SUPPLEMENTAL INFORMATION

Quinazoline-based anti-virulence compounds selectively target *Salmonella* PhoP/PhoQ signal transduction system

María Ayelén Carabajal[a,1], Christopher R. M. Asquith[b,c1],Tuomo Laitinen[d], Graham J. Tizzard[e], Lucía Yim[f], Analía Rial[f], José A. Chabalgoity[f], William J. Zuercher[c,g], Eleonora García Véscovi[a,#]

[a] Instituto de Biología Molecular y Celular de Rosario, Consejo Nacional de Investigaciones Científicas y Tecnológicas, Universidad Nacional de Rosario, Rosario, Santa Fe, Argentina

[b] Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill NC 27599, USA

[c] Structural Genomics Consortium, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

[d] School of Pharmacy, Faculty of Health Sciences, University of Eastern Finland, 70211, Kuopio, Finland

[e] UK National Crystallography Service, School of Chemistry, University of Southampton, Highfield Campus, Southampton, SO17 1BJ, UK

[f] Departamento de Desarrollo Biotecnológico, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay.

[g] Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

[1] M.A.C. and C.R.M.A. contributed equally to this work

#Corresponding author

SUPPLEMENTAL FIGURES and LEGENDS

FIGURE S1

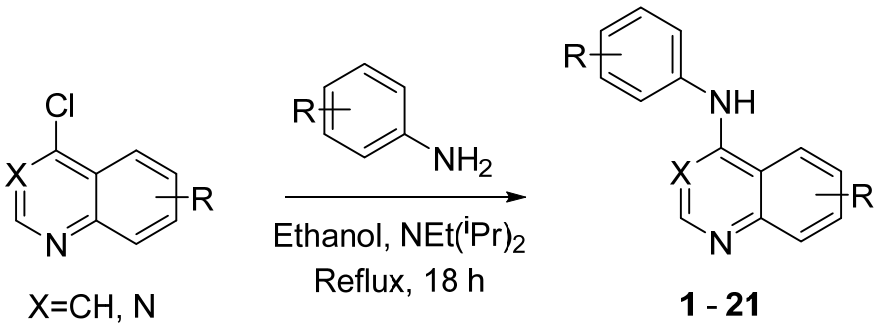


FIGURE S1. **Scheme of the general synthetic procedure.** The details of the procedure for the synthesis of 4-anilinoquin(az)olines are explained in Materials and Methods, Supplementary Methods and Supplementary Information**.**

FIGURE S2

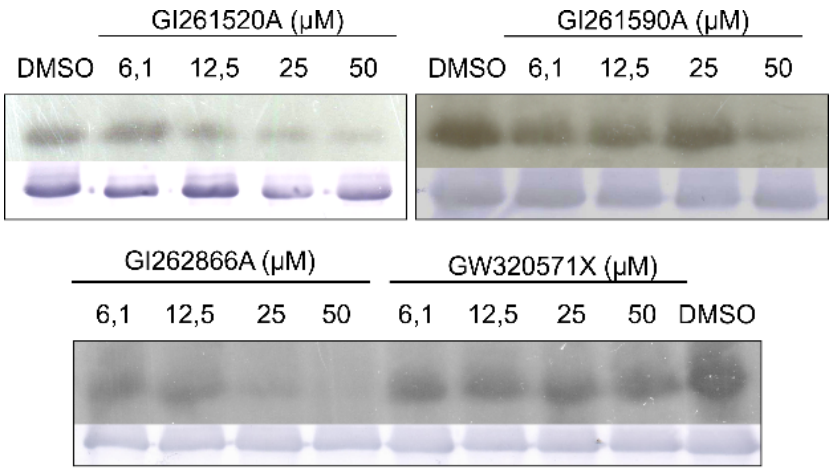
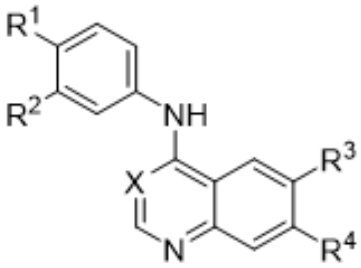


FIGURE S2. Autophosphorylation inhibitory activity of PhoQ by candidate molecules. Increasing concentrations of GI261520A, GI262866A, GI261590A or GW320571X, were added to the *Salmonella* growth medium. Membranes obtained by cell fractionation, harboring PhoQ, were incubated for 10 min at 37 °C in a reaction medium containing [γ-^{32}P] ATP, as described in Materials and Methods. The autophosphorylation reactions were analyzed by SDS-PAGE (12% polyacrylamide) and transferred to nitrocellulose, followed by autoradiography (top) or by immunodetection analysis developed with anti-PhoQ$_{Cyt}$ polyclonal antibody (bottom).

FIGURE S3



Name	X	R^1	R^2	R^3	R^4	*virK*	*pagC*	*tppB*
						% repression		
Erlotinib	N	H	C≡C	6,7 $(OCH_2CH_2OMe)_2$		1.4±8.4	12.2±10.0	20.7±7.7
11	N	H	C≡C	OH	H	15.5±4.3	22.0±1.6	0.0±11.5
12	N	H	C≡C	H	OH	21.2±15.7	20.0±13.7	0.0±6.4
13	N	H	C≡C	OH	OH	5.2±13.0	11.5±6.7	0.0±14.5
14	CH	H	C≡C	OH	H	13.9±10.1	1.1±5.3	0.0±7.8
15	CH	H	C≡C	H	OH	0.0±7.8	0.0±9.4	2.8±2.3
16	N	H	C≡C	OMe	H	7.7±2.4	2.1±5.8	15.4±9.2
17	N	H	C≡C	H	OMe	0.0±3.0	0.0±14.4	4.2±0.6
18	N	H	C≡C	OMe	OMe	0.0±9.7	0.0±7.5	15.3±11.7
19	CH	H	C≡C	OMe	OMe	0.0±16.5	15.2±2.4	0.0±11.6
20	N	OMe	H	OH	H	33.1±5.6	45.3±9.8	0.0±14.7
21	N	OMe	H	OMe	H	14.2±9.0	8.6±9.5	0.0±13.5

FIGURE S3. Probing the limits of the pharmacophore. The structure shows X, R^1, R^2, R^3 and R^4 positions that were substituted by the functional groups indicated below. Inhibition action was calculated with the β-galactosidase activity from *lacZ* transcriptional fusions to two different PhoP-activated genes (*virK* and *pagC)* and one PhoP-unrelated control reporter (*tppB*). Cells were grown overnight either in LB plus DMSO 0.25% v/v (compounds' vehicle) or 25 μM of the indicated compound. Repression percentages were calculated taking the values obtained with the sole addition of DMSO 0.25% v/v as 100%. Results are the average of three independent assays performed in duplicate.

FIGURE S4

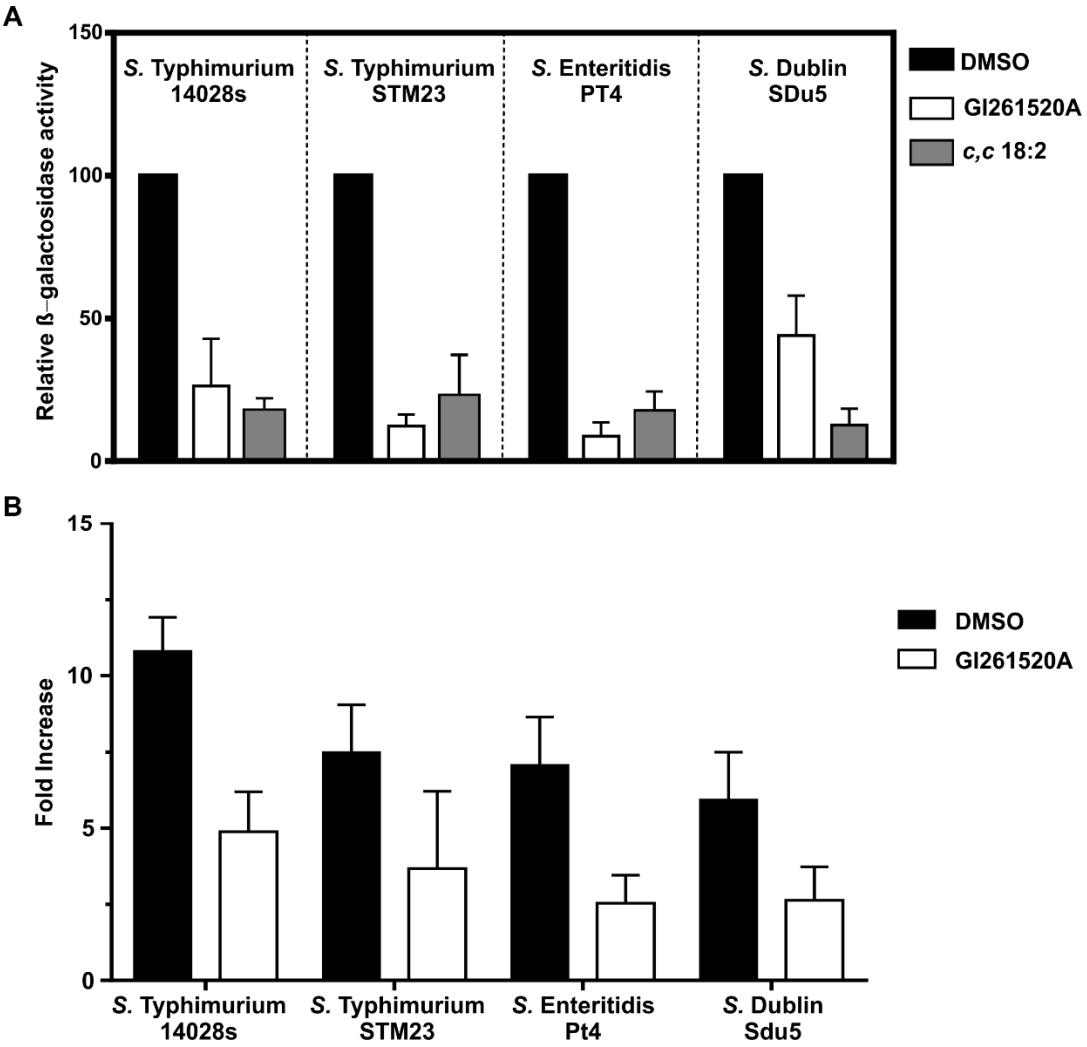


FIGURE S4. **Inhibitory action on different *Salmonella enterica* serovars**. (A) β-galactosidase activity from *pagC*::*lacZ* transcriptional fusion measured in cells grown in LB supplemented with DMSO 0.25% v/v, 25 μM of GI261520A or 1.8 mM *cis*,*cis*-9,12-octadecadienoic acid (*c*,*c*-18:2), dissolved in DMSO. β-galactosidase activity was measured as described under Materials and Methods. Results are the average of three independent assays performed in duplicate, and error bars correspond to S.D. (B) Recovery of intracellular *Salmonella* at 18 h relative to 1.5 h post infection (fold increase values) from RAW264.7 macrophages incubated with DMSO 0.25% v/v or 25 μM of GI261520A dissolved in DMSO. Results are the average of three independent assays performed in duplicate, and error bars correspond to S.D.

SUPPLEMENTARY MATERIALS AND METHODS

Chemicals and Reagents. Screening stocks (10 mM) of the first and second generations of the Published Kinase Inhibitor Set (PKIS) from GlaxoSmithKline (GSK) (1) were stored at −20ºC in

DMSO. Nitrocellulose membranes were from Amersham Biosciences. [γ-^{32}P]ATP (3000 μCi mmol^{-1}) was obtained from PerkinElmer, NEN®. Compounds were routinely dissolved in DMSO at a concentration of 25 mM or 10 mM and stored at -20 °C. Synthetic analogs of GI262866A and GI261520A were synthesized following the protocol described in Supplementary Methods. All synthetic compounds presented in this work were characterized by ^{1}H and ^{13}C NMR and were shown to be >98% pure.

Genetic and Molecular Biology Techniques. Plasmids and oligonucleotides used in this work are listed in supplemental Table S1 and all constructs were verified by DNA sequencing. DNA was introduced into bacterial strains by electroporation using a Bio-Rad *E. coli* Pulser Electroporator following the manufacturer's recommendations. *Salmonella* strains carrying *lacZ*-reporter fusion to promoters on the chromosome were carried out using Lambda Red-mediated recombination in strain *Salmonella typhimurium* 14028s (2). Plasmid pPB1190 which harbors the DNA region encoding the cytoplasmic domain of EnvZ under the control of the T5 promoter, was constructed by polymerase chain reaction (PCR), using primers #324 and #292. The product was cloned between the BamHI and HindIII sites of plasmid pQE31. PCR-derived constructions were all confirmed by PCR assays. All constructs were verified by DNA sequencing.

General procedure for the synthesis of 4-anilinoquin(az)olines:

4-{[4-(benzyloxy)phenyl]amino}quinazolin-6-ol (**GI262866A**) as a yellow solid (68 %, 220 mg, 0.640 mmol) MP 231-233 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.21 (s, 1H), 10.91 (s, 1H), 8.75 (s, 1H), 8.04 (d, J = 2.5 Hz, 1H), 7.88 (d, J = 9.0 Hz, 1H), 7.70 (dd, J = 9.1, 2.5 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.52 – 7.44 (m, 2H), 7.44 – 7.37 (m, 2H), 7.37 – 7.30 (m, 1H), 7.14 – 7.09 (m, 2H), 5.16 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.8, 157.8, 156.7, 148.1, 136.9, 131.6, 129.7, 128.5 (2C, s), 127.9, 127.7 (2C, s), 126.5, 126.2 (2C, s), 121.2, 114.92, 114.85 (2C, s), 107.1, 69.4. HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{18}N_3O_2$: 344.1399 found = 344.1386; LC t_R = 4.24 min, >98% Purity.

4-{[4-(benzyloxy)phenyl]amino}quinazolin-7-ol (**1**) as a light yellow solid (78 %, 223 mg, 0.648 mmol) MP 277-279 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.76 (s, 1H), 11.42 (s, 1H), 8.76 (d, J = 9.0 Hz, 1H), 8.74 (s, 1H), 7.59 – 7.54 (m, 2H), 7.49 – 7.44 (m, 2H), 7.42 – 7.38 (m, 2H), 7.36 – 7.28 (m, 3H), 7.12 – 7.07 (m, 2H), 5.15 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.3, 158.9, 156.6, 150.5, 140.4, 136.9, 129.7, 128.5 (2C, s), 127.9, 127.7 (2C, s), 127.1, 126.3 (2C, s), 119.4, 114.8 (2C, s), 105.8, 102.0, 69.4. HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{18}N_3O_2$: 344.1399 found = 344.1386; LC t_R = 4.33 min, >98% Purity.

4-{[4-(benzyloxy)phenyl]amino}quinazoline-6,7-diol (**2**) as a light yellow solid (69 %, 189 mg, 0.527 mmol) MP 272-274 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.32 (s, 2H), 8.53 (s, 1H), 7.91 (s, 1H), 7.64 – 7.53 (m, 2H), 7.50 – 7.44 (m, 2H), 7.43 – 7.37 (m, 2H), 7.36 – 7.30 (m, 2H), 7.20 (s,

1H), 7.10 – 7.01 (m, 2H), 5.13 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.4, 155.7, 154.3, 149.1, 147.7, 137.1, 131.2, 128.5 (2C, s), 127.8, 127.7 (2C, s), 125.4 (2C, s), 114.7 (2C, s), 107.3, 106.7, 104.9, 69.4. HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{18}N_3O_3$: 360.1348 found = 360.1335; LC t_R = 4.22 min, >98% Purity.

4-{[4-(benzyloxy)phenyl]amino}quinolin-6-ol (**3**) as a light yellow solid (64 %, 182 mg, 0.532 mmol) MP 218-220 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 10.37 (s, 1H), 8.32 (d, J = 6.8 Hz, 1H), 7.96 (d, J = 9.1 Hz, 1H), 7.91 (d, J = 2.5 Hz, 1H), 7.63 (dd, J = 9.1, 2.4 Hz, 1H), 7.58 – 7.44 (m, 2H), 7.46 – 7.22 (m, 5H), 7.23 – 7.13 (m, 2H), 6.57 (d, J = 6.8 Hz, 1H), 5.17 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.3, 156.5, 154.1, 140.1, 136.8, 132.2, 130.2, 128.5 (2C, s), 128.0, 127.8 (2C, s), 127.2 (2C, s), 124.9, 121.9, 118.7, 116.0 (2C, s), 105.4, 98.6, 69.5. HRMS m/z $[M+H]^+$ calcd for $C_{22}H_{19}N_2O_2$: 343.1447 found = 343.1433; LC t_R = 4.45 min, >98% Purity.

4-({4-[(4-fluorophenyl)methoxy]phenyl}amino)quinazolin-6-ol (**4**) as a yellow solid (76 %, 228 mg, 0.631 mmol) Decomposed >200 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 10.91 (s, 1H), 8.75 (s, 1H), 8.05 (d, J = 2.5 Hz, 1H), 7.88 (d, J = 9.0 Hz, 1H), 7.70 (dd, J = 9.0, 2.4 Hz, 1H), 7.67 – 7.54 (m, 2H), 7.57 – 7.39 (m, 2H), 7.40 – 7.17 (m, 2H), 7.17 – 7.01 (m, 2H), 5.14 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 163.0, 159.7 (d, J = 175.5 Hz), 157.8, 156.6, 148.1, 133.2 (d, J = 3.0 Hz), 131.6, 130.0 (d, J = 8.3 Hz, 2C), 129.8, 126.5, 126.2 (2C, s), 121.2 115.3 (d, J = 21.4 Hz, 2C), 114.93, 114.86 (2C, s), 107.1, 68.7. HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{17}N_3O_2F$: 362.1305 found = 362.1290; LC t_R = 4.31 min, >98% Purity.

4-({4-[(3-fluorophenyl)methoxy]phenyl}amino)quinazolin-6-ol (**5**) as a dark green solid (58 %, 174 mg, 0.482 mmol) 138-140 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (s, 1H), 10.94 (s, 1H), 8.74 (s, 1H), 8.06 (d, J = 2.5 Hz, 1H), 7.89 (d, J = 9.0 Hz, 1H), 7.71 (dd, J = 9.0, 2.4 Hz, 1H), 7.67 – 7.49 (m, 2H), 7.45 (td, J = 8.0, 6.0 Hz, 1H), 7.41 – 7.22 (m, 2H), 7.21 – 6.93 (m, 3H), 5.19 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 162.2 (d, J = 243.7 Hz), 158.8, 157.80, 156.4, 148.1, 139.9 (d, J = 7.4 Hz), 131.6, 130.5 (d, J = 8.4 Hz), 129.9, 126.5, 126.3 (2C, s), 123.5 (d, J = 2.8 Hz), 121.1, 114.9, 114.9 (2C, s) , 114.6 (d, J = 20.9 Hz), 114.2 (d, J = 21.9 Hz), 107.1, 68.6 (d, J = 1.9 Hz). HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{17}N_3O_2F$: 362.1305 found = 362.1296; LC t_R = 4.25 min, >98% Purity.

4-({4-[(2-fluorophenyl)methoxy]phenyl}amino)quinazolin-6-ol (**6**) as a dark green solid (69 %, 207 mg, 0.573 mmol) 235-237 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.23 (s, 1H), 10.91 (s, 1H), 8.75 (s, 1H), 8.05 (d, J = 2.5 Hz, 1H), 7.88 (d, J = 9.0 Hz, 1H), 7.70 (dd, J = 9.0, 2.4 Hz, 1H), 7.66 – 7.48 (m, 3H), 7.48 – 7.38 (m, 1H), 7.38 – 7.19 (m, 2H), 7.19 – 7.05 (m, 2H), 5.19 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) 160.4 (d, J = 246.1 Hz), 158.8, 157.8, 156.5, 148.1, 131.7, 130.7 (d, J = 4.1 Hz), 130.5 (d, J = 8.3 Hz), 129.9, 126.5, 126.3 (2C, s), 124.6 (d, J = 3.5 Hz), 123.7 (d, J = 14.5

Hz), 121.2, 115.4 (d, J = 21.0 Hz), 114.9, 114.8 (2C, s), 107.1, 63.8 (d, J = 3.7 Hz). HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{17}N_3O_2F$: 362.1305 found = 362.1291; LC t_R = 4.30 min, >98% Purity.

4-((4-((4-fluorobenzyl)oxy)phenyl)amino)quinazolin-7-ol (**7**) as a grey solid (58 %, 174 mg, 0.482 mmol) Decomposed >300 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.74 (s, 1H), 11.38 (s, 1H), 8.81 – 8.66 (m, 2H), 7.66 – 7.38 (m, 4H), 7.29 (dd, J = 6.9, 2.4 Hz, 2H), 7.27 – 7.15 (m, 2H), 7.14 – 7.05 (m, 2H), 5.13 (s, 2H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.2, 161.8 (d, J = 243.7 Hz), 158.9, 156.5, 150.5, 140.5, 133.2 (d, J = 3.0 Hz), 130.0 (d, J = 8.3 Hz, 2C), 129.7, 127.1, 126.3 (2C, s), 119.4, 115.3 (d, J = 21.4 Hz, 2C), 114.8 (2C, s), 105.8, 102.1, 68.7. HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{17}N_3O_2F$: 362.1305 found = 362.1291; LC t_R = 4.44 min, >98% Purity.

4-((4-((4-fluorobenzyl)oxy)phenyl)amino)quinazoline-6,7-diol (**8**) as a colourless solid (55 %, 158 mg, 0.420 mmol) 285-290 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.55 (s, 1H), 7.88 (s, 1H), 7.70 – 7.35 (m, 4H), 7.33 – 7.12 (m, 3H), 7.12 – 7.01 (m, 2H), 5.12 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.8 (d, J = 243.5 Hz), 157.4, 155.7, 154.4, 149.0, 147.7, 137.4, 133.3 (d, J = 8.3 Hz, 2C), 130.9, 130.0 (d, J = 8.4 Hz, 2C), 125.4, 115.3 (d, J = 21.3 Hz, 2C), 114.8 (s, 2C), 107.2, 106.8, 104.6, 68.7. HRMS m/z $[M+H]^+$ calcd for $C_{21}H_{17}N_3O_3F$: 378.1254 found = 378.1240; LC t_R = 4.29 min, >98%

***N*-(4-(benzyloxy)phenyl)-6-methoxyquinazolin-4-amine** (**GI261520A**) as a light yellow solid (84 %, 231 mg, 0.647 mmol) MP 270-272 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.79 (s, 1H), 8.41 (d, J = 2.6 Hz, 1H), 7.92 (d, J = 9.1 Hz, 1H), 7.72 (dd, J = 9.2, 2.5 Hz, 1H), 7.68 – 7.53 (m, 2H), 7.53 – 7.18 (m, 5H), 7.18 – 7.04 (m, 2H), 5.17 (s, 2H), 4.00 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.0 (2C, s), 156.8, 148.8, 136.9, 133.3, 129.6, 128.5 (2C, s), 127.9, 127.7 (2C, s), 126.8, 126.4 (2C, s), 121.4, 114.9 (2C, s), 114.6, 104.6, 69.4, 56.7. HRMS m/z $[M+H]^+$ calcd for $C_{22}H_{19}N_3O_2$: 357.1477 found = 358.1546; LC t_R = 4.53 min, >98% Purity.

***N*-[4-(benzyloxy)phenyl]-7-methoxyquinazolin-4-amine** (**9**) as a colourless solid (91 %, 251 mg, 0.701 mmol) MP 247-249 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.58 (s, 1H), 8.87 (d, J = 9.3 Hz, 1H), 8.81 (s, 1H), 7.71 – 7.57 (m, 2H), 7.58 – 7.12 (m, 7H), 7.13 – 6.99 (m, 2H), 5.15 (s, 2H), 3.97 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.7, 158.9, 156.7, 150.8, 140.7, 136.9, 129.6, 128.5 (2C, s), 127.9, 127.7 (2C, s), 127.0, 126.3 (2C, s), 118.7, 114.8 (2C, s), 107.1, 100.1, 69.4, 56.3. HRMS m/z $[M+H]^+$ calcd for $C_{22}H_{19}N_3O_2$: 357.1477 found = 358.1547; LC t_R = 4.49 min, >98% Purity.

***N*-(4-(benzyloxy)phenyl)-6,7-dimethoxyquinazolin-4-amine** (**10**) as a colourless solid (84 %, 217 mg, 0.561 mmol) MP 250-252 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.44 (s, 1H), 8.73 (s, 1H), 8.36 (s, 1H), 7.68 – 7.56 (m, 2H), 7.55 – 7.43 (m, 2H), 7.43 – 7.15 (m, 4H), 7.15 – 7.03 (m, 2H), 5.15 (s, 2H), 4.00 (s, 3H), 3.96 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 157.9, 156.5, 156.0, 150.0,

148.6, 137.0, 135.5, 129.9, 128.5 (2C, s), 127.9, 127.7 (2C, s), 126.3 (2C, s), 114.8 (2C, s), 107.1, 104.1, 99.8, 69.4, 57.0, 56.4. HRMS m/z $[M+H]^+$ calcd for $C_{23}H_{21}N_3O_3$: 388.1661 found = 388.1651; LC t_R = 4.55 min, >98% Purity.

4-((3-ethynylphenyl)amino)quinazolin-6-ol (**11**) as a beige solid (61%, 135 mg, 0.515 mmol) MP decomp. 230 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.33 (s, 1H), 10.98 (s, 1H), 8.85 (s, 1H), 8.11 (d, J = 2.5 Hz, 1H), 8.05 – 7.85 (m, 2H), 7.85 – 7.63 (m, 2H), 7.50 (t, J = 7.9 Hz, 1H), 7.42 (dt, J = 7.7, 1.4 Hz, 1H), 4.29 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.1, 157.9, 148.1, 137.2, 132.0, 129.5, 129.2, 127.6, 126.8, 125.3, 122.1, 121.3, 115.1, 107.2, 82.9, 81.4. HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{12}N_3O$: 262.0980; found = 262.0976; LC t_R = 3.07 min, >98% Purity.

4-((3-ethynylphenyl)amino)quinazolin-7-ol (**12**) as a beige/yellow solid (65%, 141 mg, 0.540 mmol) MP decomp. 200 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.81 (s, 1H), 11.50 (s, 1H), 8.84 (s, 1H), 8.83 – 8.78 (m, 1H), 7.86 (t, J = 1.9 Hz, 1H), 7.75 (ddd, J = 8.1, 2.2, 1.2 Hz, 1H), 7.48 (t, J = 7.9 Hz, 1H), 7.40 (dt, J = 7.7, 1.4 Hz, 1H), 7.37 – 7.28 (m, 2H), 4.28 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.5, 159.1, 150.7, 140.8, 137.2, 129.5, 129.2, 127.7, 127.3, 125.4, 122.0, 119.7, 106.1, 102.1, 82.9, 81.4. HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{12}N_3O$: 262.0980: found = 262.0973; LC t_R = 3.11 min, >98% Purity.

4-((3-ethynylphenyl)amino)quinazoline-6,7-diol (**13**) as a colourless solid (58%, 123 mg, 0.443 mmol) MP decomp. 200 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.58 (s, 1H), 7.96 (t, J = 1.8 Hz, 1H), 7.89 (s, 1H), 7.82 (ddd, J = 8.2, 2.2, 1.1 Hz, 1H), 7.40 (q, J = 9.1, 8.5 Hz, 2H), 7.27 (dt, J = 7.6, 1.3 Hz, 1H), 7.25 (s, 1H), 7.12 (s, 1H), 4.22 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.9, 154.1, 149.8, 147.5, 138.9, 129.0 (2C, s), 127.4, 125.8, 123.5, 121.8 (2C, s), 107.9, 106.4, 83.3, 80.8. HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{12}N_3O_2$: 278.0930; found = 278.0922; LC t_R = 3.10 min, >98% Purity.

4-((3-ethynylphenyl)amino)quinolin-6-ol (**14**) as a brown solid (59%, 128 mg, 0.493 mmol) MP 165-167 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 10.55 (s, 1H), 8.38 (d, J = 6.8 Hz, 1H), 8.03 (d, J = 9.1 Hz, 1H), 7.97 (d, J = 2.5 Hz, 1H), 7.67 (dd, J = 9.1, 2.4 Hz, 1H), 7.61 – 7.13 (m, 4H), 6.78 (d, J = 6.8 Hz, 1H), 4.33 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.7, 153.3, 140.2, 138.1, 132.3, 130.3, 130.1, 128.1, 125.9, 125.1, 123.2, 121.9, 119.2, 105.6, 99.3, 82.6, 81.9. HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{13}N_2O$: 261.1028; found = 261.1023; LC t_R = 3.10 min, >98% Purity.

4-((3-ethynylphenyl)amino)quinolin-7-ol (**15**) as a beige solid (56%, 122 mg, 0.468 mmol) MP 254-256 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (s, 1H), 10.55 (s, 1H), 8.38 (d, J = 6.8 Hz, 1H), 8.03 (d, J = 9.1 Hz, 1H), 7.97 (d, J = 2.5 Hz, 1H), 7.67 (dd, J = 9.1, 2.4 Hz, 1H), 7.62 – 7.49 (m, 3H), 7.49 – 7.35 (m, 1H), 6.78 (d, J = 6.8 Hz, 1H), 4.33 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.7, 153.3, 140.2, 138.1, 132.3, 130.3, 130.1, 128.1, 125.9, 125.1, 123.2, 121.9, 119.2,

105.6, 99.3, 82.6, 81.9. HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{13}N_2O$: 261.1028; found = 261.1020; LC t_R = 3.20 min, >98% Purity.

***N*-(3-Ethynylphenyl)-6-methoxyquinazolin-4-amine** (**16**) as a yellow solid (56%, 119 mg, 0.432 mmol) MP 176-178 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.89 (s, 1H), 8.56 (d, J = 2.7 Hz, 1H), 7.96 (d, J = 9.1 Hz, 1H), 7.92 (t, J = 1.8 Hz, 1H), 7.83 (ddd, J = 8.1, 2.2, 1.2 Hz, 1H), 7.73 (dd, J = 9.1, 2.6 Hz, 1H), 7.54 – 7.47 (m, 1H), 7.42 (dt, J = 7.7, 1.3 Hz, 1H), 4.29 (s, 1H), 4.02 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.2, 159.0, 148.8, 137.1, 133.5, 129.6, 129.1, 127.8, 127.1, 125.5, 122.0, 121.4, 114.8, 104.8, 82.9, 81.4, 56.9. HRMS m/z $[M+H]^+$ calcd for $C_{17}H_{14}N_3O$: 276.1137 found = 276.1127; LC t_R = 3.47 min, >98% Purity.

***N*-(3-Ethynylphenyl)-7-methoxyquinazolin-4-amine** (**17**) as a colourless solid (68 %, 241 mg, 0.874 mmol) MP 223-225 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.72 (s, 1H), 8.96 (d, J = 9.3 Hz, 1H), 8.91 (s, 1H), 7.89 (t, J = 1.9 Hz, 1H), 7.78 (ddd, J = 8.1, 2.2, 1.2 Hz, 1H), 7.59 – 7.36 (m, 4H), 4.29 (s, 1H), 3.98 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 164.9, 159.2, 150.9, 141.0, 137.1, 129.5, 129.2, 127.7, 127.2, 125.4, 122.0, 119.0, 107.3, 100.2, 82.9, 81.4, 56.3. HRMS *m/z* $[M+H]^+$ calcd for $C_{17}H_{14}N_3O$: 276.1137, found 276.1127, LC t_R = 3.34 min, >98% Purity.

***N*-(3-Ethynylphenyl)-6,7-dimethoxyquinazolin-4-amine** (**18**) as a colourless solid (74 %, 251 mg, 0.824 mmol) MP 237-239 ºC; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.48 (s, 1H), 8.85 (s, 1H), 8.38 (s, 1H), 7.88 (t, J = 1.8 Hz, 1H), 7.79 (ddd, J = 8.1, 2.2, 1.1 Hz, 1H), 7.49 (t, J = 7.8 Hz, 1H), 7.40 (dt, J = 7.7, 1.3 Hz, 1H), 7.37 (s, 1H), 4.28 (s, 1H), 4.02 (s, 3H), 3.99 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 158.1, 156.3, 150.2, 148.8, 137.4, 136.0, 129.2, 129.2, 127.6, 125.3, 122.0, 107.4, 104.1, 99.9, 82.9, 81.3, 57.0, 56.5. HRMS *m/z* $[M+H]^+$ calcd for $C_{18}H_{16}N_3O_2$: 306.1243, found 306.1230, LC t_R = 3.41 min, >98% Purity.

***N*-(3-Ethynylphenyl)-6,7-dimethoxyquinolin-4-amine** (**19**) as a light-beige solid (67%, 228 mg, 0.749 mmol) MP 232-234 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.94 (s, 1H), 8.35 (d, J=6.9 Hz, 1H), 8.25 (s, 1H), 7.60 (q, J= 1.4 Hz, 1H), 7.58–7.52 (m, 2H), 7.51–7.43 (m, 2H), 6.76 (d, J=6.9 Hz, 1H), 4.34 (s, 1H), 3.99 ppm (d, J=19.4 Hz, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 154.6, 152.9, 149.4, 139.8, 138.1, 135.3, 130.2, 130.0, 128.1, 125.9, 123.2, 111.9, 103.0, 99.7, 99.31, 82.6, 81.9, 56.9, 56.1. HRMS-ESI (m/z): $[M+H]^+$ calcd for $C_{19}H_{16}N_2O_2$ 305.1290, found 305.1278; LC: t_R = 3.75 min, purity > 98%.

4-((4-methoxyphenyl)amino)quinazolin-6-ol (**20**) as a yellow solid (87%, 193 mg, 0.722 mmol) MP 170-172 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (s, 1H), 10.90 (s, 1H), 8.75 (s, 1H), 8.04 (d, J = 2.5 Hz, 1H), 7.87 (d, J = 9.0 Hz, 1H), 7.69 (dd, J = 9.0, 2.5 Hz, 1H), 7.65 – 7.51 (m, 2H), 7.11 – 6.95 (m, 2H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.8, 157.8, 157.7, 148.2, 131.7,

129.5, 126.5, 126.2 (2C, s), 121.2, 114.9, 113.9 (2C, s), 107.1, 55.4. HRMS m/z $[M+H]^+$ calcd for $C_{15}H_{14}N_3O_2$: 268.1086; found = 268.1081; LC t_R = 2.70 min, >98% Purity

6-methoxy-*N*-(4-methoxyphenyl)quinazolin-4-amine (**21**) as a yellow solid (84%, 182 mg, 0.647 mmol) MP 270-272 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 8.79 (s, 1H), 8.45 (d, J = 2.6 Hz, 1H), 7.93 (d, J = 9.2 Hz, 1H), 7.71 (dd, J = 9.1, 2.6 Hz, 1H), 7.67 – 7.50 (m, 2H), 7.12 – 6.96 (m, 2H), 4.00 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 159.0, 157.7, 148.8, 133.2, 129.4, 126.8, 126.4 (2C, s), 121.3, 114.6, 113.9 (2C, s), 104.7, 56.8, 55.4. HRMS m/z $[M+H]^+$ calcd for $C_{16}H_{16}N_3O_2$: 282.1243; found = 282.1238; LC t_R = 3.22 min, >98% Purity.

Protein Purification Protocols. The PhoQc fusion protein was purified from the *E. coli* strain TB1 transformed with plasmid pPB1021 (3). Expression of PhoQc was achieved by addition of 0.3 mM IPTG to a log-phase cell culture in LB broth. After sonication and centrifugation, the soluble cellular extract containing the PhoQc protein was collected and subjected to amylose affinity chromatography analysis following the manufacturer's instructions (NEB, Inc.). The fusion protein with six histidine residues (EnvZc) was purified from *strain E. coli* M15 [pREP4] transformed with plasmid pPB1190. Expression of EnvZc was achieved by addition of 0.5 mM IPTG. Cells were pelleted, resuspended in sonication buffer (25 mM Tris-HCl (pH 8.0), 200 mM KCl) and subjected to sonication. Cell debris was removed by centrifugation, and the supernatant was passed through a Ni-NTA-agarose affinity column. The EnvZc protein was recovered by elution with 0.1 M imidazole and dialyzed exhaustively against 25 mM Tris-HCl (pH 8.0), 50 mM KCl. The protein profile of the purified proteins was determined by SDS-polyacrylamide gel electrophoresis (PAGE).

Cell viability assay. Cell viability was assayed by the standard colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction, as described (4). Raw 264.7 macrophages were seeded ($5x10^4$ cells/well) in a 96-well plate in medium (DMEM + 10% FCS) and pre-incubated at 37°C for 24 h before exposure to the selected compound at a final concentration of 25 µM or DMSO 0.5% v/v as control (compounds´ vehicle). The cells were further incubated for additional 18 h. After removing the supernatant, each well was washed twice with PBS, and 0.5 µg/mL MTT (Sigma M2128) in culture medium was added and incubated for 3 h in a CO_2 incubator. Next, the supernatant was discarded and the resultant formazan crystals were dissolved in DMSO absorbance intensity was measured at 570 nm and 630 nm. Cells treated with DMSO 0.25% v/v were used for the negative control and cells treated with Triton X-100 0.01% v/v for 10 min were used for the positive control. Percent of cytotoxicity was calculated with the following equation:

$$\%\ cytotoxicity = \left(1 - \frac{Abs\ Sample}{Abs\ Negative\ Control}\right) \times 100$$

Mass Spectrometry Method. Samples were analyzed with a ThermoFisher Q Exactive HF-X (ThermoFisher, Bremen, Germany) mass spectrometer coupled with a Waters Acquity H-class liquid chromatograph system. Samples were introduced via a heated electrospray source (HESI) at a flow rate of 0.6 mL/min. Electrospray source conditions were set as: spray voltage 3.0 kV, sheath gas (nitrogen) 60 arb, auxiliary gas (nitrogen) 20 arb, sweep gas (nitrogen) 0 arb, nebulizer temperature 375 degrees C, capillary temperature 380 degrees C, RF funnel 45 V. The mass range was set to 150-2000 m/z. All measurements were recorded at a resolution setting of 120,000.

Separations were conducted on a Waters Acquity UPLC BEH C18 column (2.1 x 50 mM, 1.7 uM particle size). LC conditions were set at 100 % water with 0.1 % formic acid (A) ramped linearly over 9.8 mins to 95 % acetonitrile with 0.1 % formic acid (B) and held until 10.2 mins. At 10.21 mins the gradient was switched back to 100% A and allowed to re-equilibrate until 11.25 mins. Injection volume for all samples was 3 uL.

Xcalibur (ThermoFisher, Breman, Germany) was used to analyze the data. Solutions were analyzed at 0.1 mg/mL or less based on responsiveness to the ESI mechanism. Molecular formula assignments were determined with Molecular Formula Calculator (v 1.2.3). All observed species were singly charged, as verified by unit *m/z* separation between mass spectral peaks corresponding to the ^{12}C and $^{13}C^{12}C_{c-1}$ isotope for each elemental composition.

298 **SUPPLEMENTARY TABLES**

299 **Table S1. Oligonucleotides, Strains and plasmids**

300

Oligonucleotides			
Primers	**5' -> 3' sequence**		**Reference**
pagC-P1 Fwd	CACACCGTAAATAATAAGTAGTATTAAGGAGTTGTTGTGTAGGCTGGAGCTGCTTCG		This work
pagC-P2 Rev	GGTGCGGAAGGCGAACCTTCCGCATAGCTTATGCTTTTCATATGAATATCCTCCTTA		This work
pagC-prom Fwd	CCGAACGGTGTCAAGTTTC		This work
P1Rev	CGAAGCAGCTCCAGCCTACAC		This work
#324	CGGGATCCGATGCCGTTAGTGGATCTT		This work
#292	ACGACAAAAGAGGCATAAGCTTGGG		This work
Strain or plasmid	**Stock name**	**Genotype or property**	**Source or reference**
Strains ***Salmonella* Typhimurium**			
ATCC 14028s	14028s	wild-type	ATCC
phoP	MS7953s	14028s *phoP*::Tn10	(1)
virK::lacZ	EG9532	*pcgG9283::MudJ*	(2)
pagC::lacZ	PB11970	*pagC::lacZ::Cm*	This work
pagK::lacZ	PB3111	*pbgF::MudJ*	Laboratory Stock
pipD::lacZ	EG9341	*pipD::MudJ*	(3)
pcgM::lacZ	PB3118	*pcgM::MudJ*	Laboratory Stock
pcgF::lacZ	EG9530	*pcgF9281::MudJ*	(2)
tppB::lacZ	PB3062	*tppB::MudI*	(4)

cpxP::lacZ	PB12103	*cpxP::lacZ*	(5)
virK::lacZ phoQ [pUHE21-2::*phoQ*]	PB4663	*pcgG9283::*Mud*J phoQ*::Tn10 with pEG9050	(6)
STM23 *pagC*::*lacZ*	PB12710	*pagC*::*lacZ*	This work
Other *Salmonella* strains			
S. Enteritidis PT4 *pagC*::*lacZ*	PB12711	*pagC*::*lacZ*	This work
S. Dublin SDu5 *pagC*::*lacZ*	PB12712	*pagC*::*lacZ*	This work
Escherichia coli			
K12 TB1 [pmalc::PhoQc]	PB1361	F^- ara Δ(lac-proAB) [φ80dlac Δ(lacZ)M15] rpsL(Str^R) thi hsdR	New England Biolabs
M15 [pREP4] [pQE31::EnvZc]	PB2994	*F-, Φ80ΔlacM15, thi, lac-, mtl-, recA+ , KmR*	This work
Plasmids			
pPhoQ	p9050	*reppMB1 Ampr lacIq phoQ*	(7)
pPhoQc	pPB1021	*reppMB1 Ampr lacIq malE lacZα phoQc*	(8)
pEnvZc	pPB1190	*ColE1 Ampr EnvZc*	This work

Table S2. SMILES and Labbook codes for final compounds

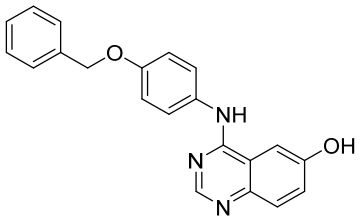
Labbook Code	Manuscript Code	SIMILES
CA439	**GI262866A**	OC1=CC2=C(NC3=CC=C(OCC4=CC=CC=C4)C=C3)N=CN=C2C=C1
CA440	**1**	OC1=CC2=NC=NC(NC3=CC=C(OCC4=CC=CC=C4)C=C3)=C2C=C1
CA443	**2**	OC1=CC2=C(NC3=CC=C(OCC4=CC=CC=C4)C=C3)N=CN=C2C=C1O
CA442	**3**	OC1=CC2=C(NC3=CC=C(OCC4=CC=CC=C4)C=C3)C=CN=C2C=C1
CA436	**4**	OC1=CC2=C(NC3=CC=C(OCC4=CC=C(F)C=C4)C=C3)N=CN=C2C=C1
CA437	**5**	OC1=CC2=C(NC3=CC=C(OCC4=CC=CC(F)=C4)C=C3)N=CN=C2C=C1
CA438	**6**	OC1=CC2=C(NC3=CC=C(OCC4=CC=CC=C4F)C=C3)N=CN=C2C=C1
CA445	**7**	OC1=CC2=C(NC3=CC=C(OCC4=CC=C(F)C=C4)C=C3)N=CN=C2C=C1O
CA444	**8**	FC(C=C1)=CC=C1COC(C=C2)=CC=C2NC3=C4C(C=C(O)C=C4)=NC=N3
CA461	**GI261520A**	COC1=CC2=C(NC3=CC=C(OCC4=CC=CC=C4)C=C3)N=CN=C2C=C1
CA459	**9**	COC1=CC2=NC=NC(NC3=CC=C(OCC4=CC=CC=C4)C=C3)=C2C=C1
CA460	**10**	COC1=CC2=C(NC3=CC=C(OCC4=CC=CC=C4)C=C3)N=CN=C2C=C1OC
CA452	**11**	OC1=CC2=C(NC3=CC=CC(C#C)=C3)N=CN=C2C=C1
CA453	**12**	OC1=CC2=NC=NC(NC3=CC=CC(C#C)=C3)=C2C=C1
CA456	**13**	OC1=CC2=C(NC3=CC=CC(C#C)=C3)N=CN=C2C=C1O
CA455	**14**	OC1=CC=C2N=CC=C(NC3=CC=CC(C#C)=C3)C2=C1
CA454	**15**	OC1=CC=C2C(NC3=CC=CC(C#C)=C3)=CC=NC2=C1
CA204	**16**	COC1=CC2=C(NC3=CC=CC(C#C)=C3)N=CN=C2C=C1
CA209	**17**	COC1=CC2=NC=NC(NC3=CC=CC(C#C)=C3)=C2C=C1
CA176	**18**	C#CC1=CC=CC(NC2=NC=NC3=CC(OC)=C(OC)C=C32)=C1
CA156	**19**	C#CC1=CC=CC(NC2=CC=NC3=CC(OC)=C(OC)C=C32)=C1
CA489	**20**	OC1=CC2=C(NC3=CC=C(OC)C=C3)N=CN=C2C=C1
CA490	**21**	COC1=CC=C(NC2=C3C=C(OC)C=CC3=NC=N2)C=C1

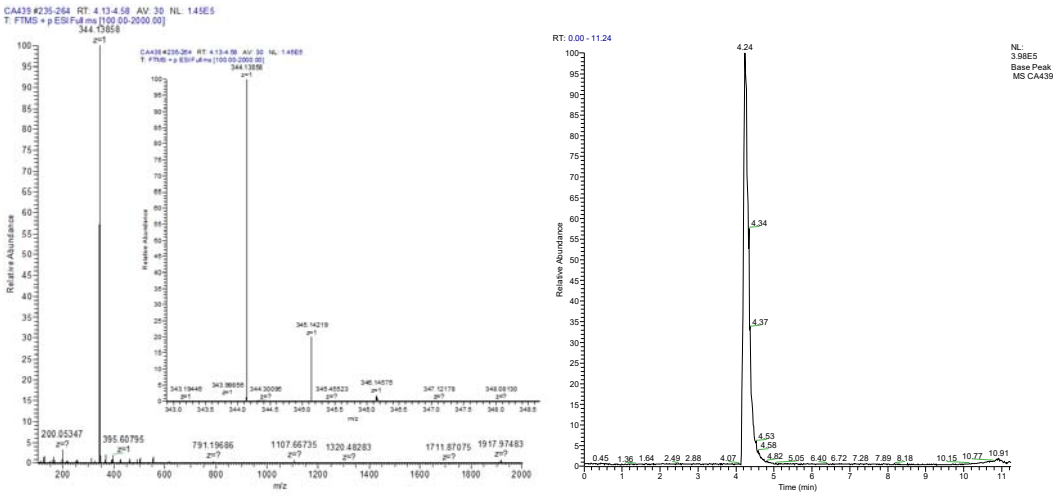
304

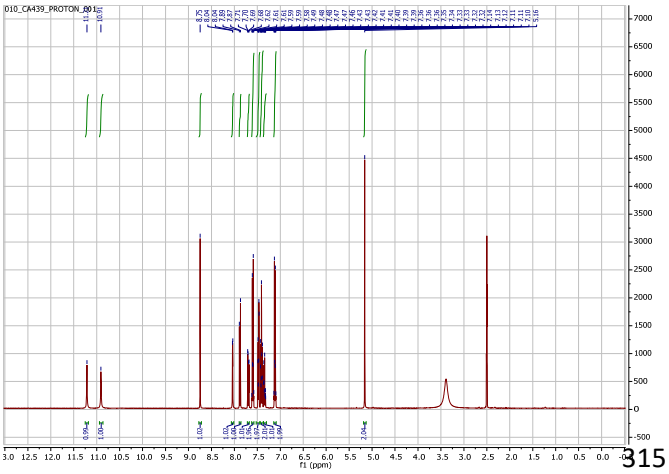
305

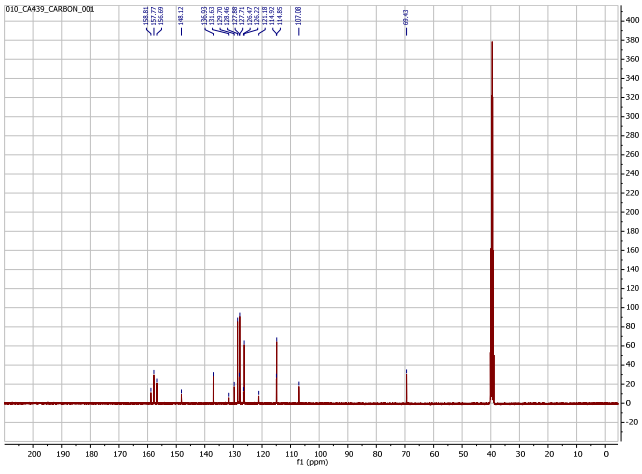
SUPPLEMENTARY INFORMATION - Compounds Characterization Spectra (GI262866A & GI261520A, 1-21)

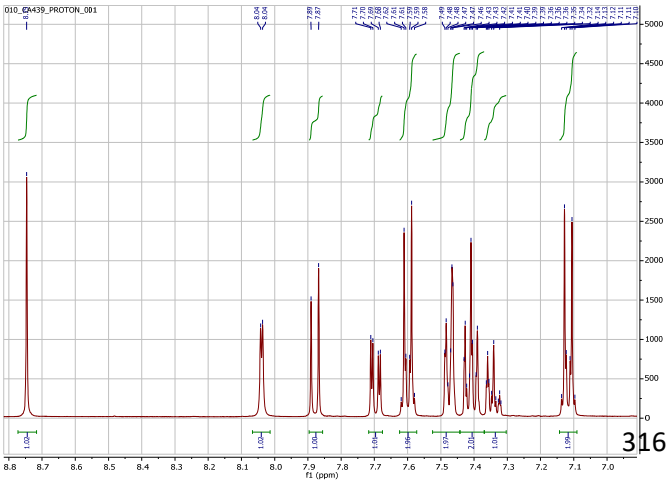
4-{[4-(benzyloxy)phenyl]amino}quinazolin-6-ol (**GI262866A**)

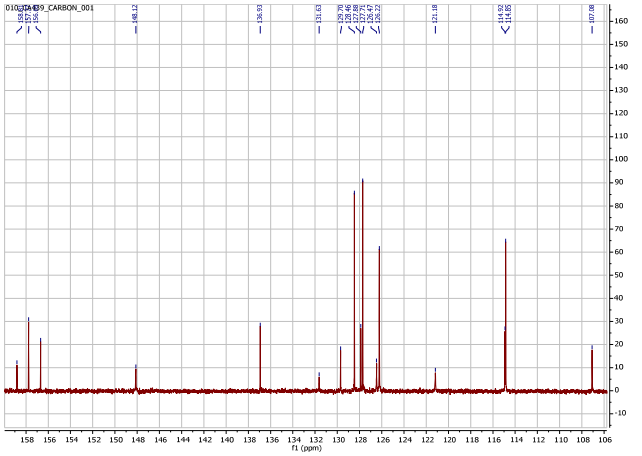




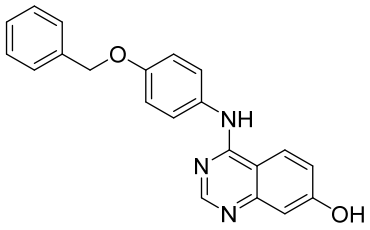


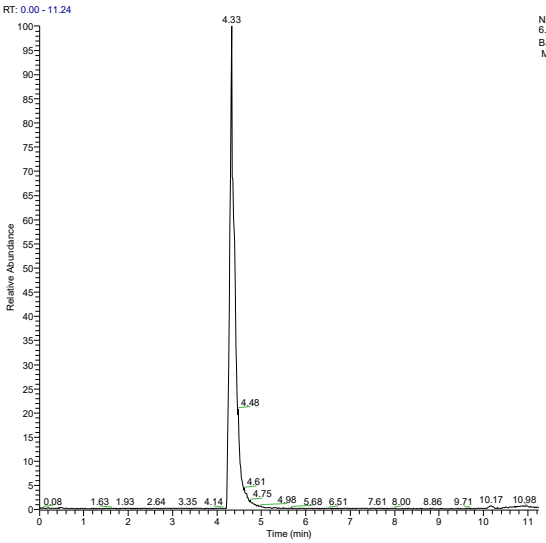


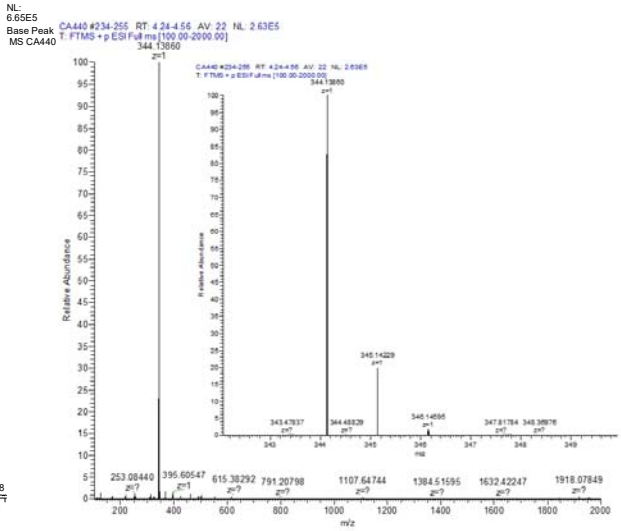


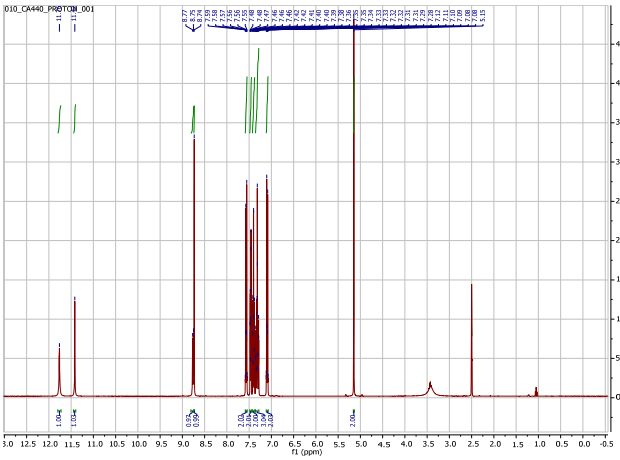


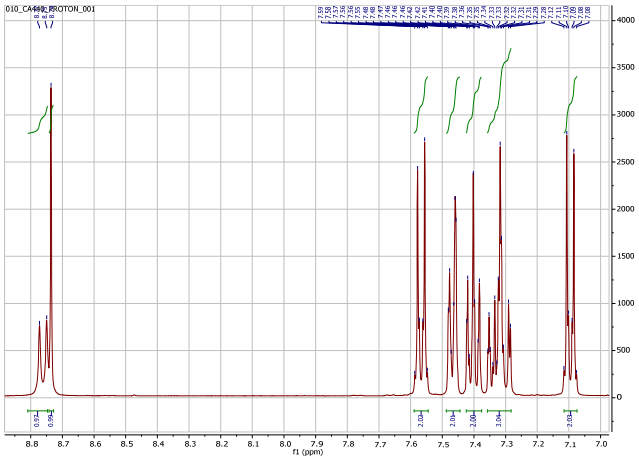
4-{[4-(benzyloxy)phenyl]amino}quinazolin-7-ol (**1**)

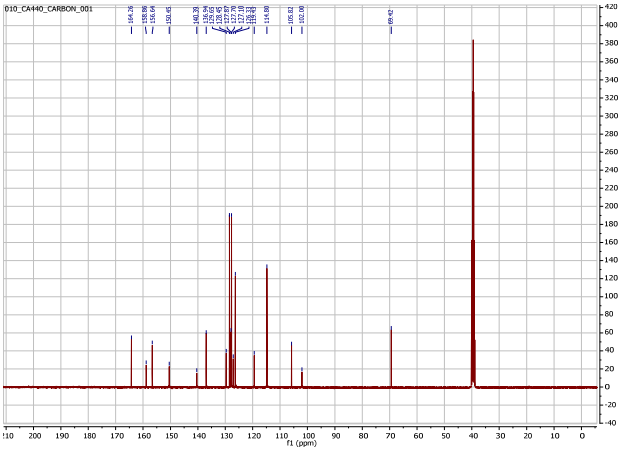


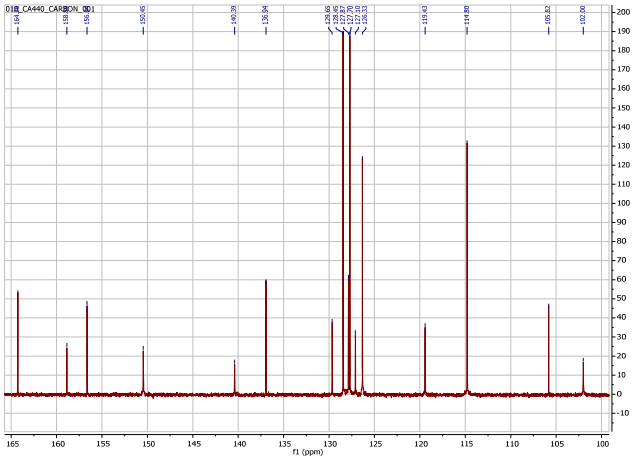






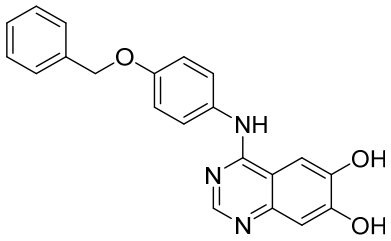


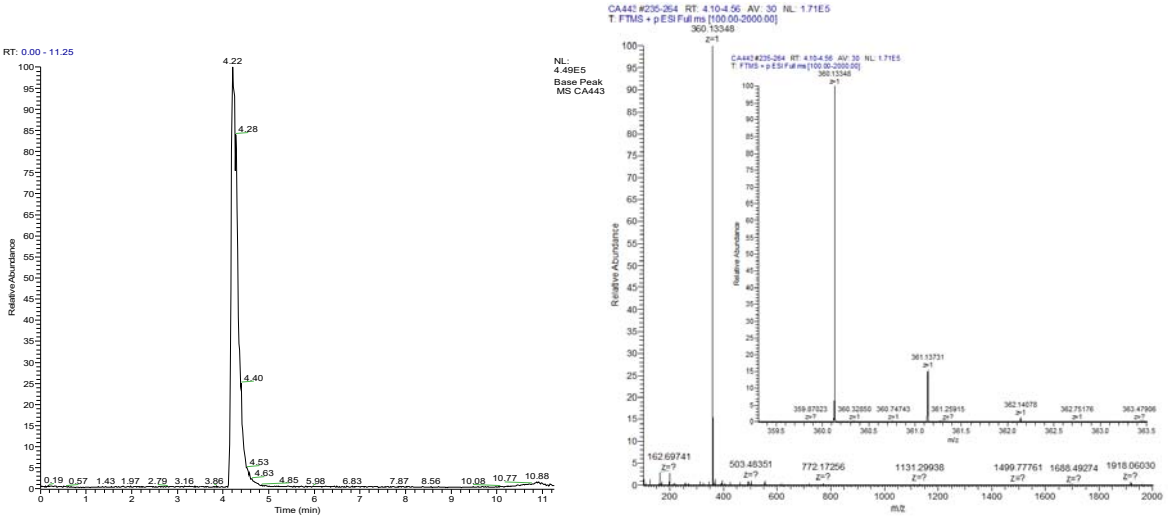
325 

326 

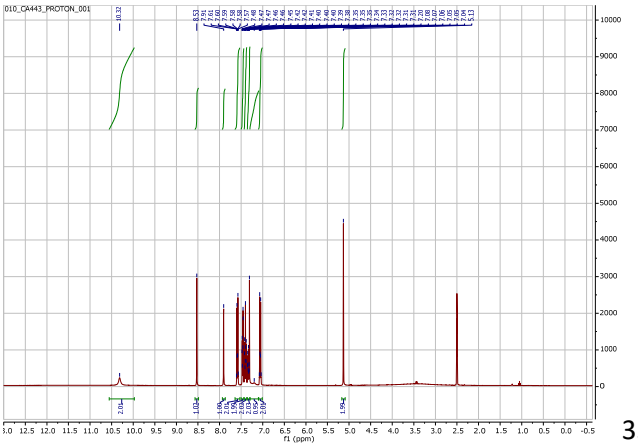
327

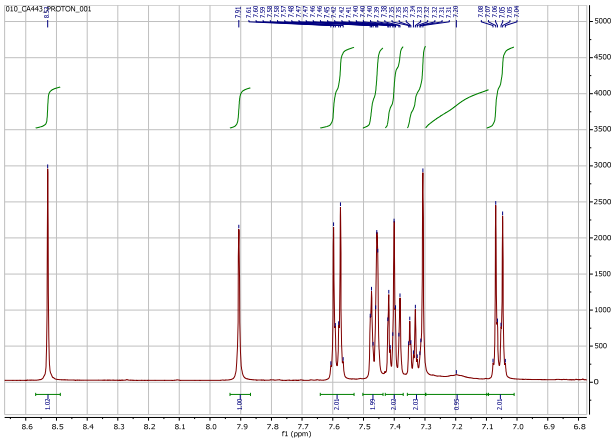
328 4-{[4-(benzyloxy)phenyl]amino}quinazoline-6,7-diol (**2**)

329 

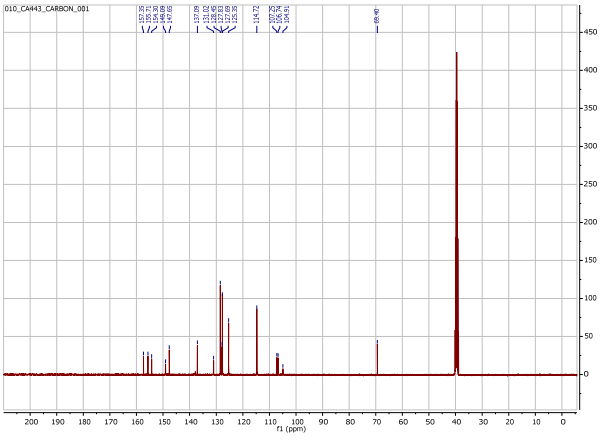
330 

331

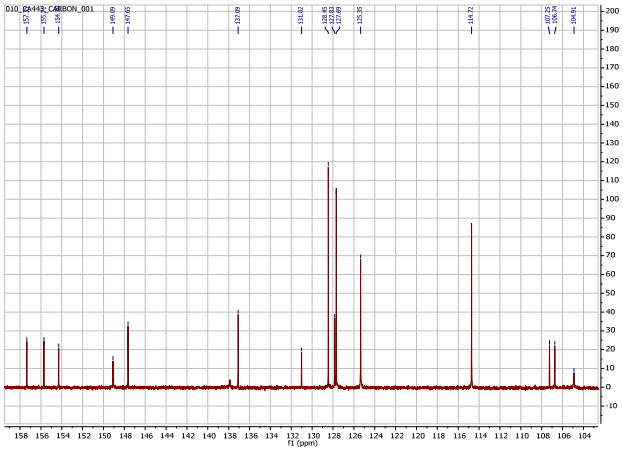
332 

333 

334



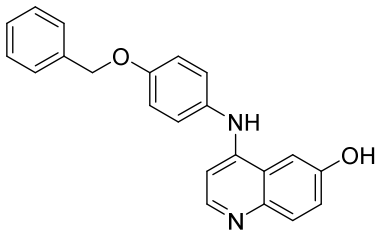
335



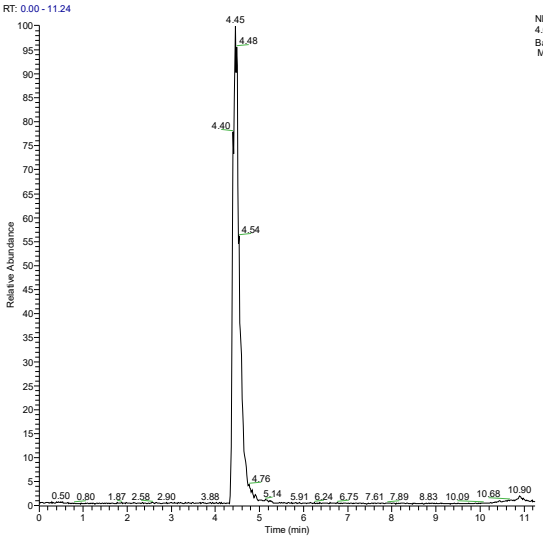
336

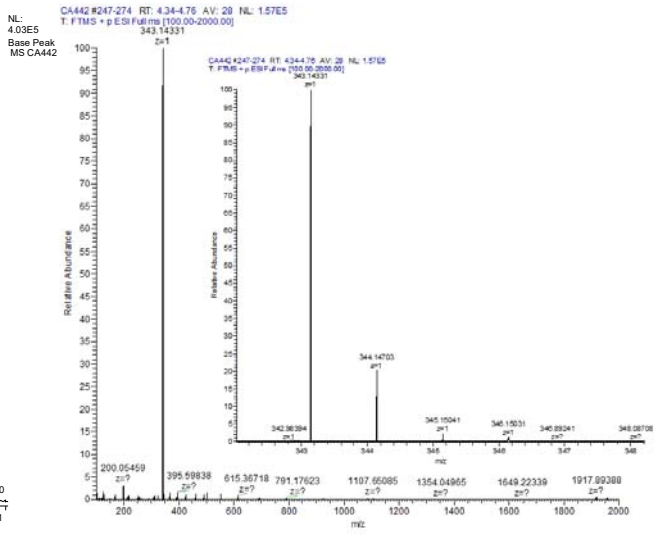
337 4-{[4-(benzyloxy)phenyl]amino}quinolin-6-ol (**3**)

338



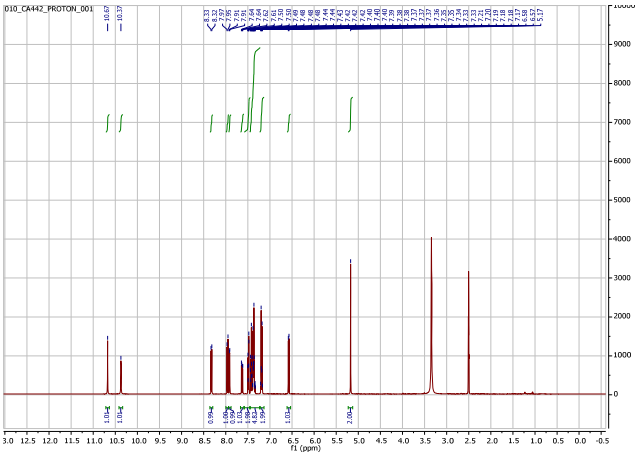
339



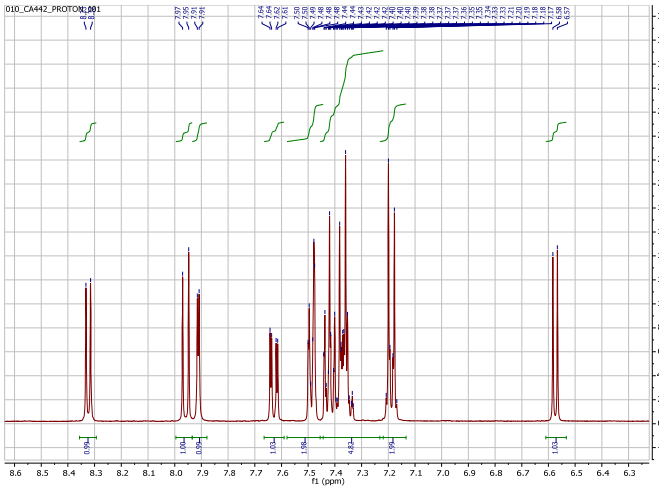


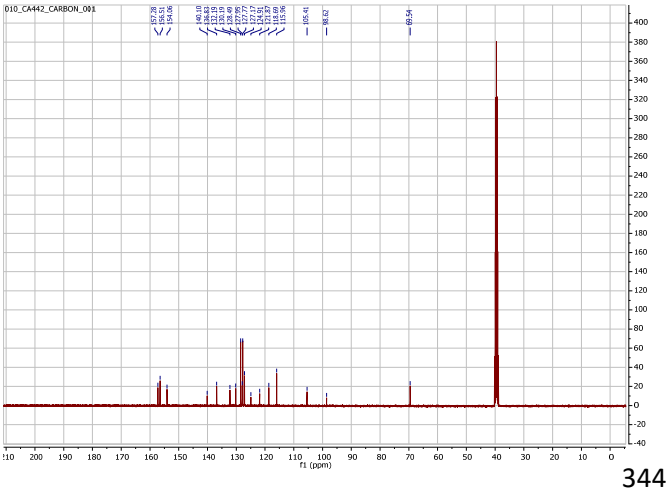
340

341

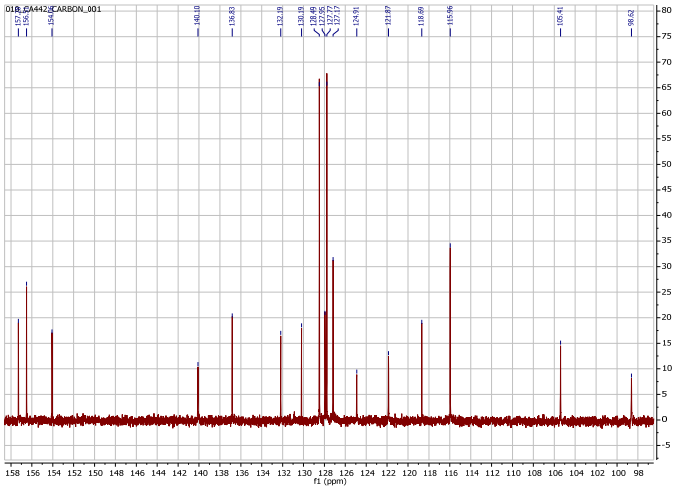


342





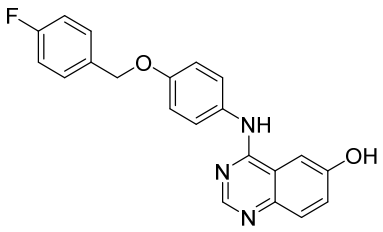
343



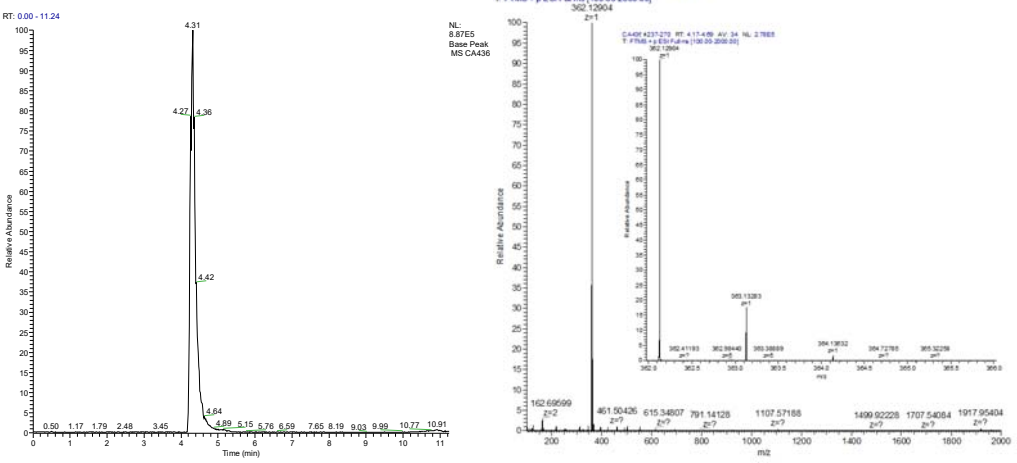
344

345

346 4-({4-[(4-fluorophenyl)methoxy]phenyl}amino)quinazolin-6-ol (**4**)

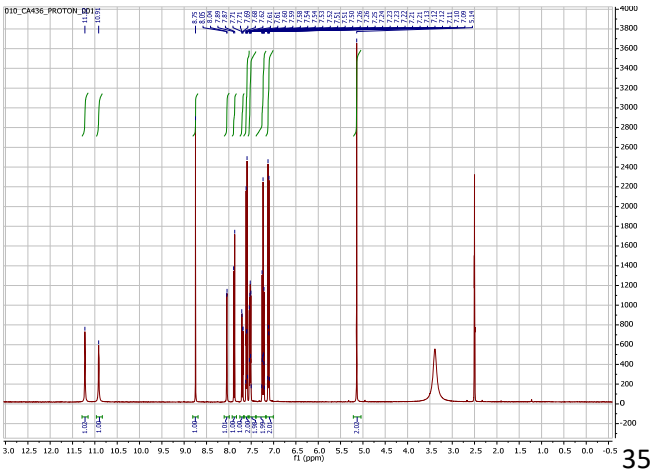


347

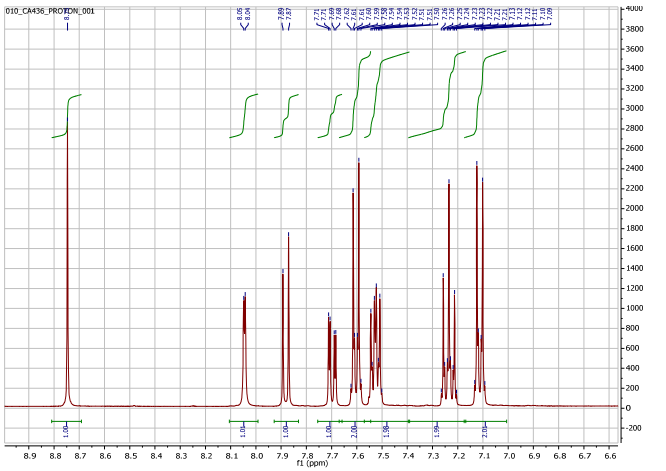


348

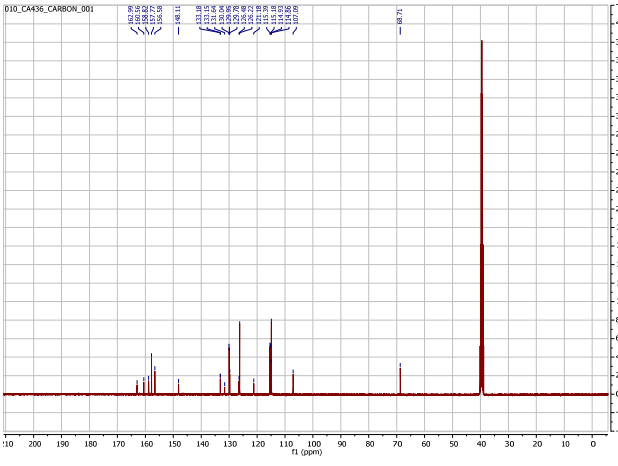
349

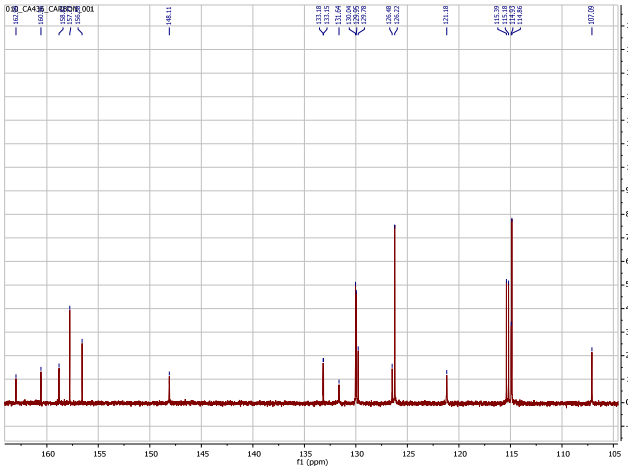


350



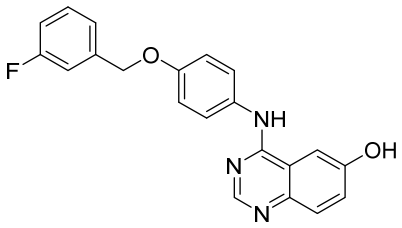
351

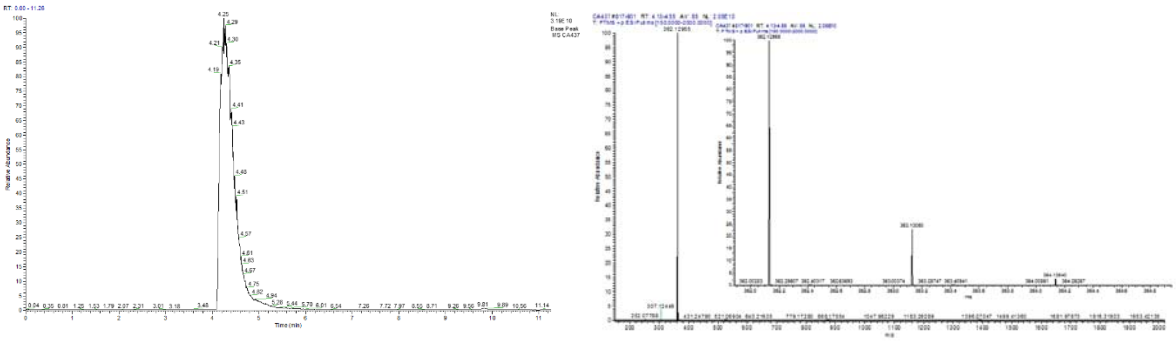
352 

353 

354

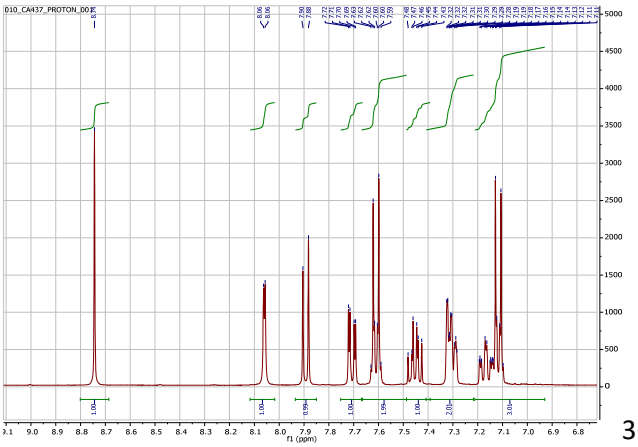
355 4-({4-[(3-fluorophenyl)methoxy]phenyl}amino)quinazolin-6-ol (**5**)

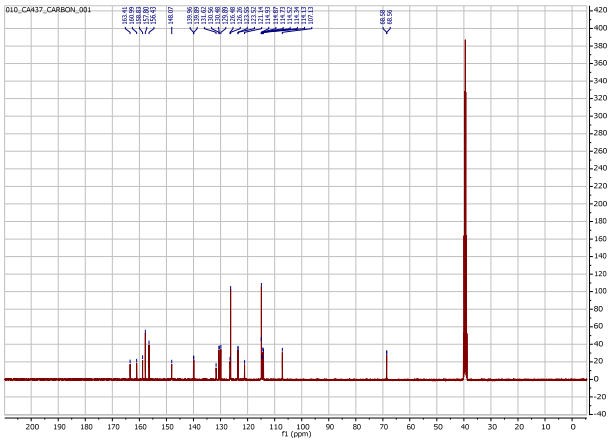
356 

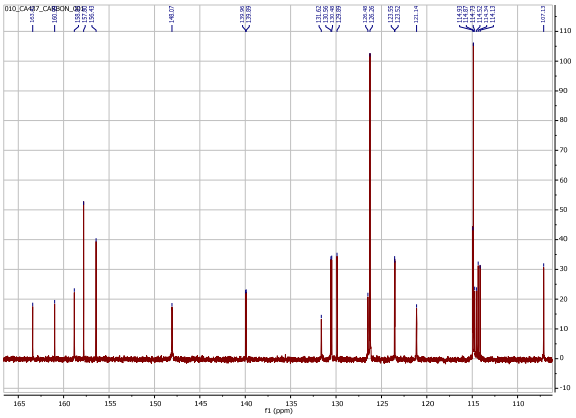
357 

358

359

360 

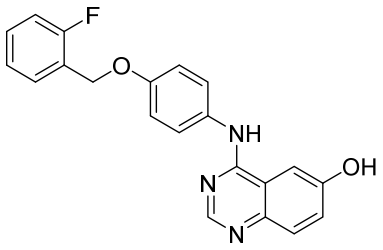
361 



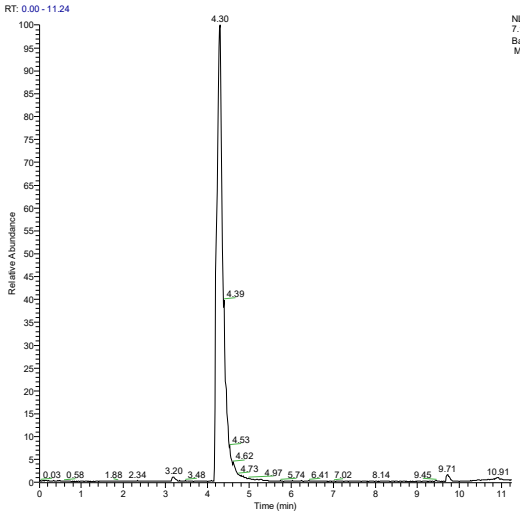
362

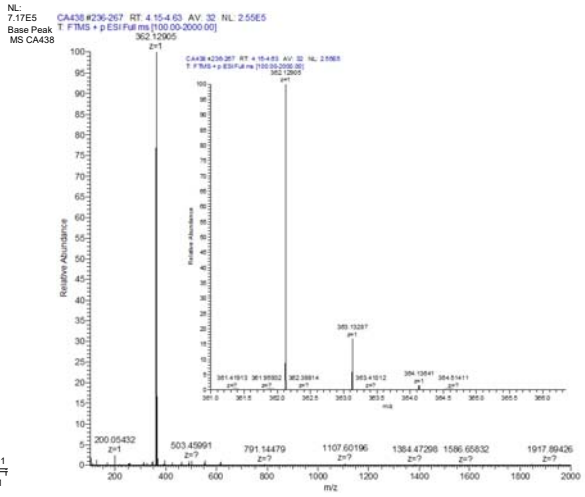
363

364 4-({4-[(2-fluorophenyl)methoxy]phenyl}amino)quinazolin-6-ol (**6**)



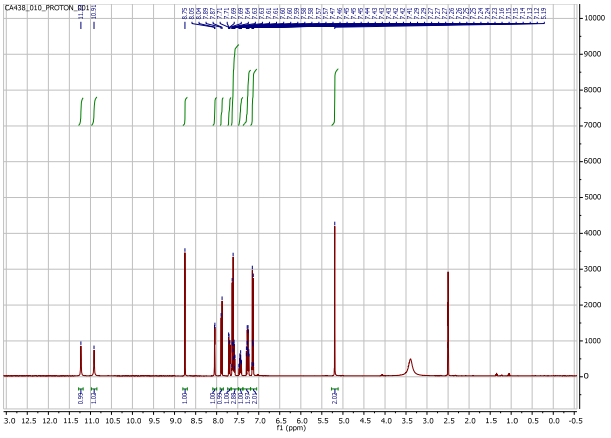
365



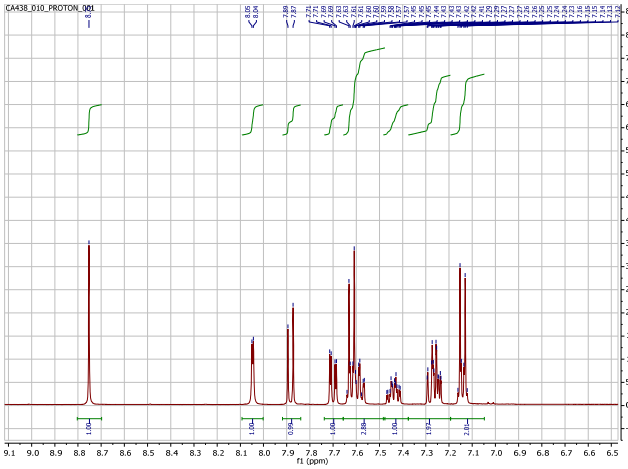


366

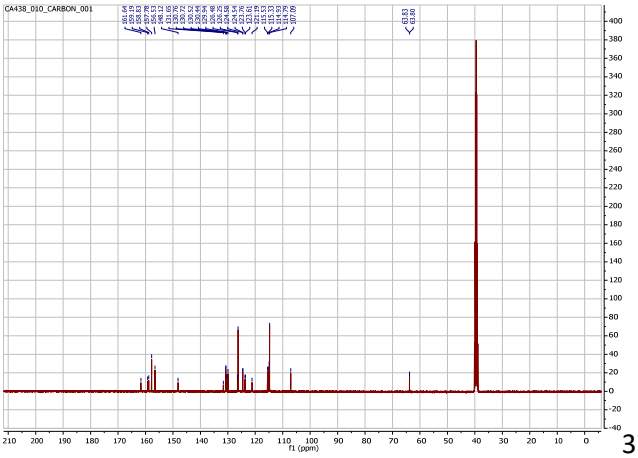
367



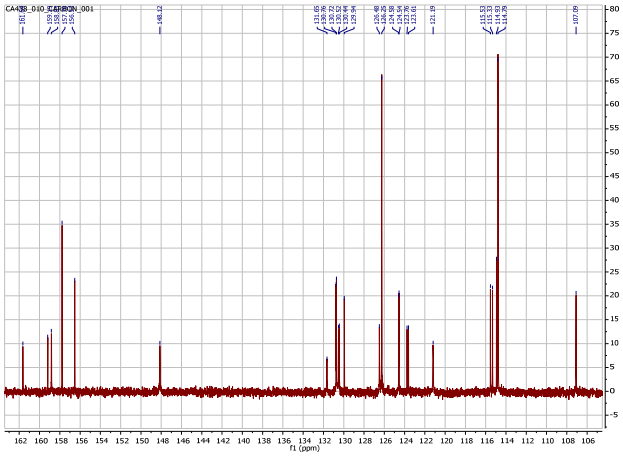
368



369



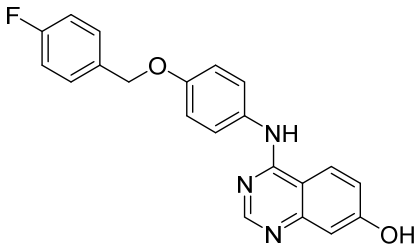
370



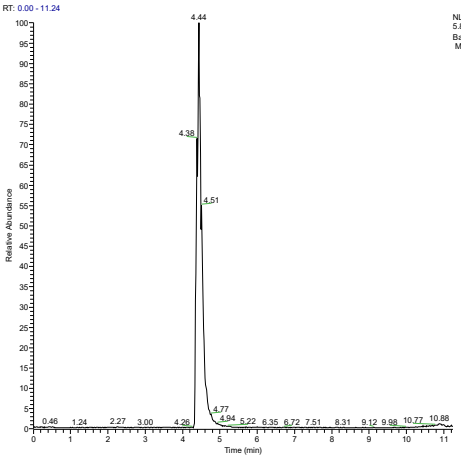
371

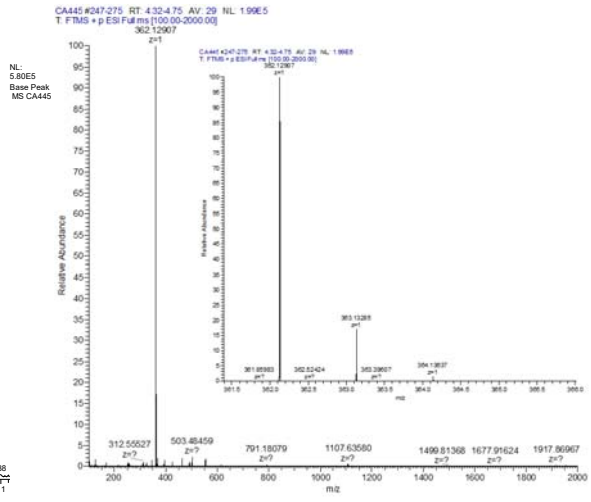
372

373 4-((4-((4-fluorobenzyl)oxy)phenyl)amino)quinazolin-7-ol (**7**)



374

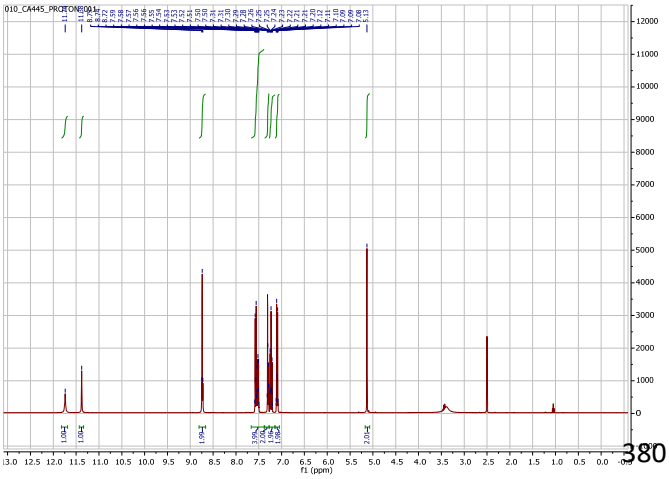




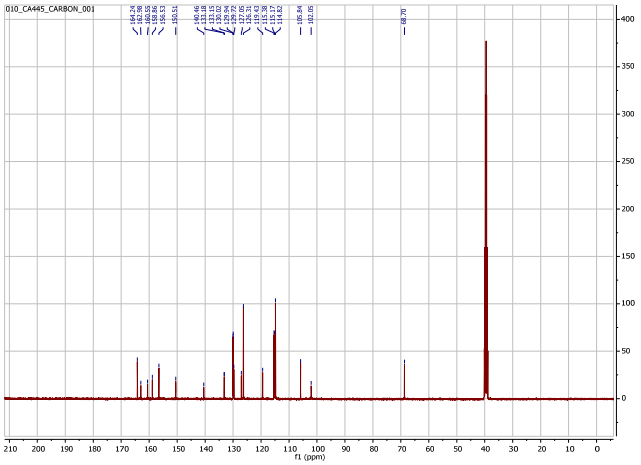
375

376

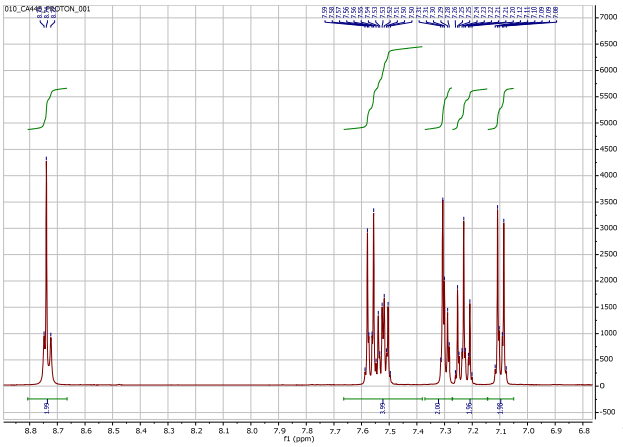
377



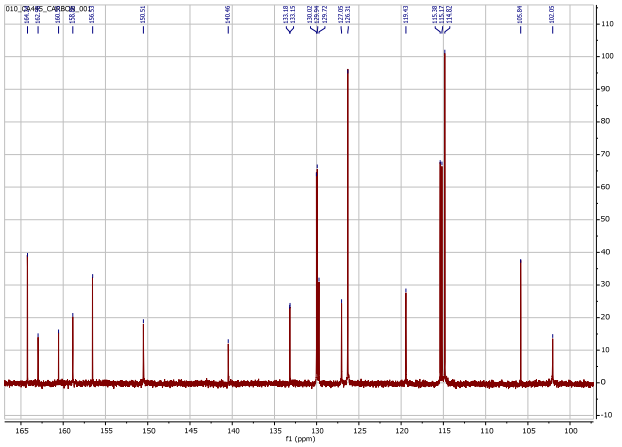
378



380

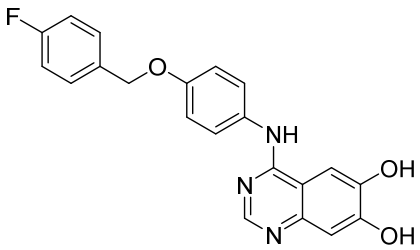


379

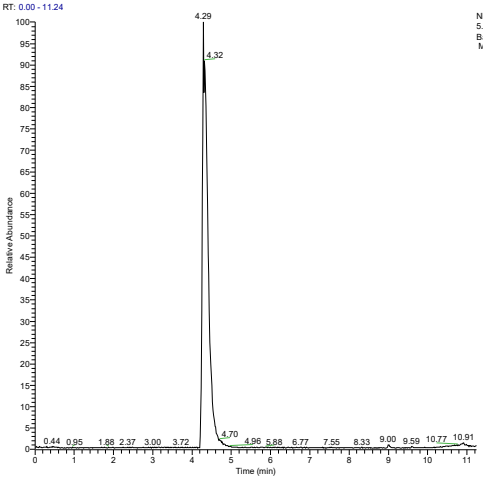


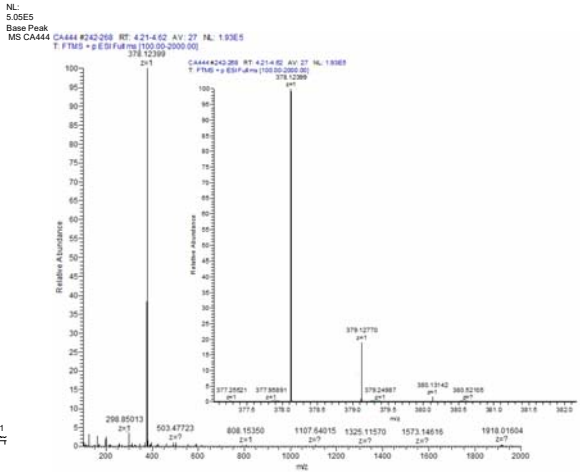
381

382 4-((4-((4-fluorobenzyl)oxy)phenyl)amino)quinazoline-6,7-diol (**8**)



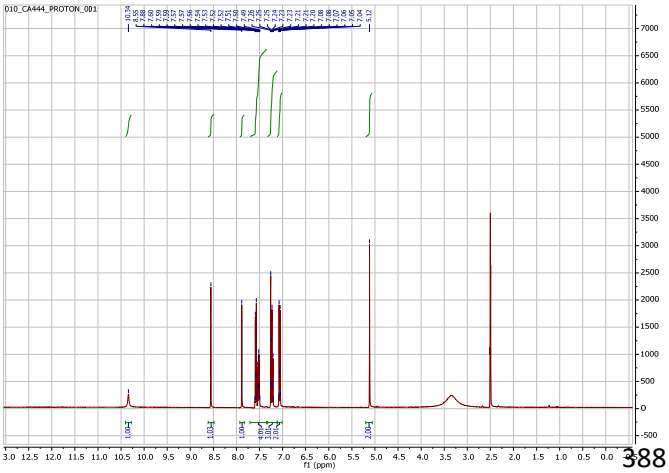
383

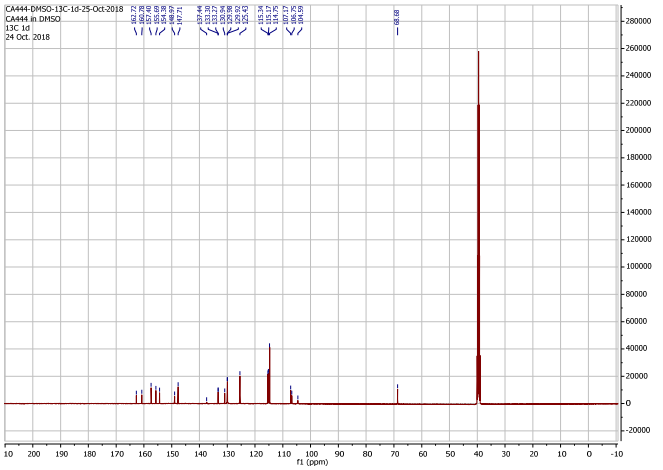


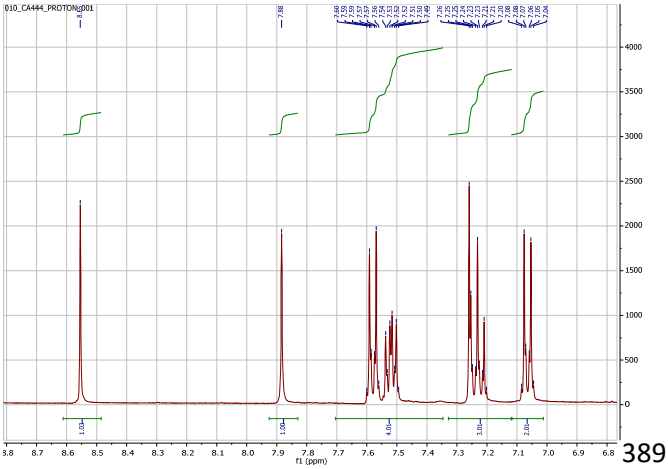


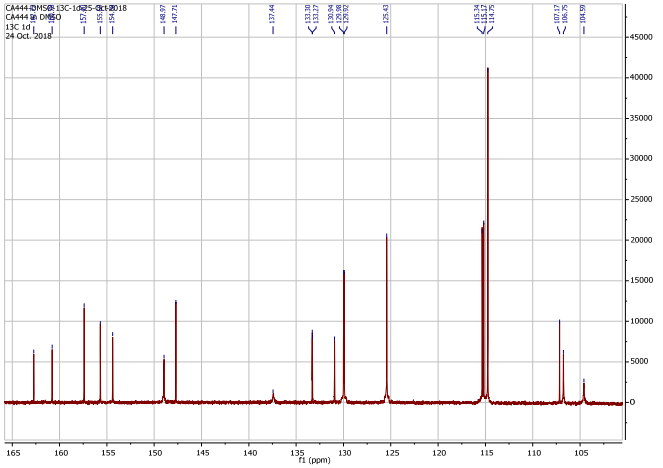
384

385

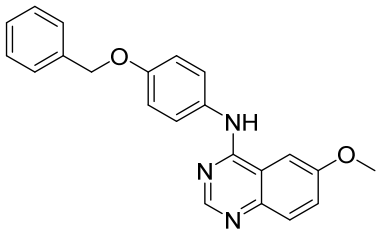
386 

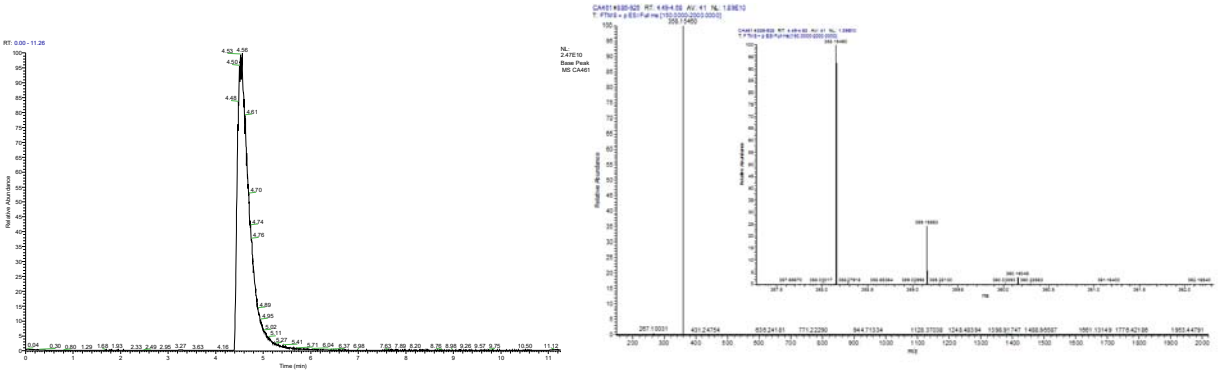
388 

387 

389 

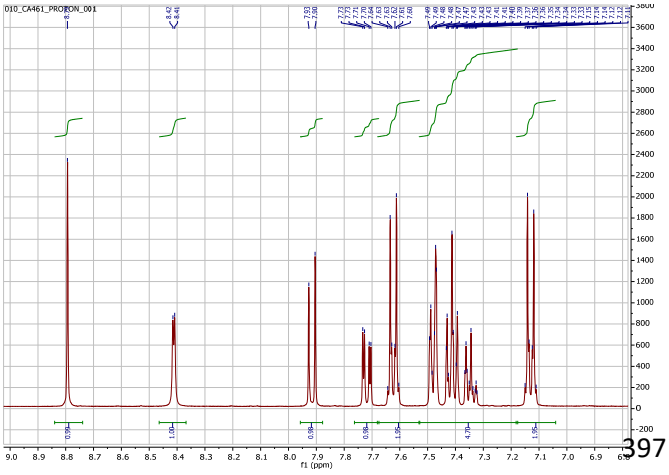
390 *N*-(4-(benzyloxy)phenyl)-6-methoxyquinazolin-4-amine (**GI261520A**)

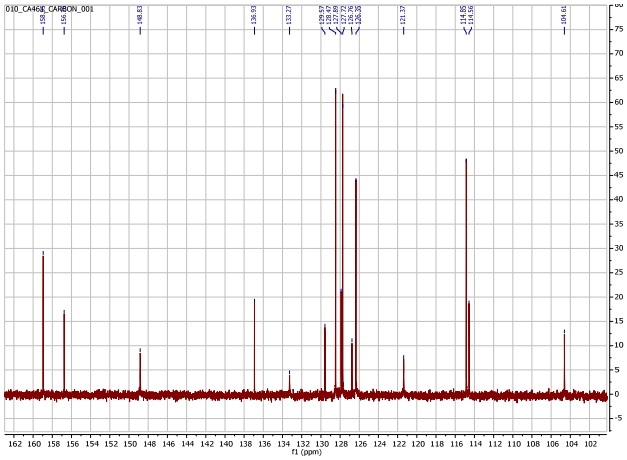
391 

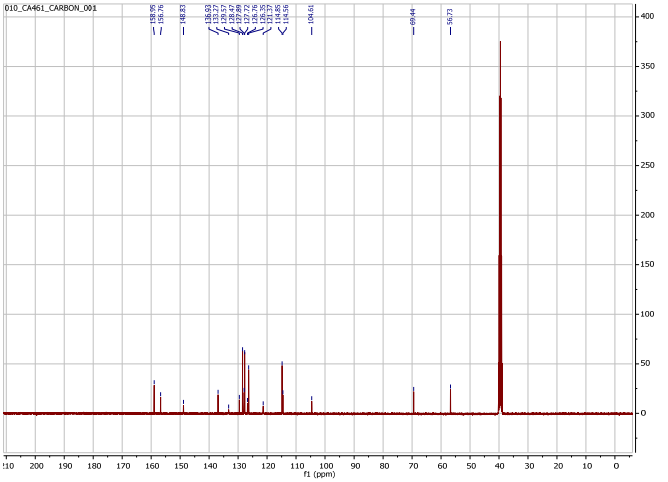
392 

393

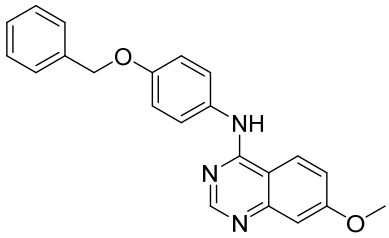
394

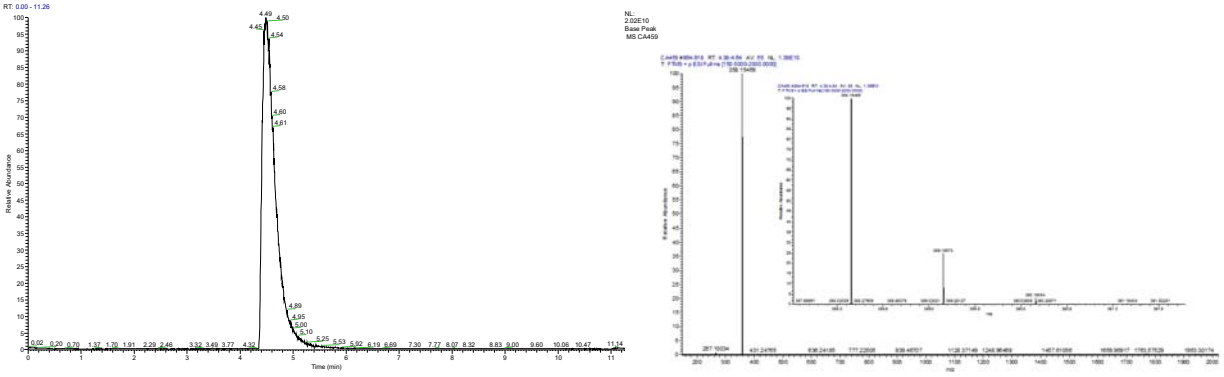
395 

397 

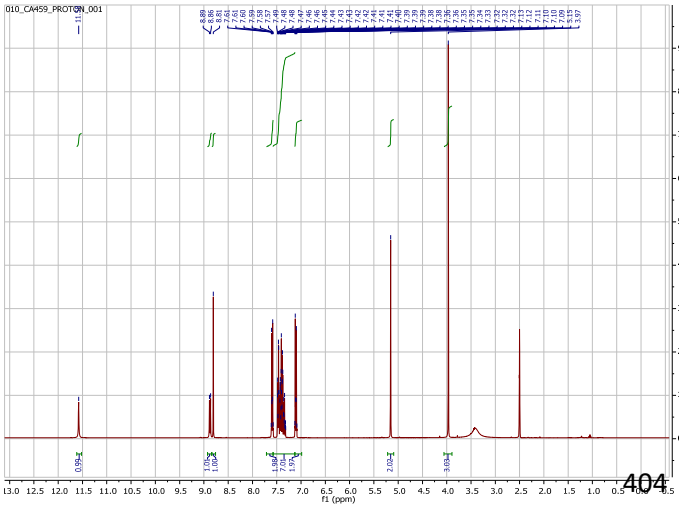
396 

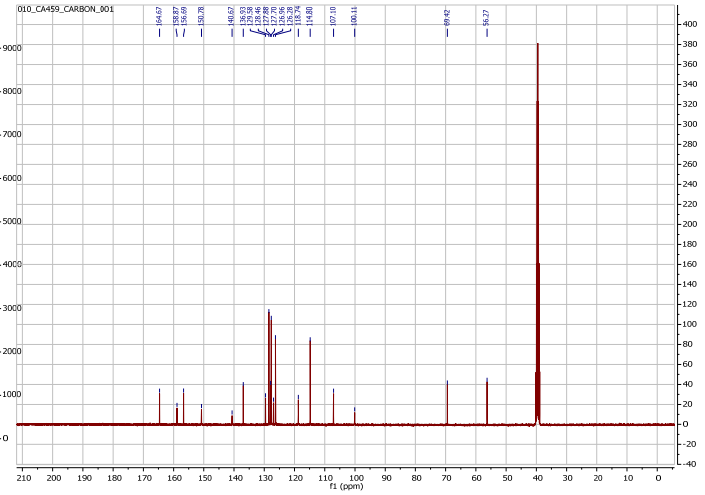
398 *N*-[4-(benzyloxy)phenyl]-7-methoxyquinazolin-4-amine (**9**)

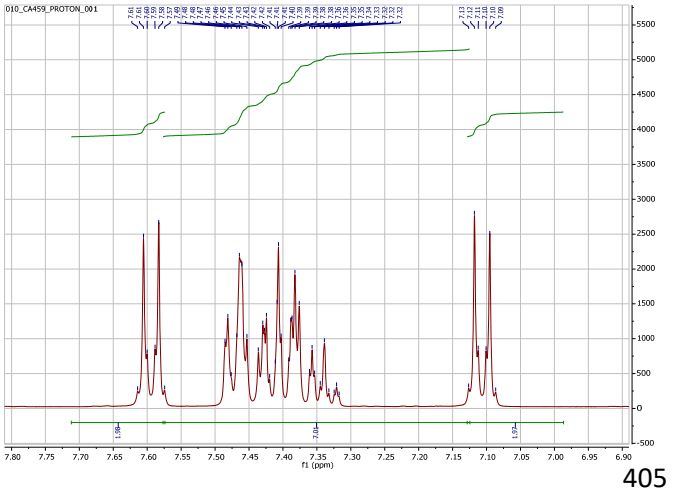
399 

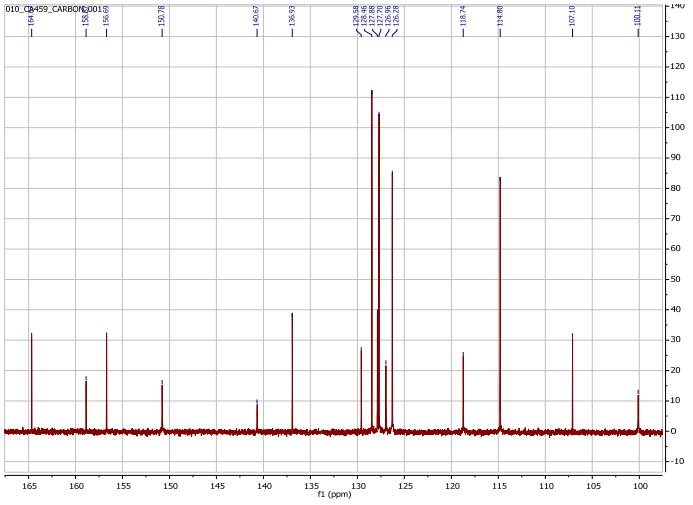
400 

401

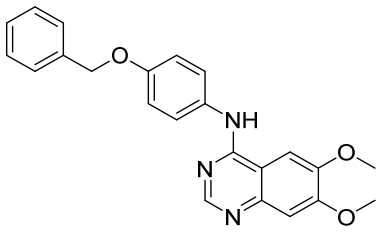
402 

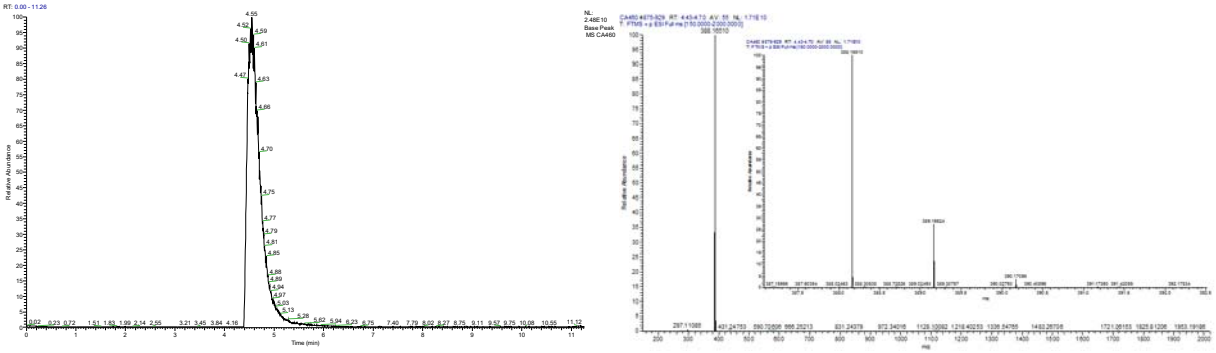
404 

403 

405 

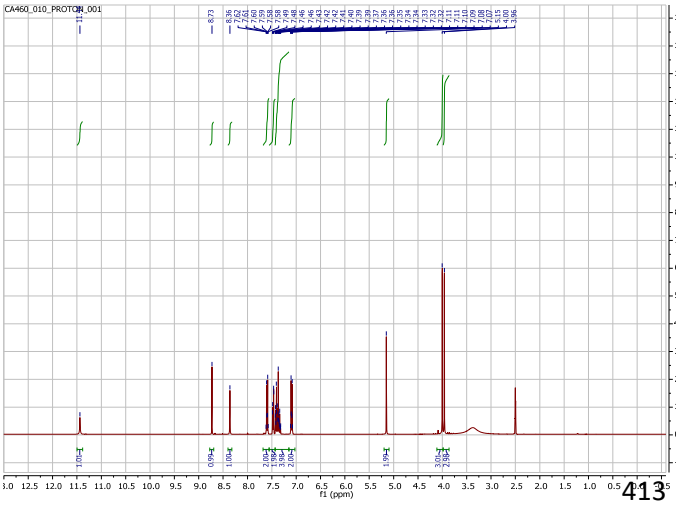
406 *N*-(4-(benzyloxy)phenyl)-6,7-dimethoxyquinazolin-4-amine (**10**)

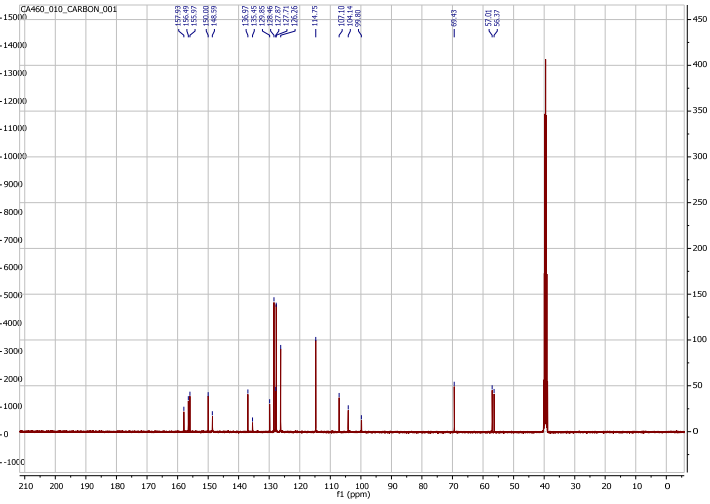
407 

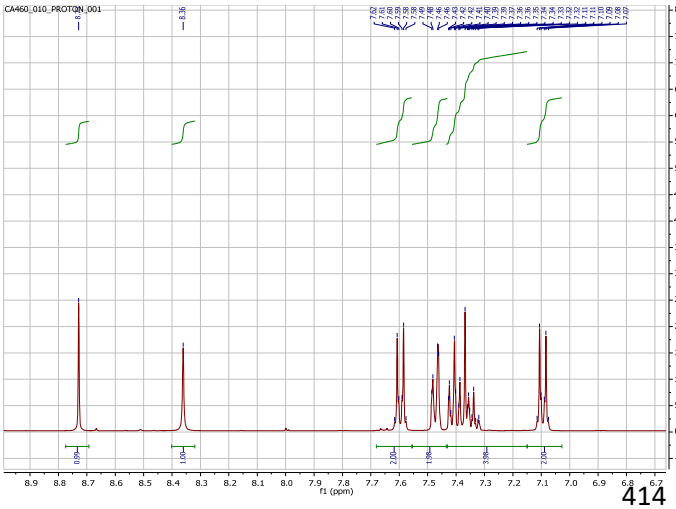
408 

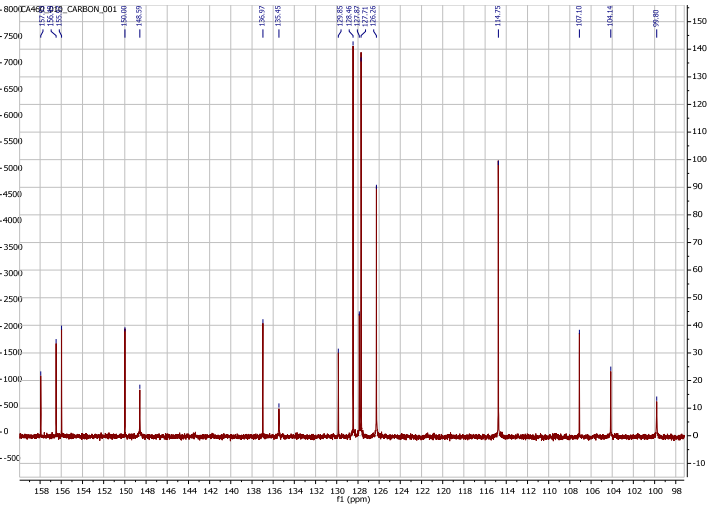
409

410

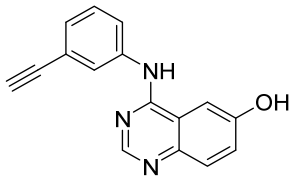
411 

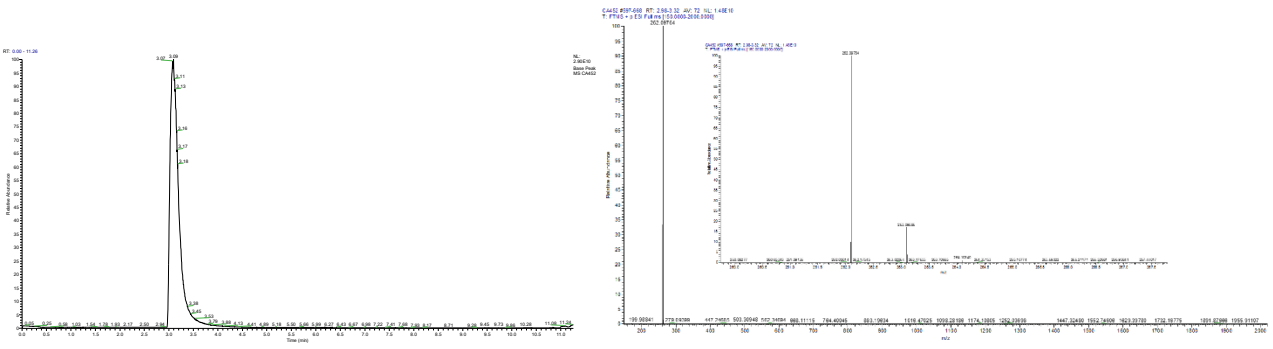
413 

412 

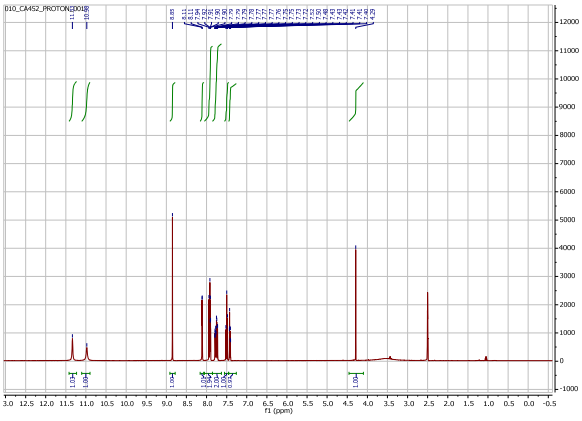
414 

415 4-((3-ethynylphenyl)amino)quinazolin-6-ol (**11**)

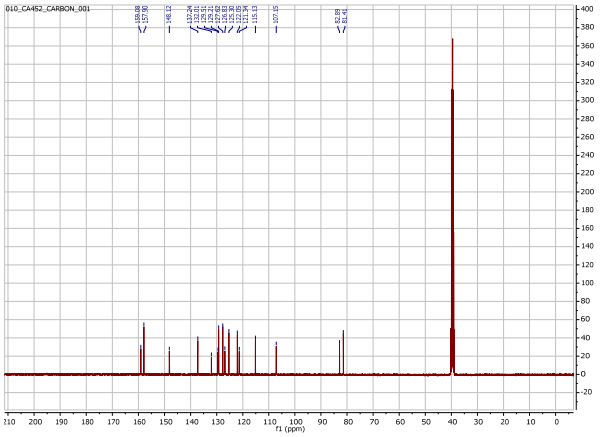
416 

417 

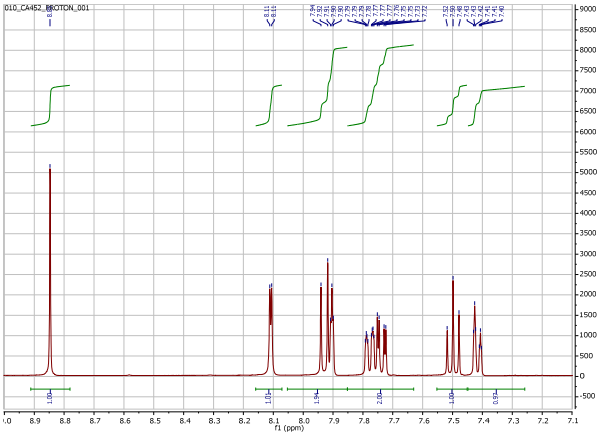
418



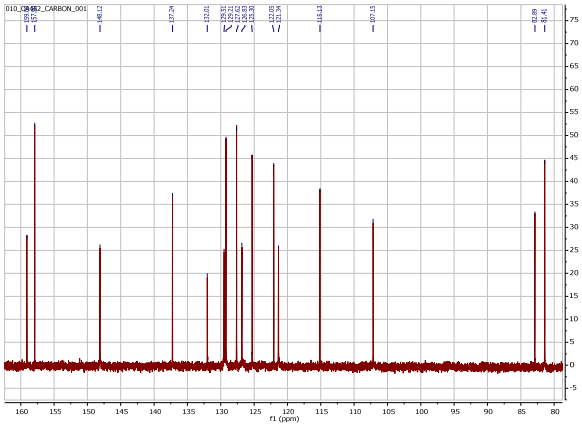
420



419



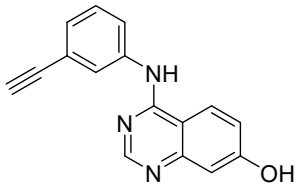
421



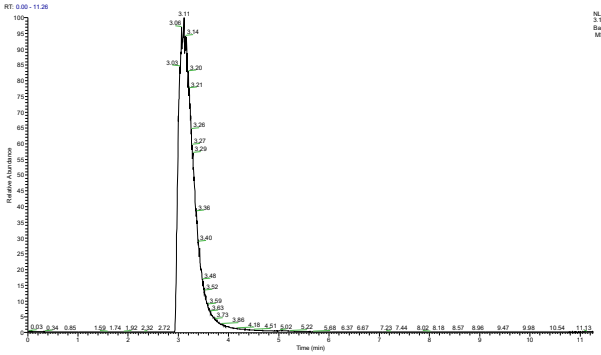
422

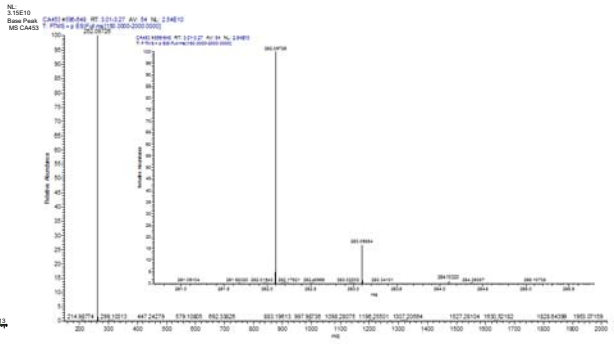
423 4-((3-ethynylphenyl)amino)quinazolin-7-ol (**12**)

424



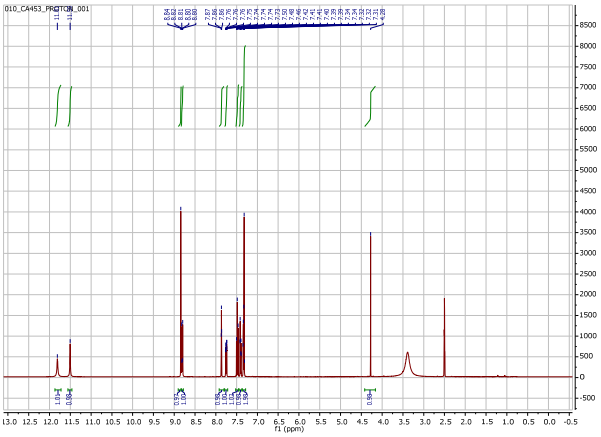
425



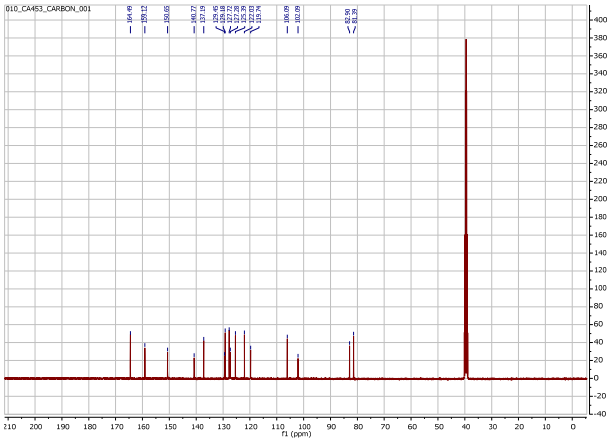


426

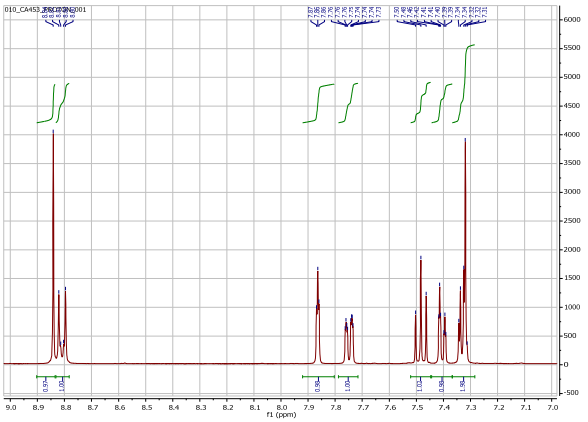
427



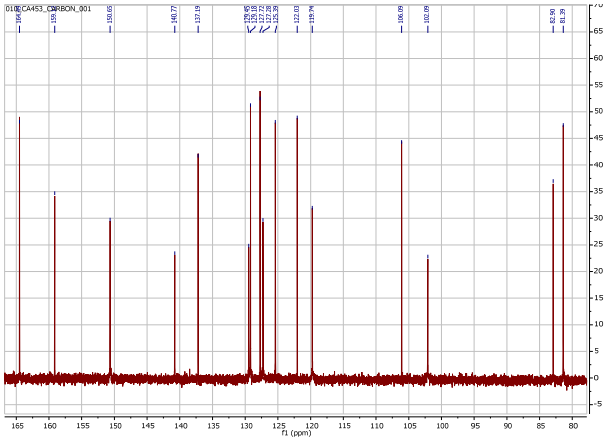
429



428



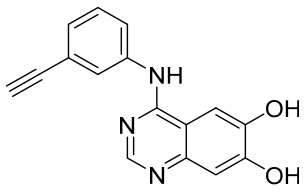
430



431

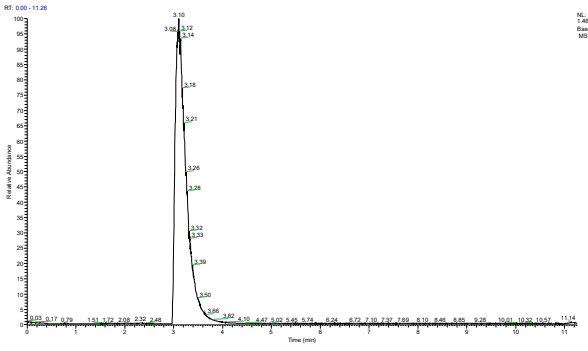
432 4-((3-ethynylphenyl)amino)quinazoline-6,7-diol (**13**)

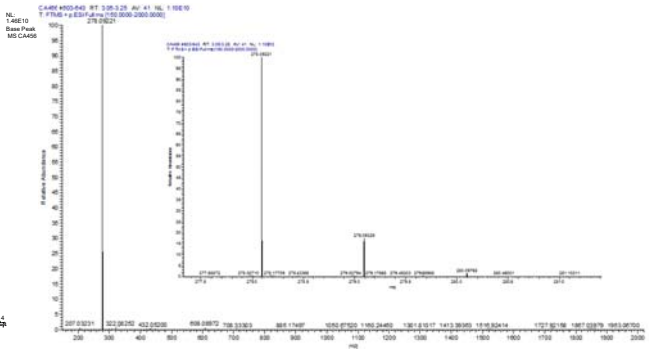
433



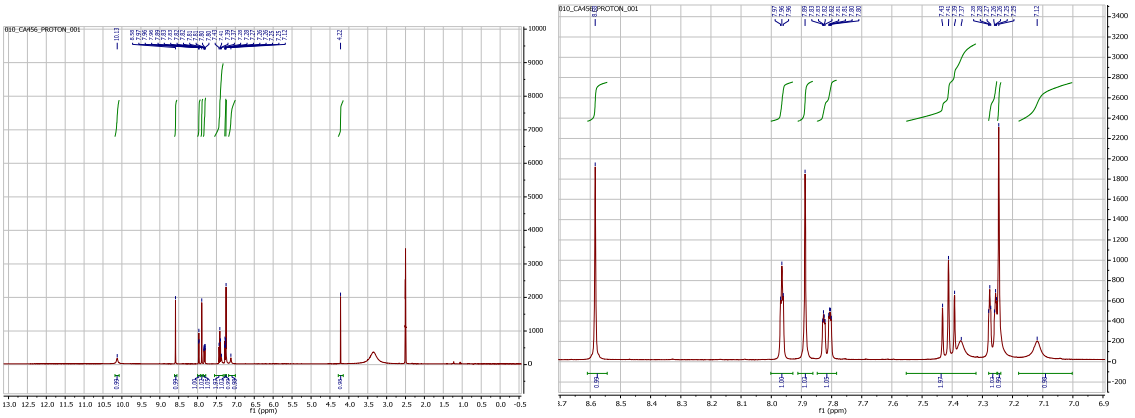
434

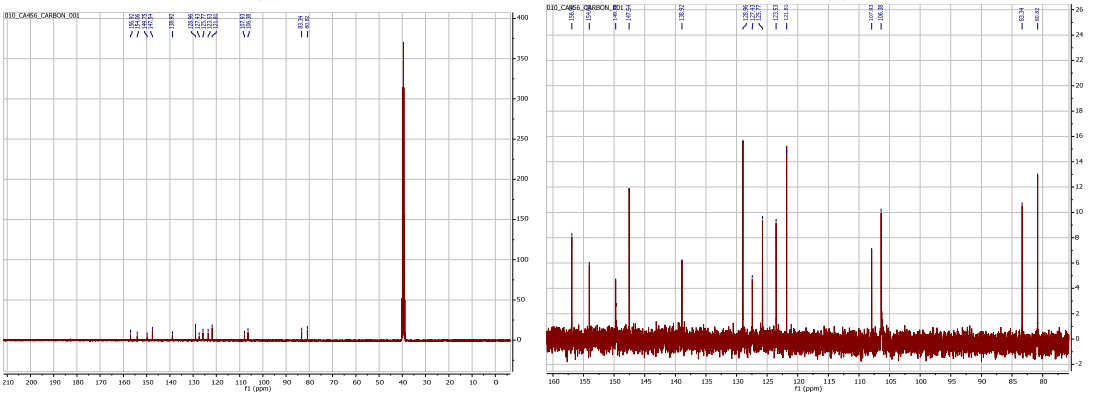
435



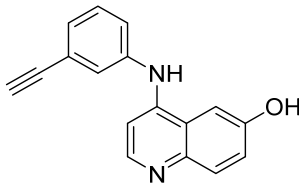


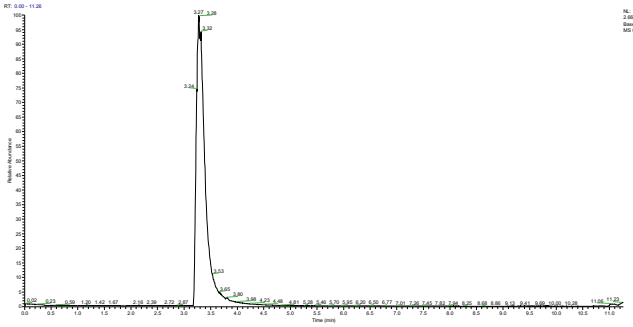
436

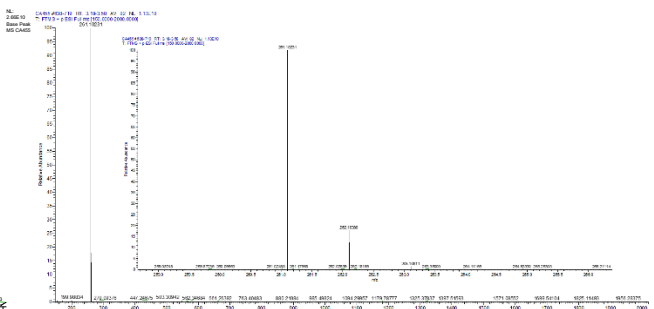


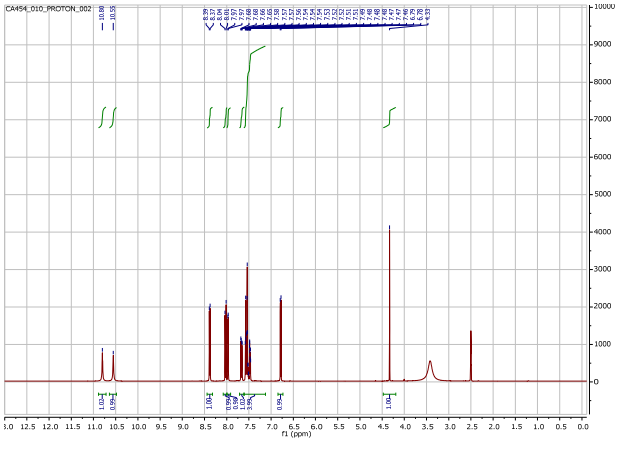


4-((3-ethynylphenyl)amino)quinolin-6-ol (**14**)

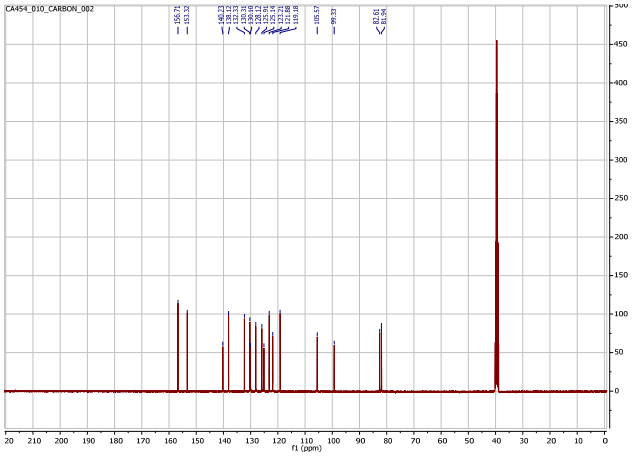






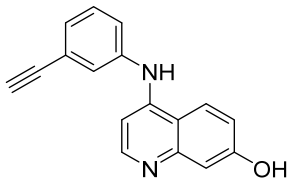


445



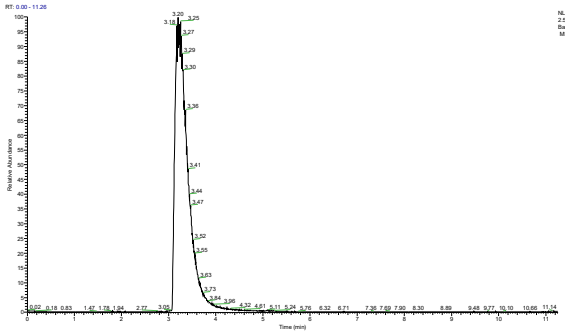
446

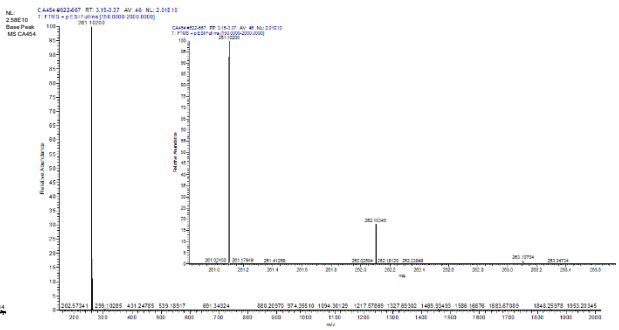
447 4-((3-ethynylphenyl)amino)quinolin-7-ol (**15**)



448

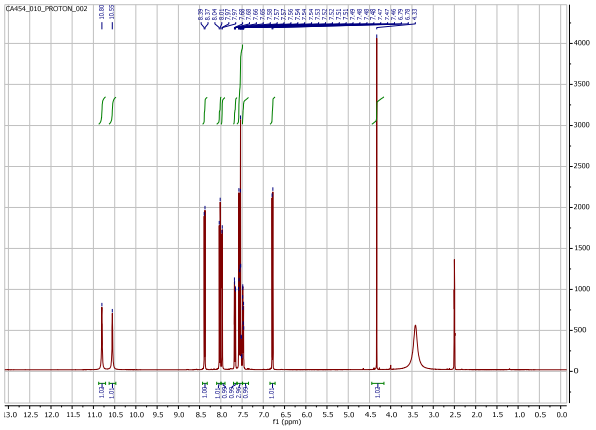
449



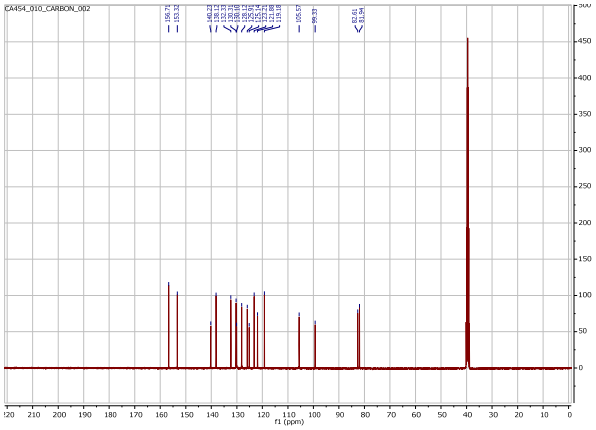


450

451



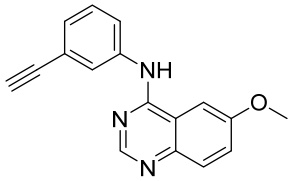
452



453

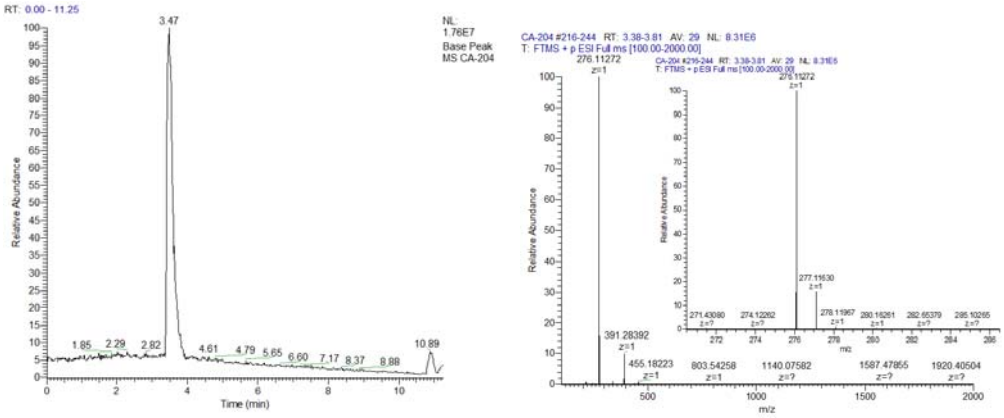
454

455 *N*-(3-ethynylphenyl)-6-methoxyquinazolin-4-amine (**16**)

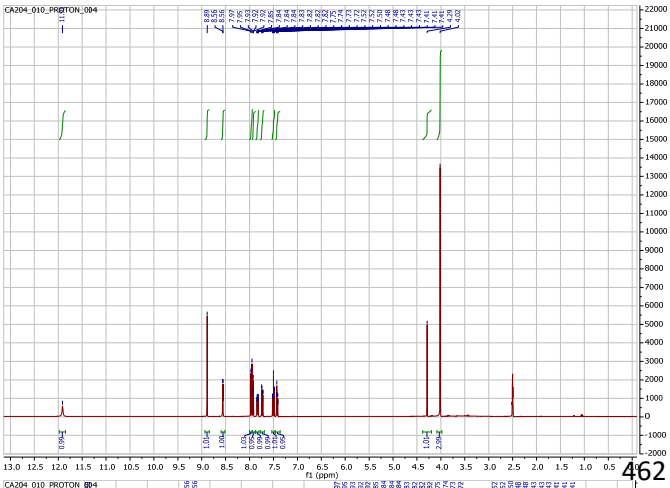


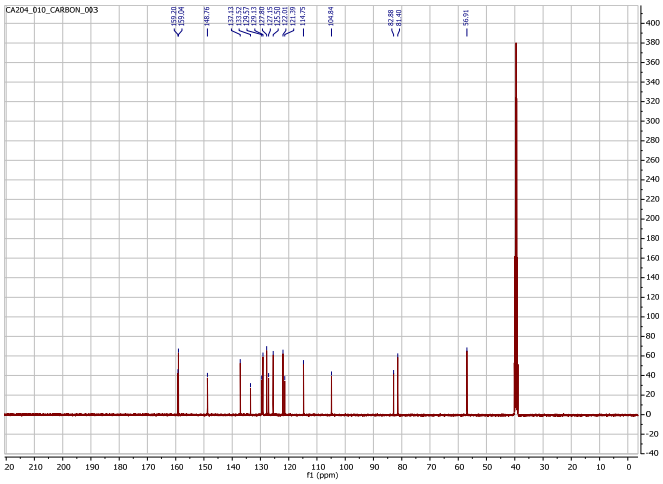
456

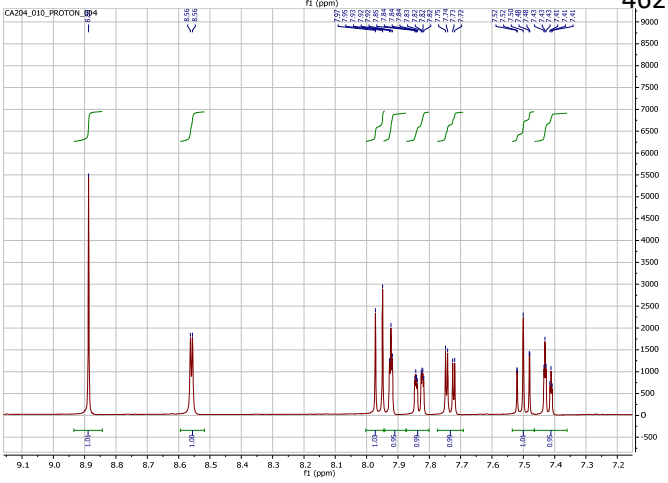
457

458 

459

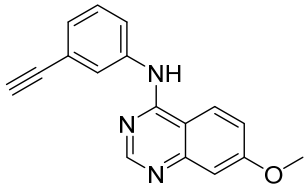
460 

462 

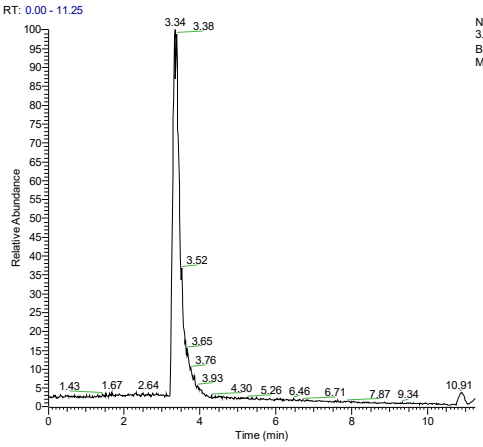
461 

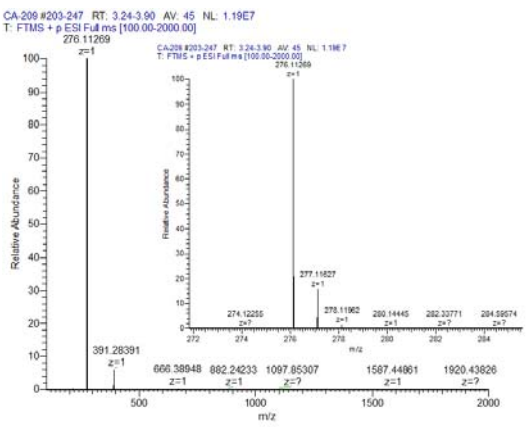
463

464 *N*-(3-ethynylphenyl)-7-methoxyquinazolin-4-amine (**17**)



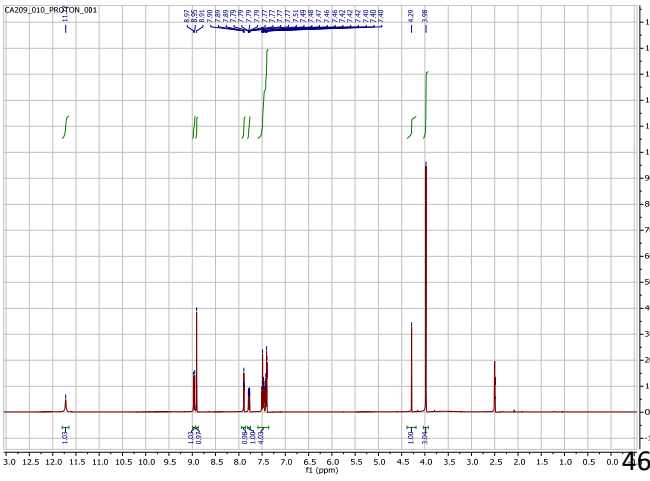
465



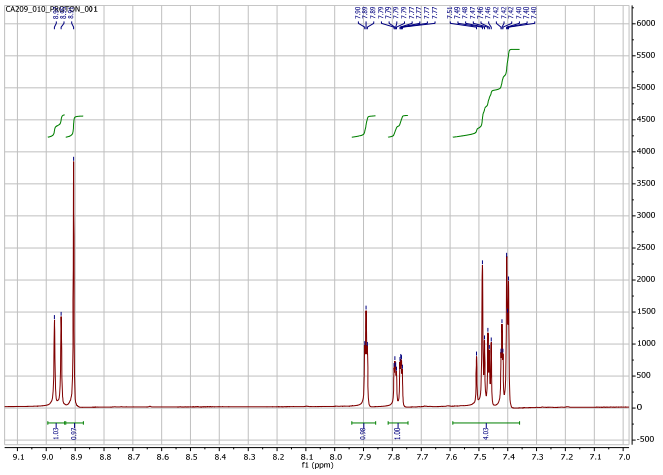


466

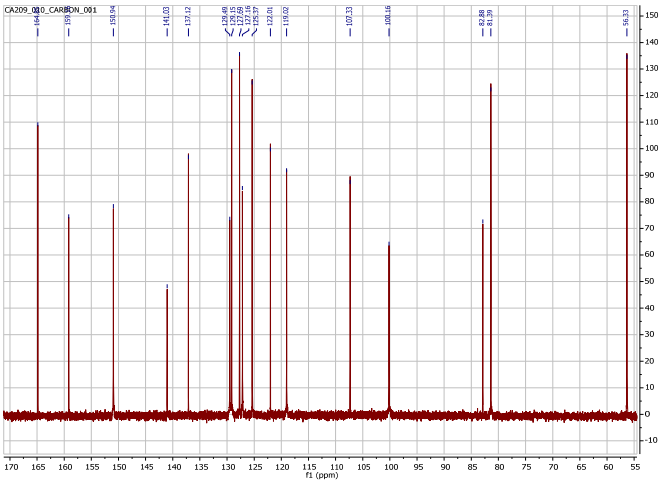
467



468

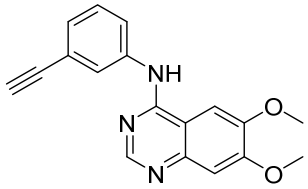


469

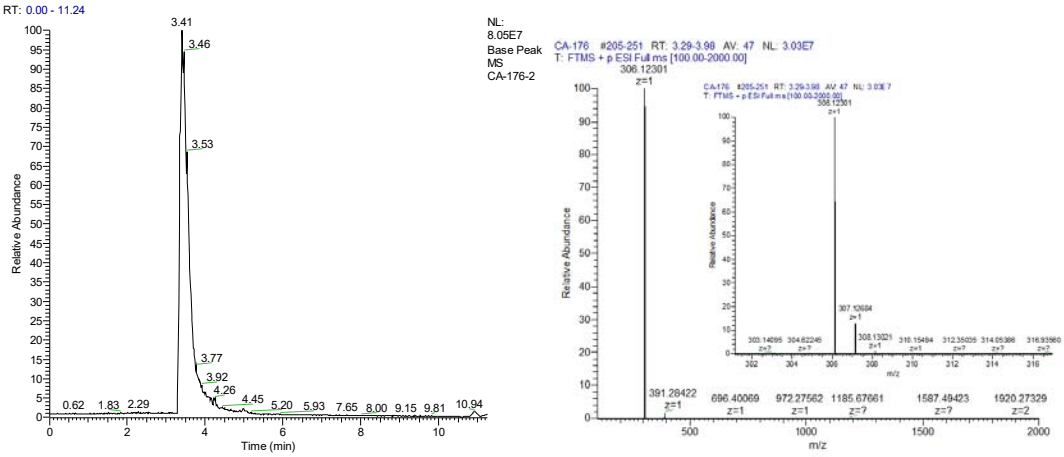


470

471 *N*-(3-ethynylphenyl)-6,7-dimethoxyquinazolin-4-amine (**18**)



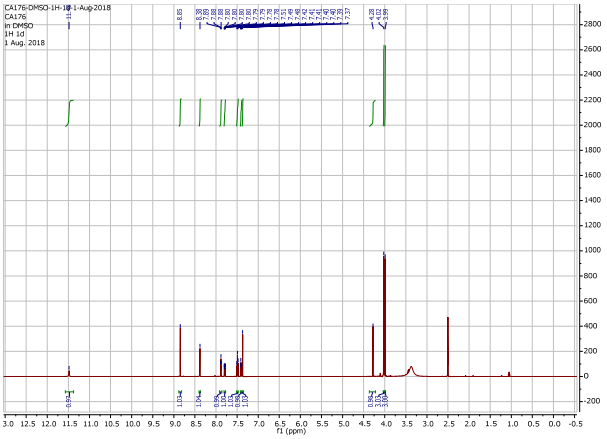
472



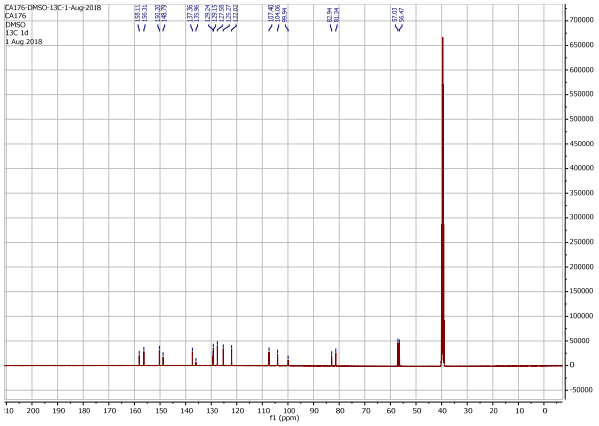
473

474

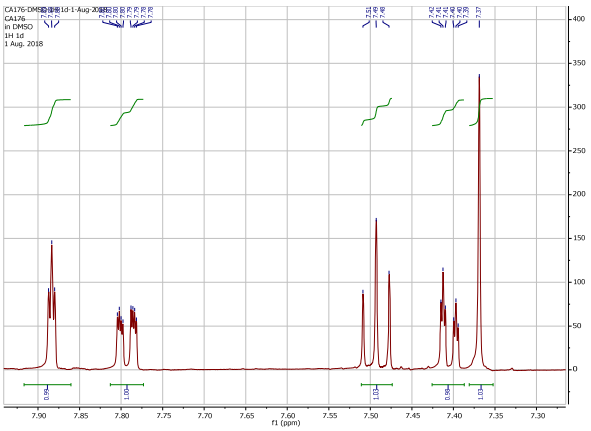
475



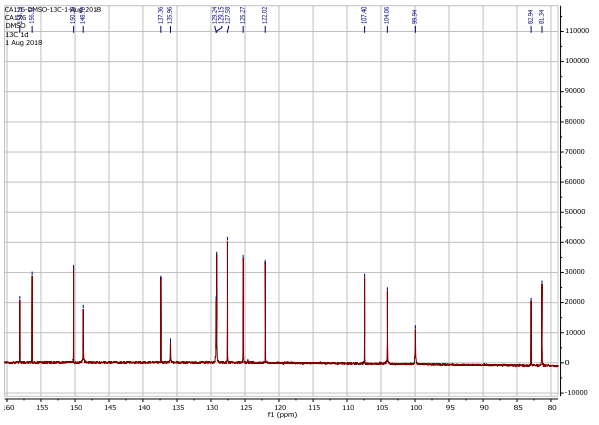
476



478



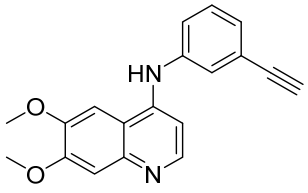
477



479

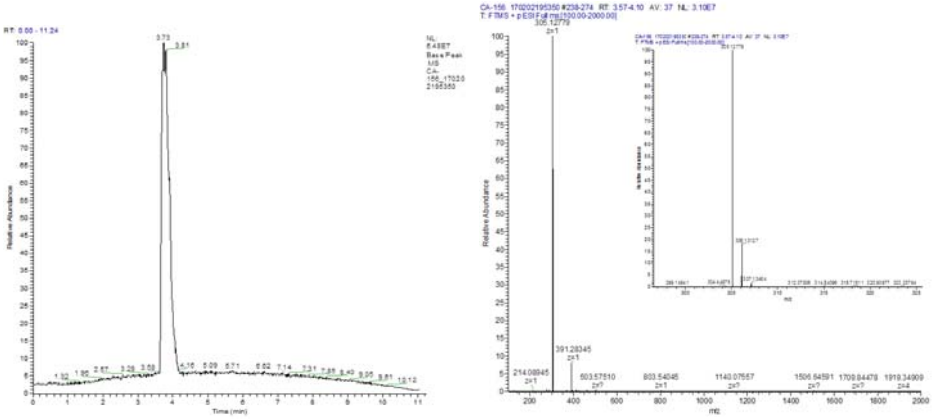
480

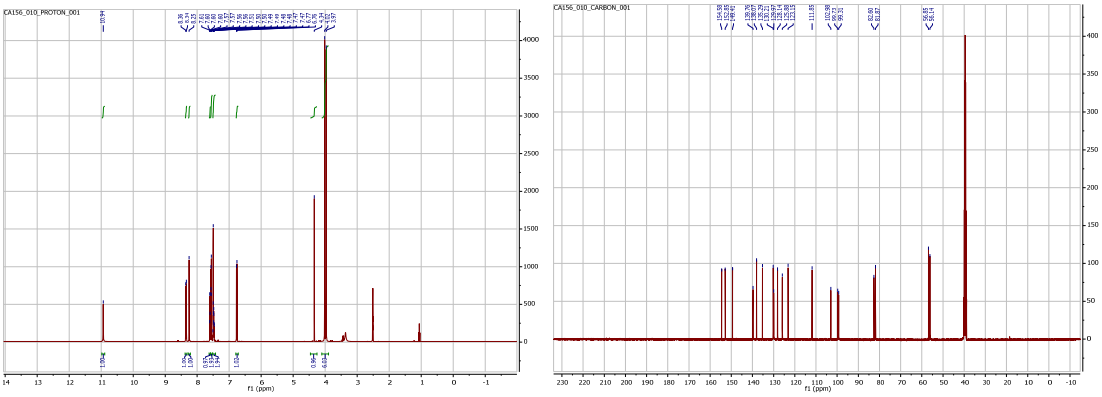
481 *N*-(3-Ethynylphenyl)-6,7-dimethoxyquinolin-4-amine (**19**)



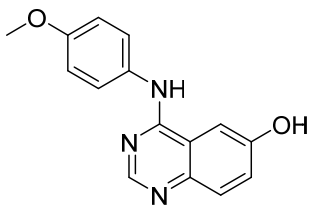
482

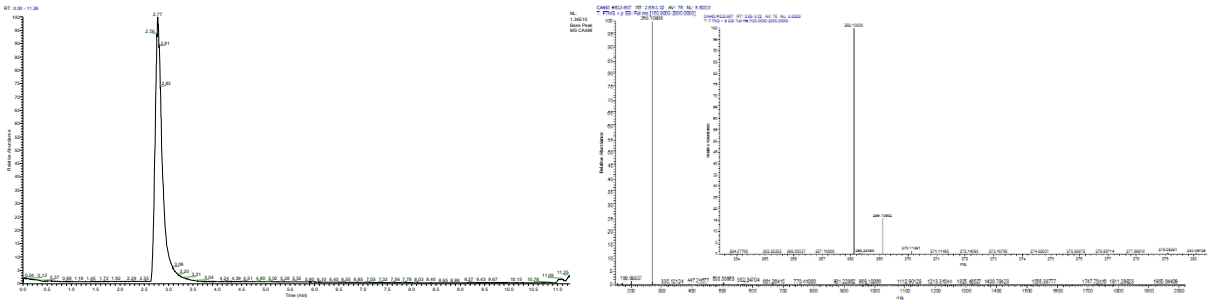
483



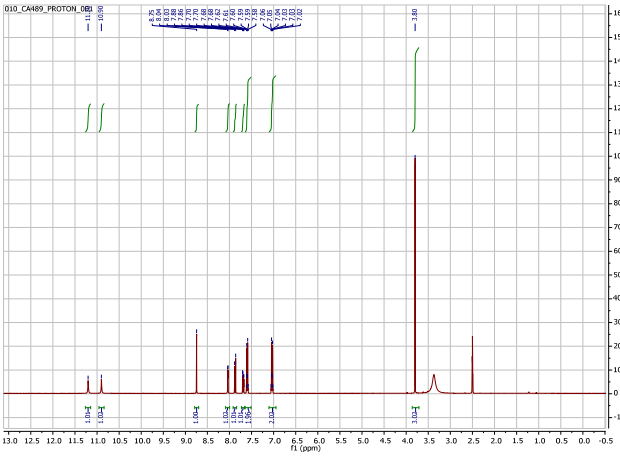


4-((4-methoxyphenyl)amino)quinazolin-6-ol (**20**)

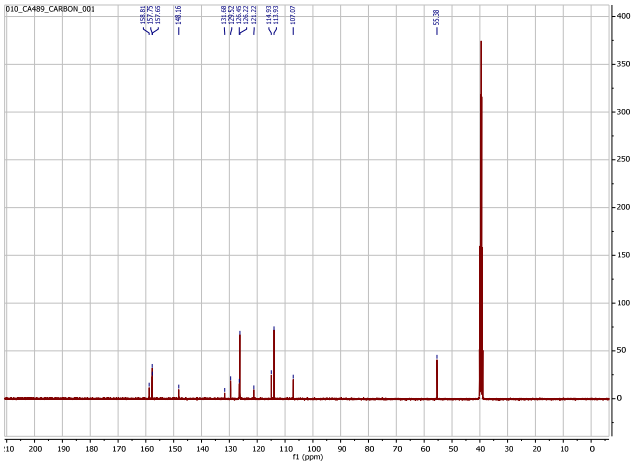




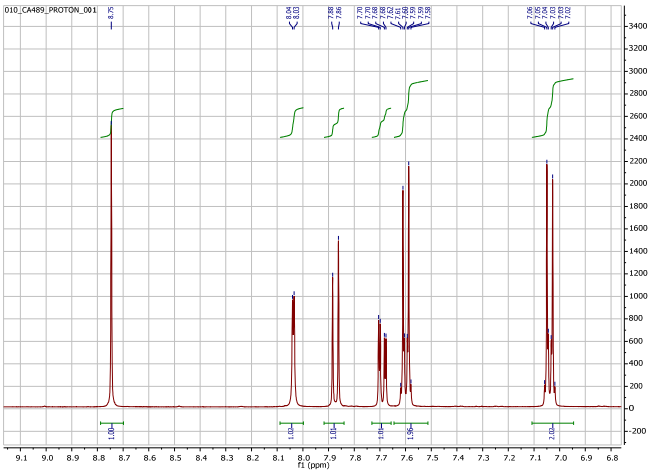
493



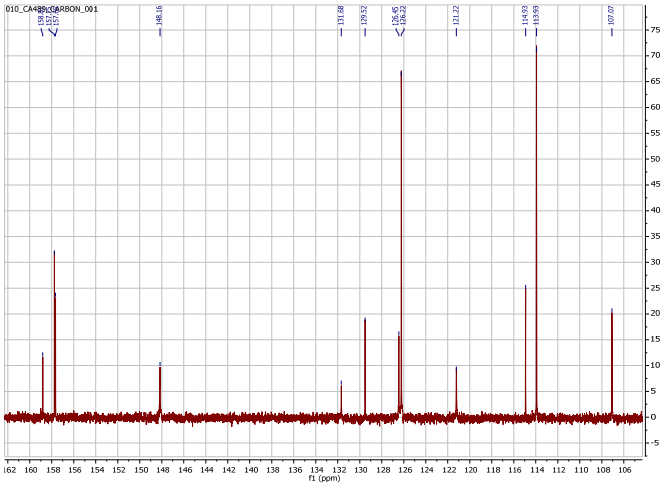
495



494



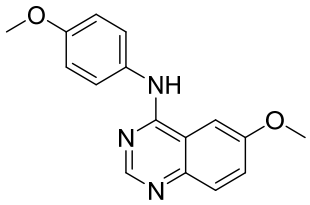
496



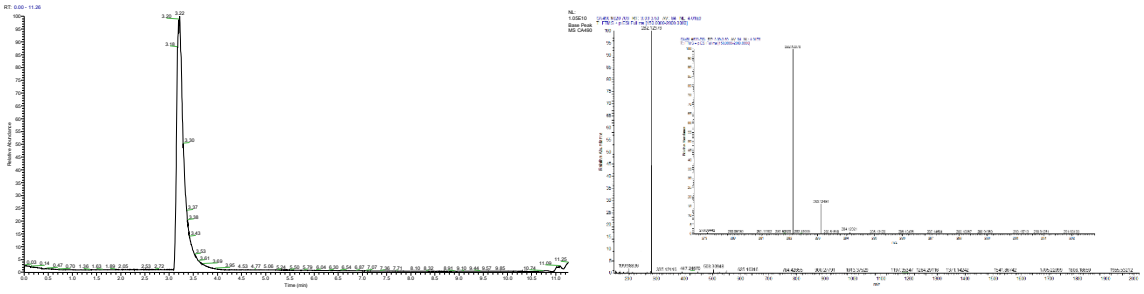
497

498 6-methoxy-*N*-(4-methoxyphenyl)quinazolin-4-amine (**21**)

499

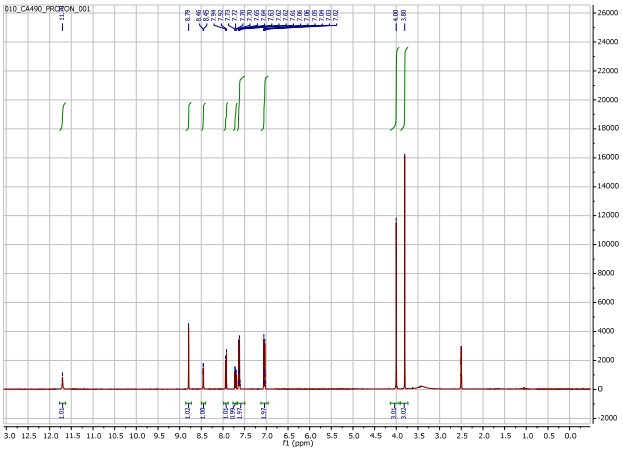


500

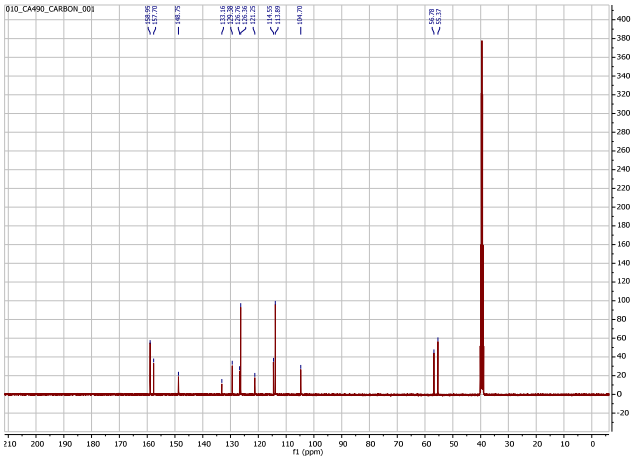


501

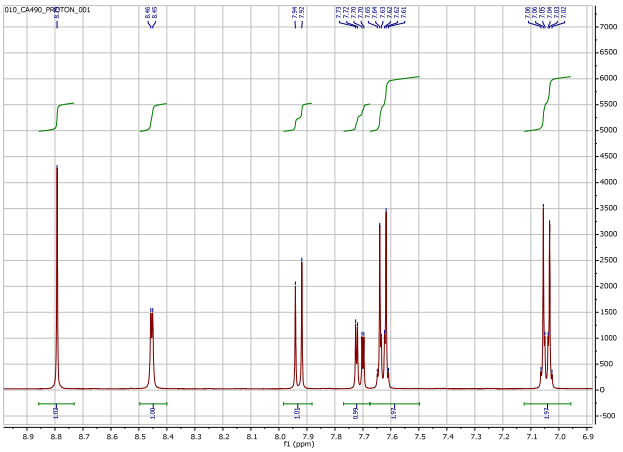
502



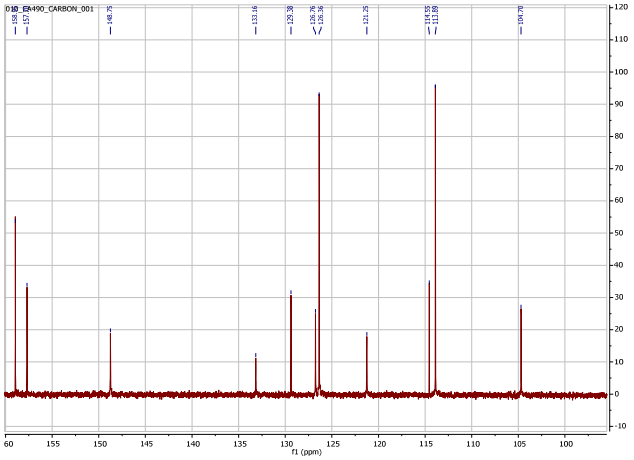
504



503

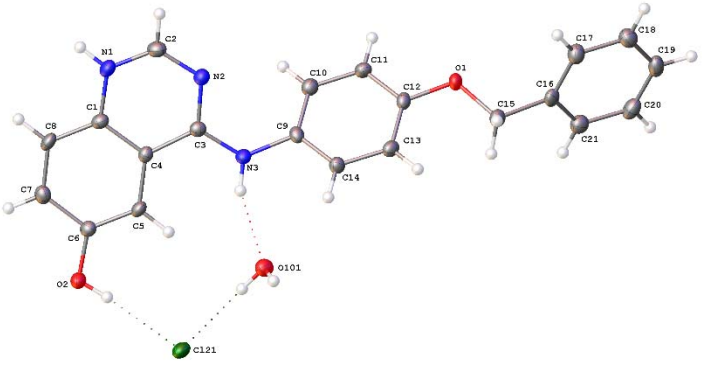


505



506 **Crystal Data and Experimental for GI261520A (2017ncs0878q)**

507



508

Experimental. Single yellow lath-shaped crystals of **2017ncs0878q** were recrystallised from DCM by slow evaporation. A suitable crystal 0.20×0.04×0.01 mm^3 was selected and mounted on a MITIGEN holder in perfluoroether oil on a Rigaku FRE+ equipped with VHF Varimax confocal mirrors and an AFC12 goniometer and HyPix 6000 detector. The crystal was kept at a steady T = 100(2) K during data collection. The structure was solved with the **ShelXT** (9, 10)structure solution program using the Intrinsic Phasing solution method and by using **Olex2** (11) as the graphical interface. The model was refined with version 2018/3 of **ShelXL** (9, 10) using Least Squares minimisation.

The crystallographic data for GI262866A has been deposited with the Cambridge Crystallographic Data Centre with the deposition number 1953813 and is freely available on request.

SUPPLENTARY MATERIAL REFERENCES

1. Fields PI, Swanson RV, Haidaris CG, Heffron F. 1986. Mutants of Salmonella typhimurium that cannot survive within the macrophage are avirulent. Proc Natl Acad Sci U S A 83:5189-93.
2. Garcia Vescovi E, Soncini FC, Groisman EA. 1996. Mg2+ as an extracellular signal: environmental regulation of Salmonella virulence. Cell 84:165-74.
3. Soncini FC, Garcia Vescovi E, Solomon F, Groisman EA. 1996. Molecular basis of the magnesium deprivation response in Salmonella typhimurium: identification of PhoP-regulated genes. J Bacteriol 178:5092-9.
4. Gibson MM, Ellis EM, Graeme-Cook KA, Higgins CF. 1987. OmpR and EnvZ are pleiotropic regulatory proteins: positive regulation of the tripeptide permease (tppB) of Salmonella typhimurium. Mol Gen Genet 207:120-9.
5. Pezza A, Pontel LB, Lopez C, Soncini FC. 2016. Compartment and signal-specific codependence in the transcriptional control of Salmonella periplasmic copper homeostasis. Proc Natl Acad Sci U S A 113:11573-11578.
6. Viarengo G, Sciara MI, Salazar MO, Kieffer PM, Furlan RL, Garcia Vescovi E. 2013. Unsaturated long chain free fatty acids are input signals of the Salmonella enterica PhoP/PhoQ regulatory system. J Biol Chem 288:22346-58.
7. Soncini FC, Vescovi EG, Groisman EA. 1995. Transcriptional autoregulation of the Salmonella typhimurium phoPQ operon. J Bacteriol 177:4364-71.
8. Castelli ME, Garcia Vescovi E, Soncini FC. 2000. The phosphatase activity is the target for Mg2+ regulation of the sensor protein PhoQ in Salmonella. J Biol Chem 275:22948-54.
9. Sheldrick GM. 2015. Crystal structure refinement with SHELXL. Acta Crystallogr C Struct Chem 71:3-8.
10. Sheldrick GM. 2015. SHELXT - integrated space-group and crystal-structure determination. Acta Crystallogr A Found Adv 71:3-8.
11. Dolomanov OV, Bourhis LJ, Gildea RJ, Howard JAK, Puschmann H. 2009. OLEX2: a complete structure solution, refinement and analysis program. Journal of Applied Crystallography doi:https://doi.org/10.1107/S0021889808042726.