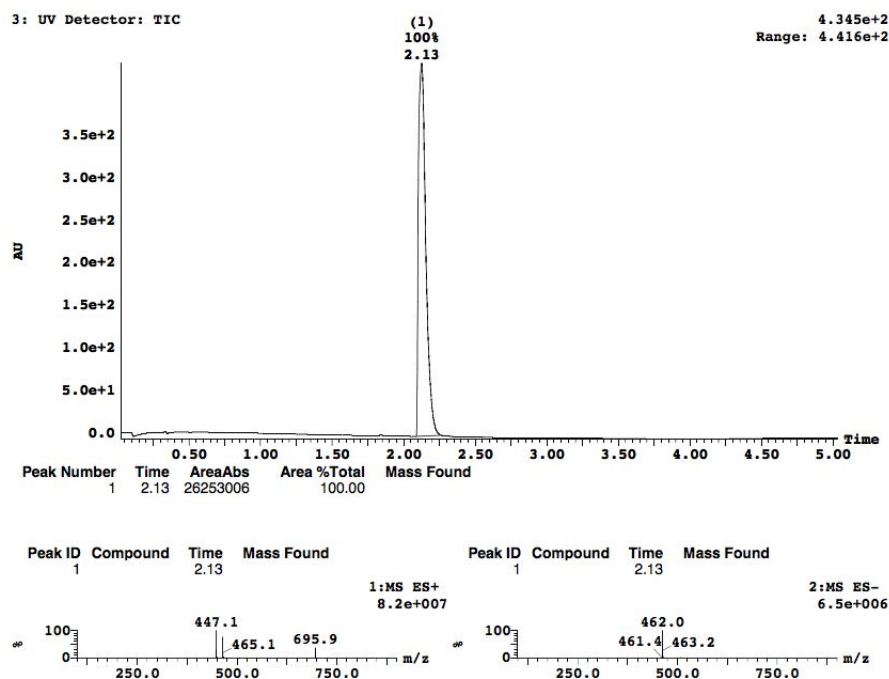
5-(4-(Isopropylsulfonyl)phenyl)-3-(3-(4-(((tetrahydro-2H-pyran-4-yl)amino)methyl)phenyl)isoxazol-5-yl)pyrazin-2-amine (16)



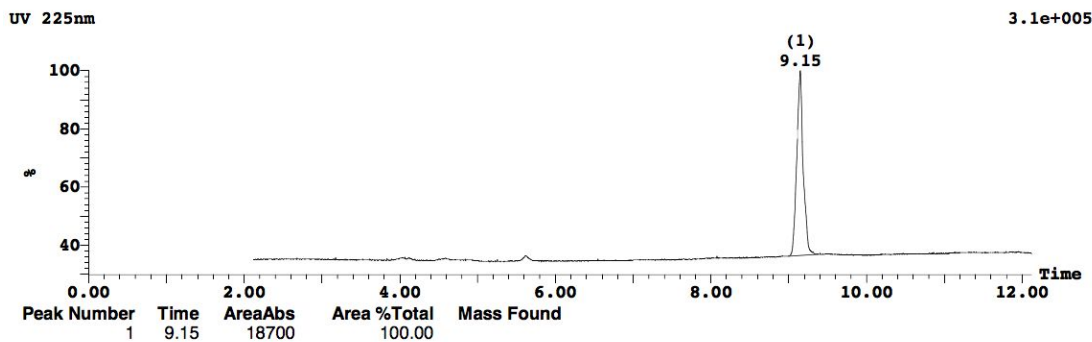
3-[3-[4-[(1S)-1-Aminoethyl]phenyl]isoxazol-5-yl]-5-(4-isopropylsulfonylphenyl)pyrazin-2-amine (17)



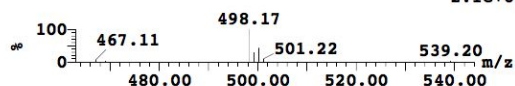
3-[3-[4-[(1R)-1-Aminoethyl]phenyl]isoxazol-5-yl]-5-(4-isopropylsulfonylphenyl)pyrazin-2-amine (18)



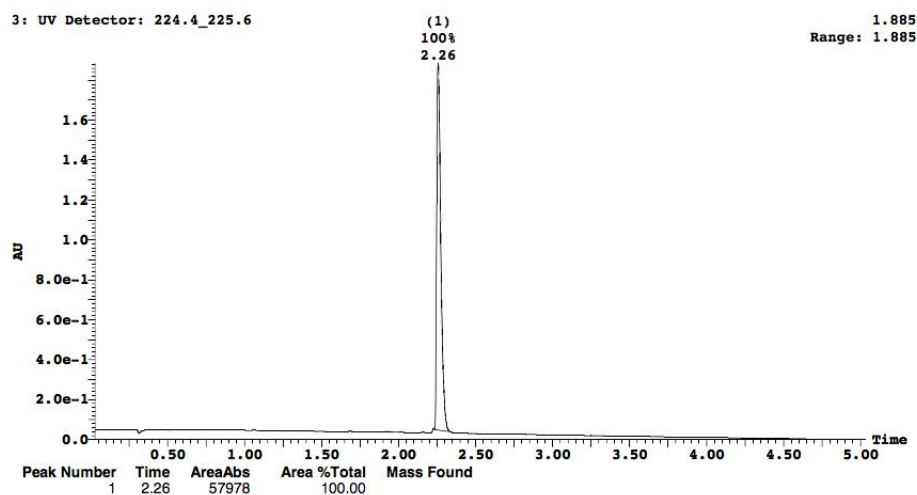
3-(3-(3-Chloro-4-((methylamino)methyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (19)



Peak ID Compound Time Mass Found
 1 9.15
 Combine (904:911-(882:885+939:943)) 1:MS ES+
 2.1e+007

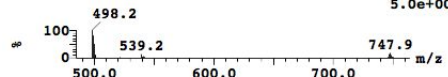


3-(3-(2-Chloro-4-((methylamino)methyl)phenyl)isoxazol-5-yl)-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (20)



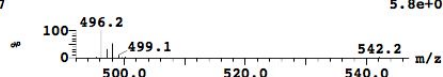
Peak ID Compound Time Mass Found
 1 2.26

1:MS ES+
 5.0e+007



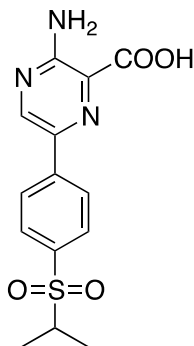
Peak ID Compound Time Mass Found
 1 2.26

2:MS ES-
 5.8e+005



Preparation of key intermediates

3-Amino-6-(4-(isopropylsulfonyl)phenyl)pyrazine-2-carboxylic acid (22).

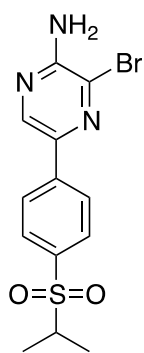


To a suspension of methyl 3-amino-6-bromo-pyrazine-2-carboxylate **21** (18.8 g, 81 mmol) in acetonitrile/water (4/1, 220 mL) at room temperature was added (4-isopropylsulfonylphenyl)boronic acid (20 g, 85 mmol), followed by K_3PO_4 (34.4 g, 162 mmol) and $Pd[P(tBu)_3]_2$ (414 mg, 0.81 mmol). The reaction mixture was heated at 60 °C for 2 h. The reaction mixture was left to cool by standing and then diluted with EtOAc (500 mL) and water (500 mL). The aqueous layer was separated and further extracted with EtOAc (2 x 200 mL). The organic extracts were combined and washed with brine (2 x 200 mL), dried over $MgSO_4$, filtered, and solvents removed *in vacuo* to leave a crude product as yellow solid, which was then triturated with ether and isolated by filtration to give methyl 3-amino-6-(4-(isopropylsulfonyl)phenyl)pyrazine-2-carboxylate as a white solid (26.5 g, 97% Yield). 1H NMR (400 MHz, $DMSO-d_6$) δ 9.04 (s, 1H), 8.26 (d, *J* 8.4 Hz, 2H), 7.95 (d, *J* 8.4 Hz, 2H), 7.67 (br s, 2H), 3.45 (m, *J* 6.8 Hz, 1H), 3.34 (s, 3H), 1.18 (d, *J* 6.8 Hz, 6H); MS (ES+) *m/z* 336.2 (*M* + *H*)⁺.

To a suspension of methyl 3-amino-6-(4-isopropylsulfonylphenyl)pyrazine-2-carboxylate (1.5 g, 4.47 mmol) in THF (6 mL) was added LiOH (3 mL of 3 M, 9.0 mmol). The mixture was stirred for 2 h at rt., and then concentrated *in vacuo*. The resulting oil was diluted with water (100 mL) and acidified to pH3 with 1M HCl. The aqueous layer was extracted

with CH₂Cl₂ (2 x 50 mL) and the combined organic extracts were dried over MgSO₄ and concentrated *in vacuo*, affording compound **18** as a yellow solid (1.44 g, 96% Yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.25 (br s, 1H), 9.01 (s, 1H), 8.35 (d, *J* 8.5 Hz, 2H), 7.92 (d, *J* 8.5 Hz, 2H), 7.72 (s, 2H), 3.45 (m, *J* 6.8 Hz, 1H), 1.18 (d, *J* 6.8 Hz, 6H); MS (ES+) *m/z* 322.1 (M + H)⁺.

3-Bromo-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (24).

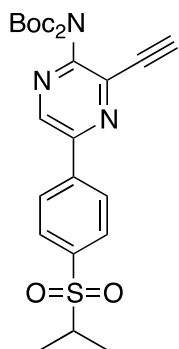


5-Bromopyrazin-2-amine **23** (5 g, 28.74 mmol), (4-isopropylsulfonylphenyl)boronic acid (7.87 g, 34.49 mmol) and K₃PO₄ (12.2 g, 57.48 mmol) were combined in acetonitrile (100 mL)/water (25 mL) and Pd[P(*t*Bu)₃]₂ (734 mg, 1.44 mmol) was added. The reaction mixture was heated at 60 °C for 1 hour, then cooled to ambient temperature and diluted with EtOAc (200 mL) and water (200 mL). The aqueous layer was further extracted with EtOAc (200 mL x 3). The combined organic extracts were dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was triturated with CH₂Cl₂ and isolated by filtration to give 5-(4-(isopropylsulfonyl) phenyl)pyrazin-2-amine as an orange solid (7.36 g, 87% Yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.67 (s, 1H), 8.20 (d, *J* 8.5 Hz, 2H), 8.00 (s, 1H), 7.87 (d, *J* 8.5 Hz, 2H), 6.86 (s, 2H), 3.43 (m, *J* 6.8 Hz, 1H), 1.17 (d, *J* 6.8 Hz, 6H); MS (ES+) *m/z* 278.2 (M + H)⁺.

N-Bromosuccinimide (3.88 g, 21.79 mmol) was added to a stirred solution of 5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (6.43 g, 21.79 mmol) in *N,N*-dimethyl

formamide (40 mL) at ambient temperature under nitrogen. The reaction was stirred at this temperature for 3 hours then slowly added to water (200 mL). The resultant precipitate was isolated by filtration and dried under vacuum to give 3-bromo-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine **20** as a brown solid (6.88 g, 84% yield). ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 8.76 (s, 1H), 8.17 (dd, J 7.0 and 1.6 Hz, 2H), 7.89 (dd, J 7.0 and 1.6 Hz, 2H), 7.17 (s, 2H), 3.44 (m, J 6.8 Hz, 1H), 1.17 (d, J 6.8 Hz, 6H); MS (ES+) m/z 358.1 ($\text{M} + \text{H}$) $^+$.

***N,N*-Bis-*tert*-butoxycarbonyl-3-ethynyl-5-(4-(isopropylsulfonyl)phenyl)pyrazin-2-amine (25).**



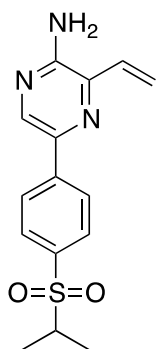
(Trimethylsilyl)acetylene (113 mg, 163 μL , 1.15 mmol) was added at 0 $^\circ\text{C}$, dropwise, to a solution of 3-bromo-5-(4-isopropylsulfonylphenyl)pyrazin-2-amine **24** (316 mg, 0.89 mmol), triethylamine (449 mg, 618 μL , 4.43 mmol), copper(I)iodide (20.3 mg, 0.11 mmol) and $\text{Pd}(\text{PPh}_3)_4$ (51.3 mg, 0.04 mmol) in *N,N*-dimethyl formamide (1.5 mL). The temperature was allowed to warm to rt and stirred for 1 hour. The solution was added dropwise to a mixture of EtOAc (20 mL)/water (20mL), and the organic layer was separated and washed with brine (20 mL \times 3), dried over MgSO_4 , filtered and then concentrated *in vacuo*. The residue was purified by chromatography on silica, using a EtOAc/ CH_2Cl_2 gradient as eluent, to provide 5-(4-isopropylsulfonylphenyl)-3-(2-trimethylsilylethynyl) pyrazin-2-amine (268 mg, 81% Yield) as an off-white solid. ^1H

NMR (400 MHz, DMSO- d_6) δ 8.60 (s, 1H), 8.05 (d, J 8.5 Hz, 2H), 7.74 (d, J 8.5 Hz, 2H), 6.81 (s, 2H), 3.29 (m, J 6.8 Hz, 1H), 1.03 (d, J 6.8 Hz, 6H), 0.15 (s, 9H); MS (ES+) m/z 374.2 (M + H)⁺.

To a solution of 5-(4-isopropylsulfonylphenyl)-3-(2-trimethylsilylethynyl)pyrazin-2-amine (260 mg, 0.70 mmol) in dichloromethane (8 mL) was added *tert*-butoxycarbonyl *tert*-butyl carbonate (456 mg, 480 μ L, 2.09 mmol) at ambient temperature, followed by triethylamine (141 mg, 194 μ L, 1.39 mmol) and *N,N*-dimethylaminopyridine (8.5 mg, 0.07 mmol). The brown solution was stirred at ambient temperature over 18 hours, then washed with brine (30 mL x3), dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by chromatography on silica, using a EtOAc/CH₂Cl₂ gradient as eluent, to give *tert*-butyl *N-tert*-butoxycarbonyl-*N*-[5-(4-isopropylsulfonylphenyl)-3-(2-trimethylsilylethynyl)pyrazin-2-yl]carbamate as a colourless oil (400mg, 100% Yield). ¹H NMR (400 MHz, DMSO- d_6) δ 9.33 (s, 1H), 8.45 (d, J 8.7 Hz, 2H), 8.04 (d, J 8.7 Hz, 2H), 4.03 (m, J 6.8 Hz, 1H), 1.36 (2s, 18H), 1.20 (d, J 6.8 Hz, 6H), 0.26 (s, 9H); MS (ES+) m/z 574.3 (M + H)⁺.

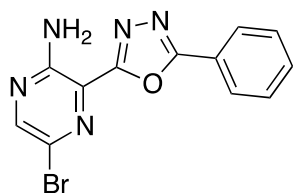
tert-Butyl *N-tert*-butoxycarbonyl-*N*-[5-(4-isopropylsulfonylphenyl)-3-(2-trimethylsilylethynyl)pyrazin-2-yl]carbamate (345 mg, 0.60 mmol) in methanol (7 mL) was stirred at ambient temperature and treated with sodium carbonate (3.45 mL of 2 M, 6.90 mmol). The suspension was stirred at ambient temperature for 1 hour, then diluted with EtOAc (30 mL)/water (30 mL). The phases were separated and the organic layer was washed with brine (30 mL x3), dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was triturated with petroleum ether to give *tert*-butyl *N-tert*-butoxycarbonyl-*N*-[3-ethynyl-5-(4-isopropylsulfonylphenyl)pyrazin-2-yl]carbamate **21** as a colourless solid (293 mg, 97% Yield). ¹H NMR (400 MHz, DMSO- d_6) δ 9.35 (s, 1H), 8.43 (d, J 8.2 Hz, 2H), 8.03 (d, J 8.2 Hz, 2H), 5.00 (s, 1H), 3.50 (m, J 6.7 Hz, 1H), 1.37 (s, 18H), 1.18 (d, J 6.7 Hz, 6H); MS (ES+) m/z 502.2 (M + H)⁺.

5-(4-(Isopropylsulfonyl)phenyl)-3-vinylpyrazin-2-amine (26).



To a solution of 3-bromo-5-(4-isopropylsulfonylphenyl)pyrazin-2-amine **24** (456 mg, 1.28 mmol), triethylamine (324 mg, 446 μ L, 3.20 mmol) and potassium trifluoro(vinyl)boranuide (171 mg, 1.28 mmol) in propanol (2 mL) was added Pd(dppf)Cl₂ (156 mg, 0.21 mmol). The mixture was heated in a microwave for 40 minutes at 100 °C. The reaction mixture was concentrated *in vacuo* and the residue was partitioned between CH₂Cl₂ (10 mL) and water (10 mL). The aqueous layer was further extracted with CH₂Cl₂ (10 mL) and the combined organic extracts was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified by chromatography on silica, using a CH₂Cl₂/MeOH gradient as eluent, yielding 5-(4-(isopropylsulfonyl)phenyl)-3-vinylpyrazin-2-amine **22** as an orange solid (220 mg, 57% Yield). MS (ES+) *m/z* 304.3 (M + H)⁺.

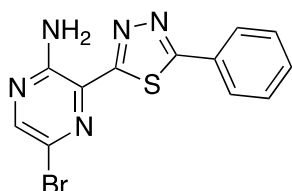
5-Bromo-3-(5-phenyl-1,3,4-oxadiazol-2-yl)pyrazin-2-amine (28).



To a suspension of 3-amino-6-bromo-pyrazine-2-carboxylic acid **27** (1.07 g, 4.57 mmol) and benzohydrazide (621 mg, 4.57 mmol) in acetonitrile (15 mL) was added PPh₃Br₂ (9.25 g, 21.91 mmol) at room temperature. The reaction mixture was stirred at rt for an hour

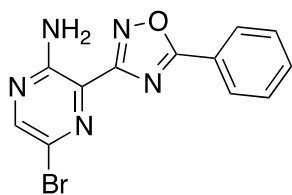
before being cooled in an ice bath. DIPEA (2.95 g, 3.97 mL, 22.83 mmol) was added slowly and the mixture was stirred for an hour at 0 °C. The resulting precipitate was filtered, washed with MeCN (10 mL) and dried to give 5-bromo-3-(5-phenyl-1,3,4-oxadiazol-2-yl)pyrazin-2-amine **28** as a yellow solid (1.22 g, 80% Yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.50 (s, 1H), 8.16-8.18 (m, 2H), 7.86 (br s, 2H), 7.76-7.69 (m, 3H); MS (ES+) *m/z* 319.9 (M + H)⁺.

5-Bromo-3-(5-phenyl-1,3,4-thiadiazol-2-yl)pyrazin-2-amine (29).



A mixture of 3-amino-6-bromo-pyrazine-2-carboxylic acid **27** (1.07 g, 4.56 mmol) and benzothiohydrazide (755 mg, 4.56 mmol) were suspended in acetonitrile (25 mL) and cooled in an ice bath. Dibromo(triphenyl)phosphorane (4.82 g, 11.41 mmol) was added and the reaction mixture was stirred for 3 hours at room temperature. The resulting precipitate was isolated by filtration, washed with acetonitrile/diethyl ether (1/1, 10 mL), then water/acetonitrile (1/1, 10 mL), and dried to afford 5-bromo-3-(5-phenyl-1,3,4-thiadiazol-2-yl)pyrazin-2-amine **29** as a brown solid. (1.67 g, 39% Yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.37 (s, 1H), 8.03-8.11 (m, 2H), 7.95 (br s, 2H), 7.56-7.62 (m, 3H) MS (ES+) *m/z* 335.9 (M + H)⁺.

5-Bromo-3-(5-phenyl-1,2,4-oxadiazol-3-yl)pyrazin-2-amine (31).



3-Amino-6-bromo-pyrazine-2-carbonitrile **30** (1.5 g, 7.54 mmol) was dissolved in methanol (50 mL) and cooled to 0 °C. Hydroxylamine hydrochloride (524 mg, 7.54 mmol) and triethylamine (763 mg, 1 mL, 7.54 mmol) were added and the reaction allowed to warm to ambient temperature. After stirring for 3 hours, the resulting precipitate was isolated by filtration and washed with methanol (5 mL) to give 3-amino-6-bromo-N'-hydroxy-pyrazine-2-carboxamidine as a beige solid (1.33 g, 76% Yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.38 (s, 1H), 8.15 (s, 1H), 7.64 (br s, 2H), 5.89 (s, 2H); MS (ES+) *m/z* 234.0 (M + H)⁺.

3-Amino-6-bromo-N'-hydroxy-pyrazine-2-carboxamidine (1.33 g, 5.73 mmol) was suspended in CH₂Cl₂ (20 mL) and triethylamine (638 mg, 879 μL, 6.31 mmol) was added followed by benzoyl chloride (886 mg, 732 μL, 6.31 mmol). The reaction was stirred at ambient temperature for 16 hours then concentrated *in vacuo*. The residue was triturated from methanol and the precipitate isolated by filtration to give 3-amino-N'-(benzoyloxy)-6-bromopyrazine-2-carboximidamide as an off-white solid (1.79 g, 93% Yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.33 (s, 1H), 8.29 (m, 2H), 7.90 (br s, 2H), 7.71 (m, 1H), 7.57 (t, J 7.8 Hz, 2H), 7.15 (br s, 2H); MS (ES+) *m/z* 338.0 (M + H)⁺.

3-Amino-N'-(benzoyloxy)-6-bromopyrazine-2-carboximidamide (1.66 g, 4.94 mmol) was added to stirred polyphosphonic acid (20 mL) at 70 °C and the reaction mixture was stirred for 7 hours, then cooled to ambient temperature and diluted with water (50 mL). The mixture was neutralized by the portionwise addition of solid sodium bicarbonate. The resultant precipitate was isolated by filtration and dried under vacuum at 40 °C to give 5-bromo-3-(5-phenyl-1,2,4-oxadiazol-3-yl)pyrazin-2-amine **31** as a fawn solid (1.38 g, 88% Yield). ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.43 (s, 1H), 8.28 - 8.26 (m, 2H), 7.80 - 7.75 (m, 1H), 7.71 - 7.67 (m, 2H), 7.49 (s, 2H); MS (ES+) *m/z* 320.0 (M + H)⁺.

Enzyme assays

Ability of compounds to inhibit ATR, ATM or DNA-PK kinase activity was tested using a radiometric-phosphate incorporation assay, as described in Table 4 and below.

A stock solution was prepared consisting of the appropriate buffer, kinase and target peptide. To this was added the compound of interest, at varying concentrations in DMSO to a final DMSO concentration of 7 %. Assays were initiated by addition of an appropriate [γ - ^{33}P] ATP solution and incubated at 25 °C. Assays were stopped, after the desired time-course, by addition of phosphoric acid and ATP to a final concentration of 100 mM and 0.66 μM , respectively. Peptides were captured on a phosphocellulose membrane, prepared as per manufacturer's instructions, and washed six times with 200 μL 100 mM phosphoric acid, prior to addition of 100 μL scintillation cocktail and scintillation counting on a 1450 Microbeta Liquid Scintillation Counter (Perkin Elmer). Dose-response data was analyzed using GraphPad Prism software (Version 3.0cx for Macintosh, GraphPad Software).

Table 4: Parameters for kinase activity assays

assay	[kinase] (nM) ^d	target peptide ^e	[peptide] (mM) ^d	[ATP] (mM) ^d	μCi [γ - ^{33}P] ATP per nmol ATP	time- course (hours) ^f
ATR ^a	5	ASELPASQPQPFSAKKK	0.8	0.01	0.8	24
ATM ^b	50	DPSVEPPLSQETFSDKKK	0.02	0.015	2.1	24
DNAPK ^c	4	EPPLSQEAFADLWKKK	0.8	0.11	0.3	2

Assay buffers: ^a 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 1 mM DTT; ^b 10 mM HEPES (pH 7.5), 5 mM MgCl₂, 5 mM MnCl₂, 100 mM NaCl, 0.1 % BSA, 1 mM DTT; ^c 50 mM HEPES (pH 7.5), 10 mM MgCl₂, 100 mM KCl, 0.2 mM

EGTA, 0.1 mM EDTA, 0.01 % BSA, 5 µg/mL calf thymus DNA, 1 mM DTT. ^d Final assay concentration. ^e Primary amino acid sequence. ^f The duration of the enzyme assay was guided by the activity of the enzyme; for both ATR and ATM low enzyme activity necessitated an extended assay to ensure sufficient activity for detection. In all cases (data not reported) previous studies had been run to ensure linearity of the reaction progress curves throughout the duration of the enzyme assay.

Cellular assays

Cell lines were purchased from ATCC and maintained according to the distributor's instructions. Cell assays were performed using exponentially growing cultures. For H2AX phosphorylation analysis using immunofluorescence (IF) microscopy, cells were fixed in 4% formaldehyde, permeabilized with 0.5% Triton X-100 and stained with mouse H2AX pS139 antibody (Upstate), AlexaFluor 488 goat anti-mouse antibody (Invitrogen) and Hoechst (Invitrogen). The cells were then analyzed using the BD Pathway 855 bioimager and BD Attovision software. Cell density was analyzed using the CellTiter 96 AQueous Cell Proliferation (MTS) assay (Promega). Cells were plated in 96 well plates and allowed to adhere overnight. The following day, compounds were added at the indicated concentrations in a final volume of 200 µl and the cells were then incubated for 96 h. MTS reagent (40 µl) was then added and 1 h later absorbance at 490 nm was measured using a SpectraMax Plus 384 plate reader (Molecular Devices). Synergy and antagonism were assessed using Macsynergy software.