Quinolin-2-one-pyrimidine hybrids acting as potent reversal agents on the P-gp-mediated multidrug resistance: insights into structure-activity relationships and the binding mode

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Table S1.

Sum of charge density values (∑ρ(r) atomic units) at the bond critical points between P-gp and ligands with different levels of activity. These values are presented as a function of the different transmembrane (TMH) domains of the receptor.

TMH	Compound						
	doxorubicin	tariquidar	**3**	**5a**	**5c**	**7b**	**8**
TMH 1	0.002593	0.008888	0.025958	0.004151	0.003587	0.002870	-
TMH 2	-	-	-	-	-	-	-
TMH 3	0.031275	-	0.015295	-	-	-	-
TMH 4	0.038774	0.042914	0.021078	0.043760	0.014509	0.054797	0.011676
TMH 5	0.015611	0.066937	0.012882	0.015997	0.038896	0.017987	0.018174
TMH 6	0.085523	0.085568	0.140271	0.077235	0.067866	0.063648	-
TMH 7	-	0.032670	-	0.018476	0.030696	0.025487	-
TMH 8	-	-	-	-	-	-	0.043847
TMH 9	-	-	-	-	-	-	0.055857

TMH 10	0.015653	0.002017	0.015958	0.000133	0.046388	0.000616	0.000587
TMH 11	-	-	0.022084	-	-	-	-
TMH 12	0.059860	0.029192	-	0.042835	0.041567	0.094637	0.030535
TOTAL	*0.249289*	*0.268186*	*0.253526*	*0.202587*	*0.243509*	*0.260042*	*0.160676*

TMH: transmembrane helice.

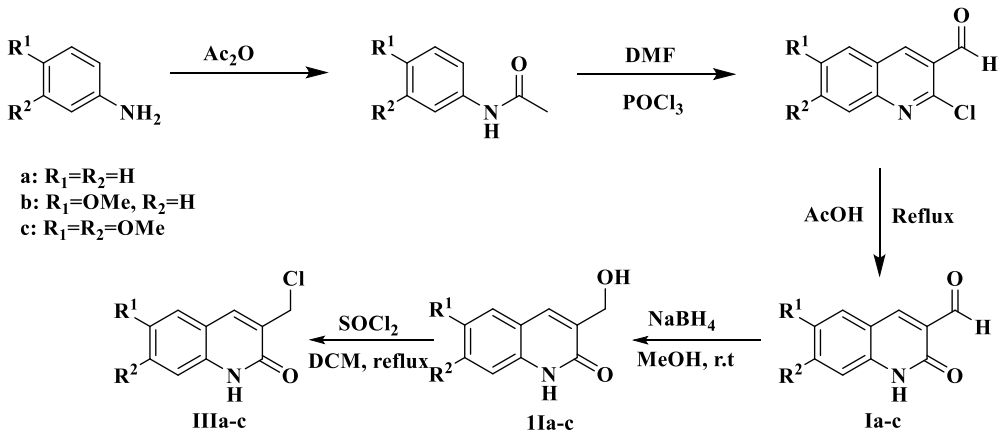
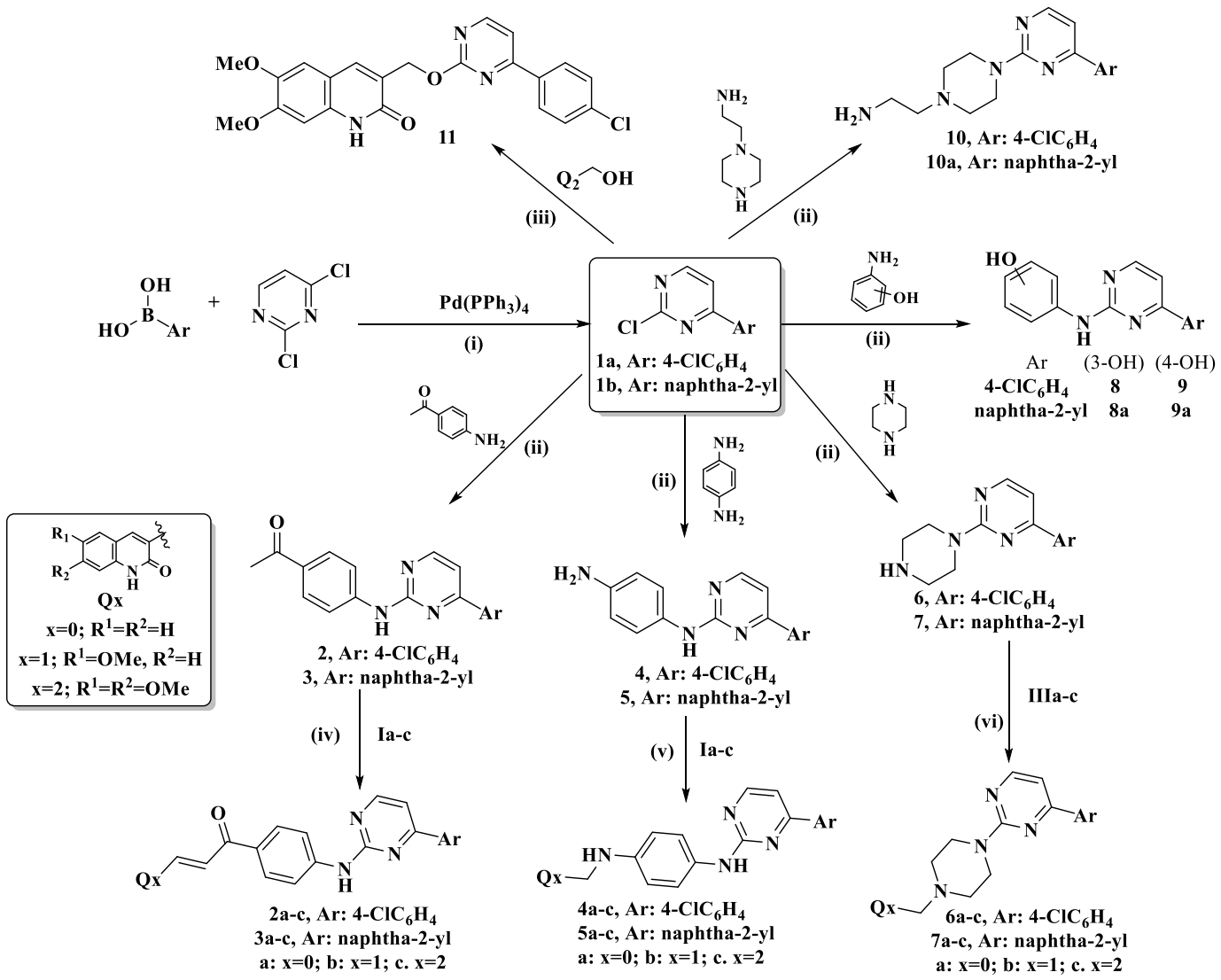


Figure S1. Synthesis of quinoline precursors **I-III**(**a-c**) [1].



Conditions: (i) K_2CO_3, acetonitrile, Δ; **(ii)** 1.4-dioxane, MW, 120 ºC**; (iii)** K_2CO_3, 1.4-dioxane, MW, 120 ºC**; (iv)** KOH, MeOH, reflux; **(v) a)** MeOH, AcOH (cat), 60 ºC, 2-12 h; **b)** addition portionwise of $NaBH_4$, RT; **(vi)** DMF/Et_3N, reflux

Figure S2. Synthesis of 2-substituted amino-4-arylpyrimidines **2-11** and **8a-10a** and their quinoline hybrids **2-7(a-c)** [2].

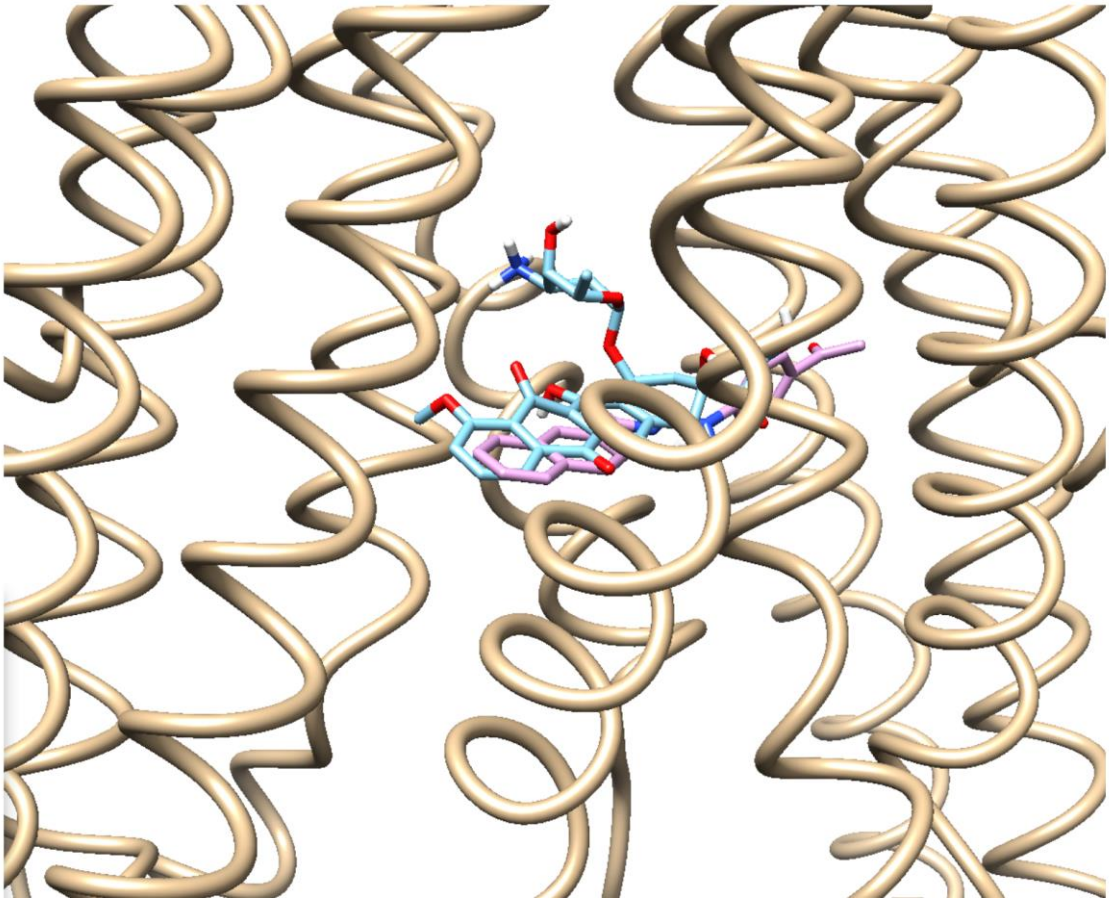


Figure S3. Superimposed structures of the most stable docked poses of doxorubicin (cyan) and compound **3** (pink).

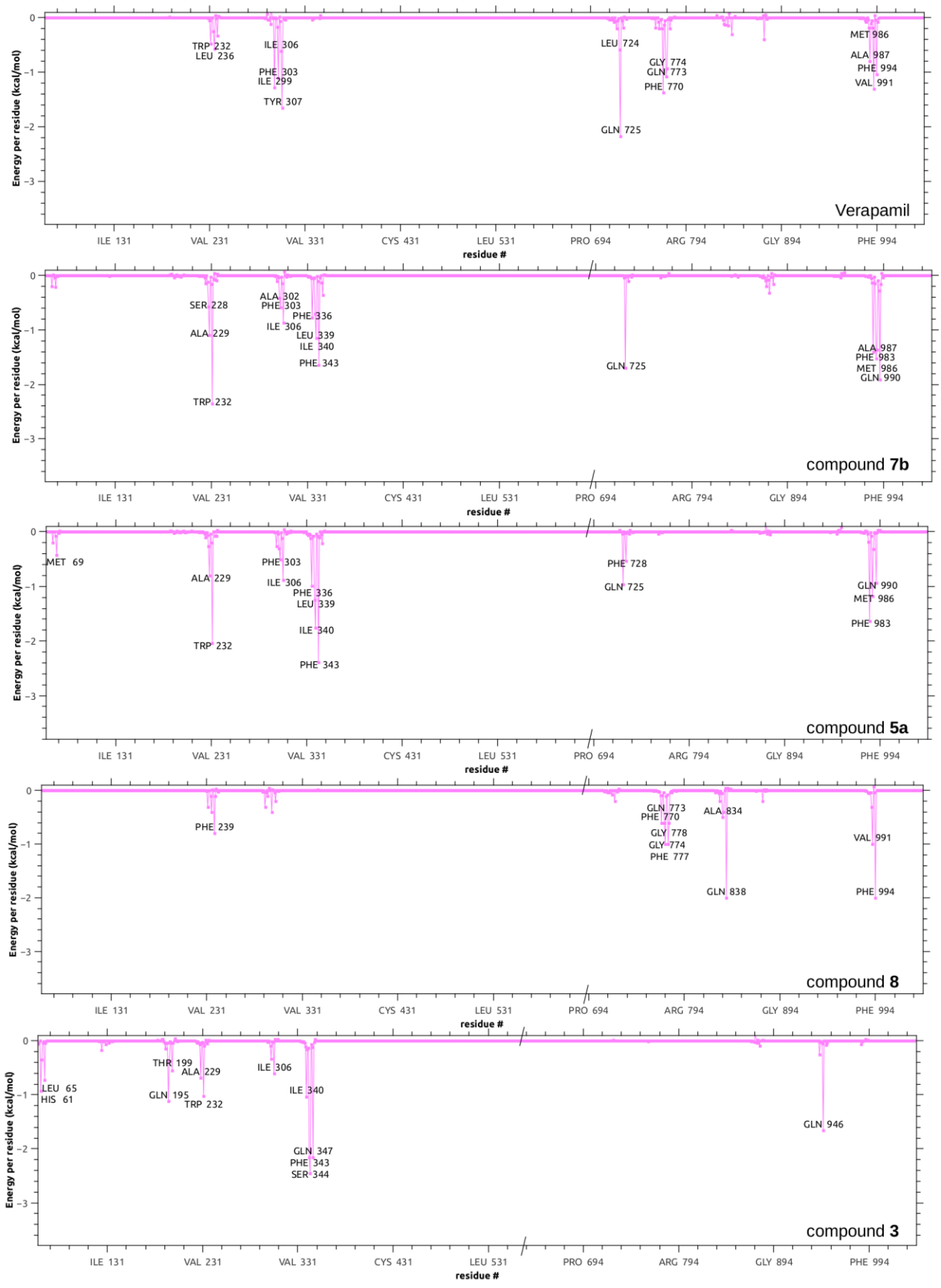


Figure S4. Energy decomposition on a per residue basis, same as main text in Fig. 8.

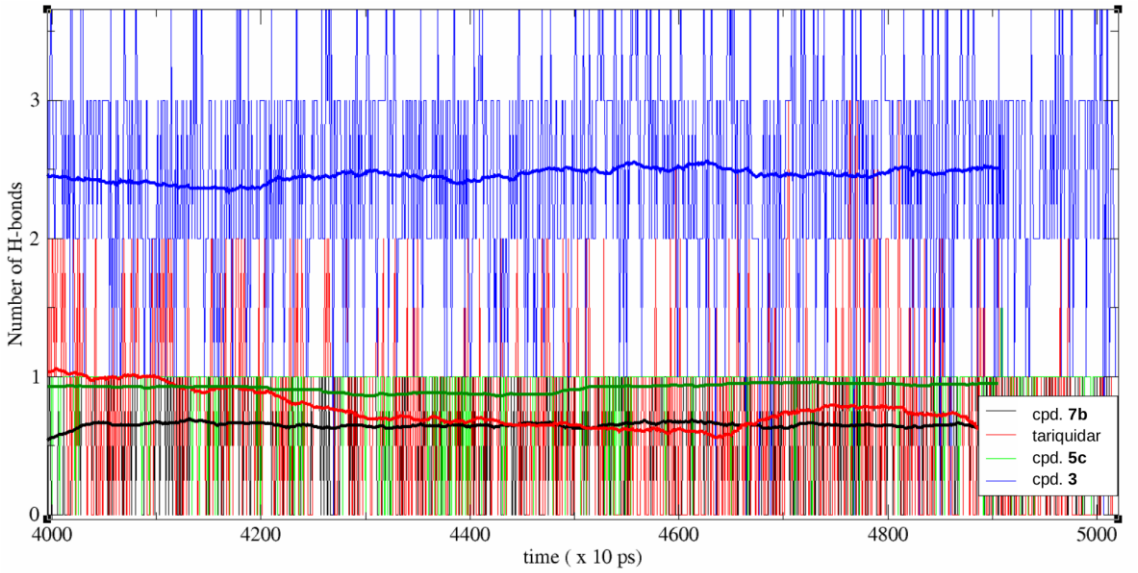
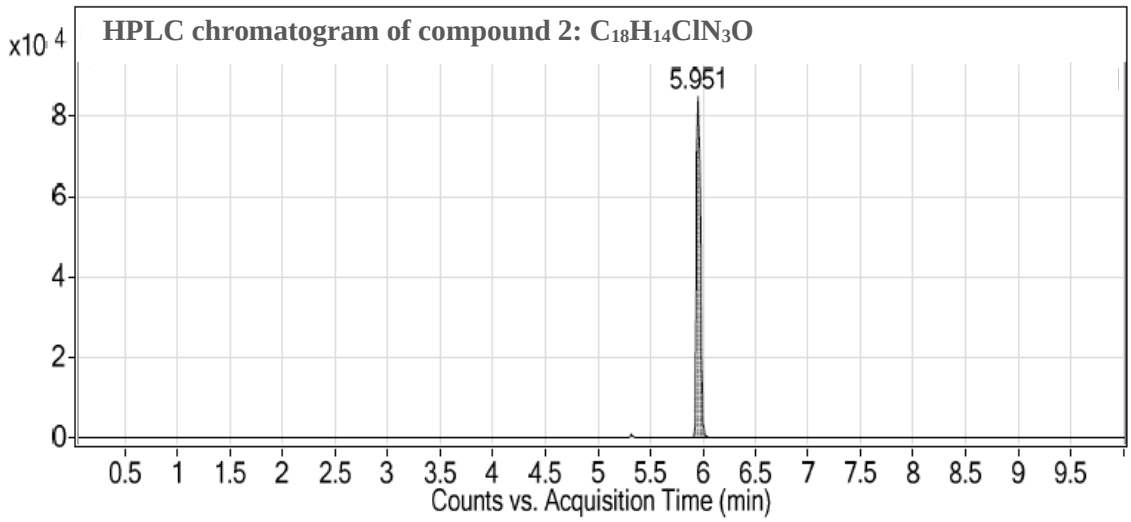


Figure S5. Number of H-bonds in time for tariquidar and the subject compounds **3**, **5c** and **7b**. Running average over 200 ps in bold lines.

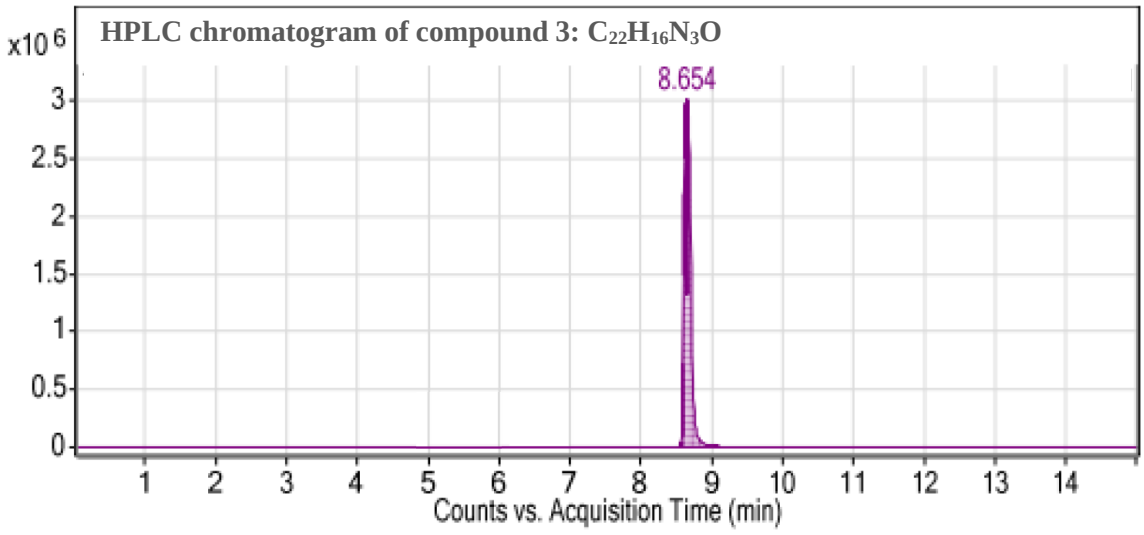
Spectroscopic Data.

Melting points were collected using a Brastead Electrothermal 9100 melting point apparatus, and the acquired data are uncorrected. Chemical shifts (δ) are given in ppm and coupling constants (J) are given in Hz. The following abbreviations are used for multiplicities: s = singlet, d = doublet, t = triplet, q= quartet, and m = multiplet. HPLC analysis was performed on an Agilent-1200 instrument equipped with a Agilent ZORBAX Extend-C18 (2.1 mm x 50 mm x 1.8 µm) PN 727700-902 column eluted with a gradient of CH_3CN/H_2O (10%, with 0.1% of formic acid) to CH_3CN (100% with 0.1% of formic acid) at a flow rate of 0.4 mL/min. High-resolution mass spectra (HRMS) were recorded on an Agilent Technologies QTOF 6520B spectrometer via an electrospray ionization (ESI).

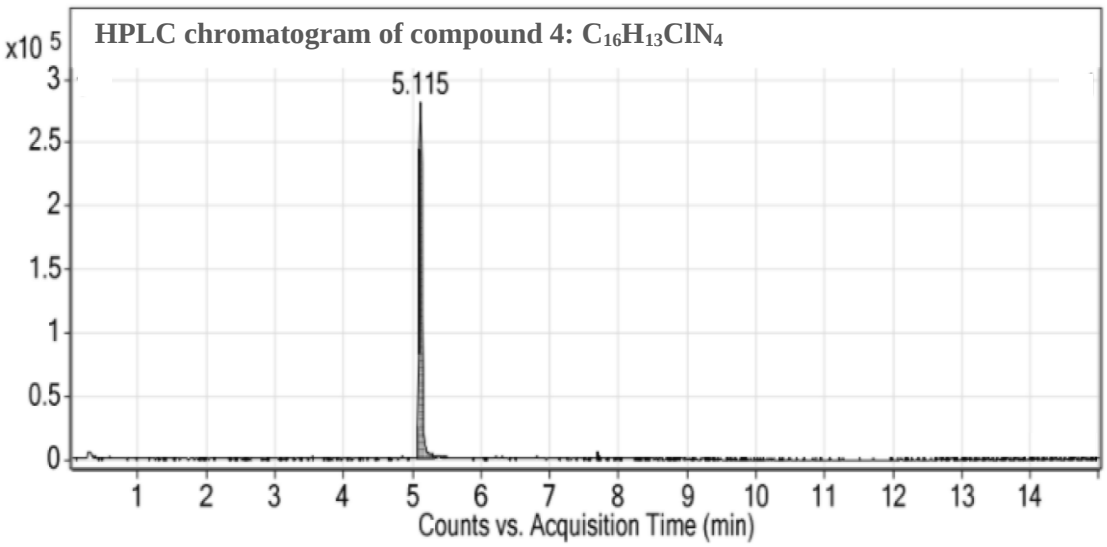
1-(4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenyl)ethan-1-one (**2**). Yellow solid, mp: 201-202 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.49 (s, 3H, CH_3), 7.49 (d, J = 5.3 Hz, 1H, CH), 7.60 (d, J = 8.8 Hz, 2H, H-Ar), 7.91 (d, J = 9.1 Hz, 1H, H-Ar), 7.91 (d, J = 9.1 Hz, 1H, H-Ar), 8.18 (d, J = 8.8 Hz, 2H, H-Ar), 8.61 (d, J = 5.2 Hz, 1H, CH), 10.13 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{18}H_{14}ClN_3O$, 324.0904; found, 324.0897.



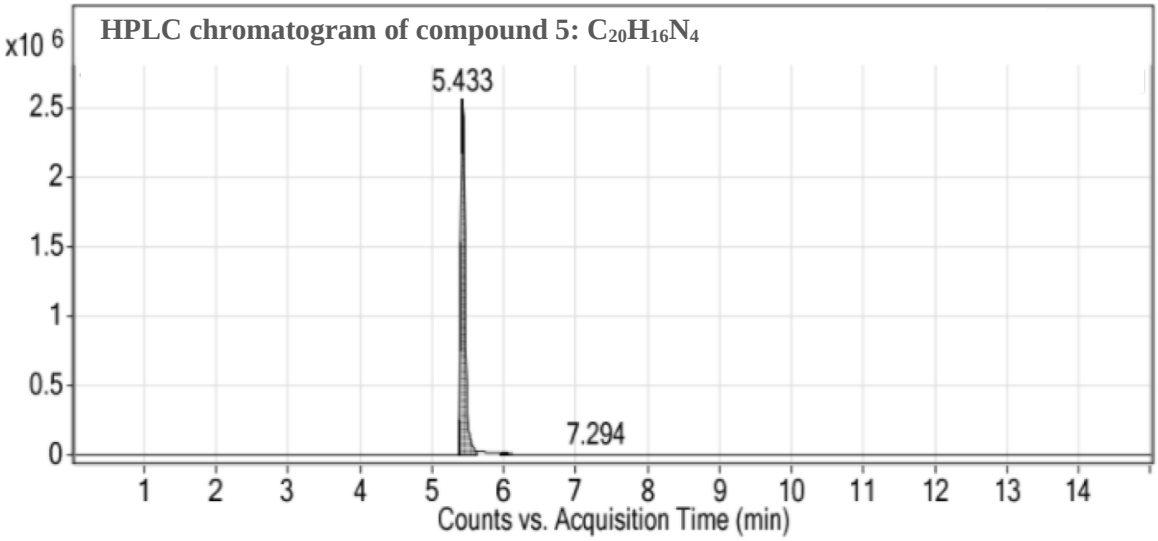
1-(4-((4-(Naphthalen-2-yl)pyrimidin-2-yl)amino)phenyl)ethenone (**3**). Yellow solid, mp: 130-131 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.53 (s, 3H, CH_3), 7.62 - 7.56 (m, 2H), 7.65 (d, J = 5.2 Hz, 1H), 8.15 - 7.89 (m, 7H), 8.30 (dd, J = 8.6, 1.7 Hz, 1H), 8.66 (d, J = 5.2 Hz, 1H), 8.78 (s, 1H), 10.18 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{22}H_{16}N_3O$, 340.1450 found, 340.1442.



N^{1}*-(4-(4-Chlorophenyl)pyrimidin-2-yl)benzene-1,4-diamine* (**4**). Yellow solid, mp: 161 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.76 (s, 2H, NH_2), 6.55 (d, J = 8.6 Hz, 2H, H-Ar), 7.23 (d, J = 5.1 Hz, 1H, CH), 7.37 (d, J = 8.6 Hz, 2H, H-Ar), 7.58 (d, J = 8.5 Hz, 2H, H-Ar), 8.12 (d, J = 8.5 Hz, 2H, H-Ar), 8.43 (d, J = 5.1 Hz, 1H, CH), 9.14 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{16}H_{13}ClN_4$, 297.0906; found, 297.0902.

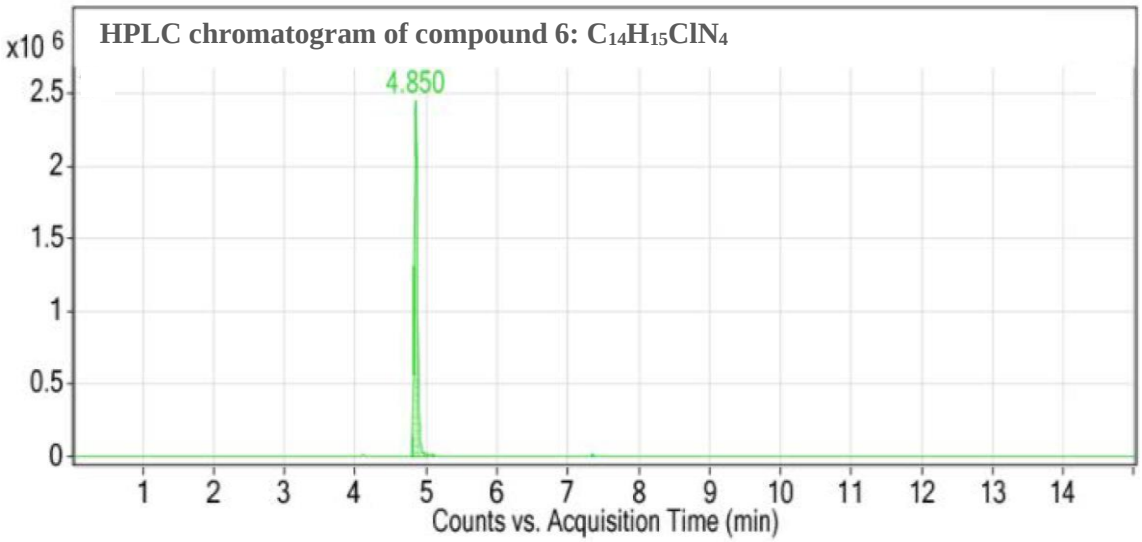


N[1]*-(4-(naphthalen-2-yl)pyrimidin-2-yl)benzene-1,4-diamine* (**5**). Yellow solid, mp: 130-131 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.76 (s, 2H, NH_2), 6.58 (d, J = 8.8 Hz, 2H, H-Ar), 7.39 (d, J = 5.2 Hz, 1H, CH), 7.44 (d, J = 8.8 Hz, 2H, H-Ar), 7.68 - 7.52 (m, 2H), 8.00 - 7.93 (m, 1H), 8.09 - 8.00 (m, 2H), 8.24 (dd, J = 8.6, 1.8 Hz, 1H, CH), 8.47 (d, J = 5.2 Hz, 1H, CH), 8.70 (d, J = 1.2 Hz, 1H, CH), 9.18 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{20}H_{16}N_4$, 313,1453; found, 313,1446.

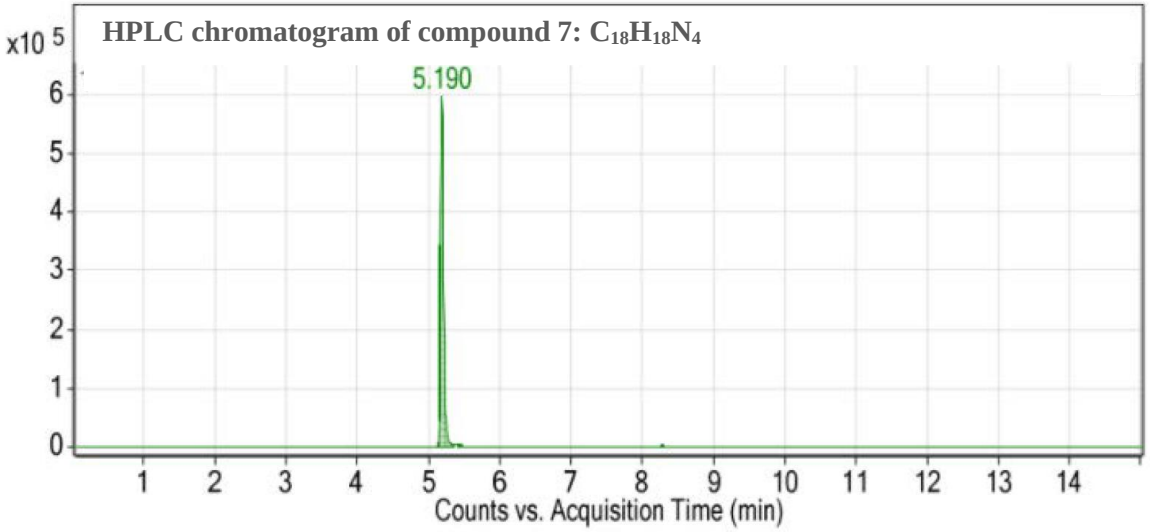


4-(4-chlorophenyl)-2-(piperazin-1-yl)pyrimidine (**6**). White solid, mp: 89-90 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.28 - 2.74 (m, 4H), 4.17 - 3.73 (m, 4H), 6.88 (d, J = 5.1 Hz, 1H, CH), 7.42 (d, J = 8.7 Hz, 2H, H-Ar), 7.98 (d, J = 8.7 Hz, 2H, H-Ar), 8.37 (d, J = 5.1

Hz, 1H, CH), NH not detected. ESI-QTOF (positive ionization) M+H calcd. for $C_{14}H_{15}ClN_4$, 275.1063; found, 275.1058.

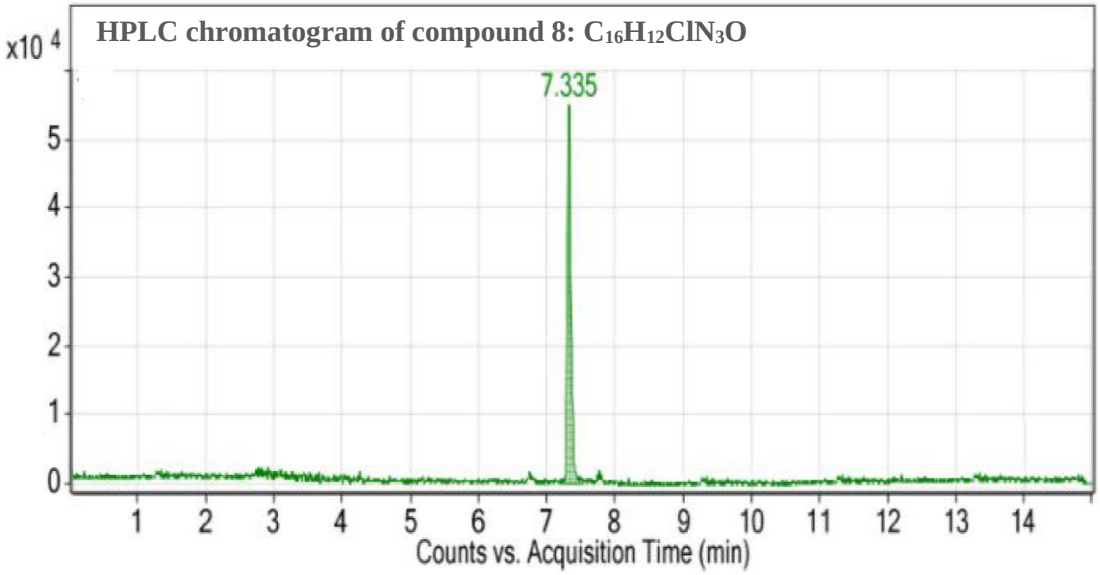


4-(naphthalen-2-yl)-2-(piperazin-1-yl)pyrimidine (**7**). White solid, mp: 112 - 113 C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.05 (br, 4H, CH_2), 4.00 (br, 4H, CH_2), 7.11 (d, J = 5.1 Hz, 1H, CH), 7.71 - 7.45 (m, 2H), 8.06 - 7.86 (m, 3H), 8.19 (dd, J = 8.5, 1.4 Hz, 1H, CH), 8.44 (d, J = 5.0 Hz, 1H, CH), 8.56 (s, 1H, CH), NH not detected. ESI-QTOF (positive ionization) M+H calcd. for $C_{18}H_{18}N_4$, 291.1610; found, 291.1604.

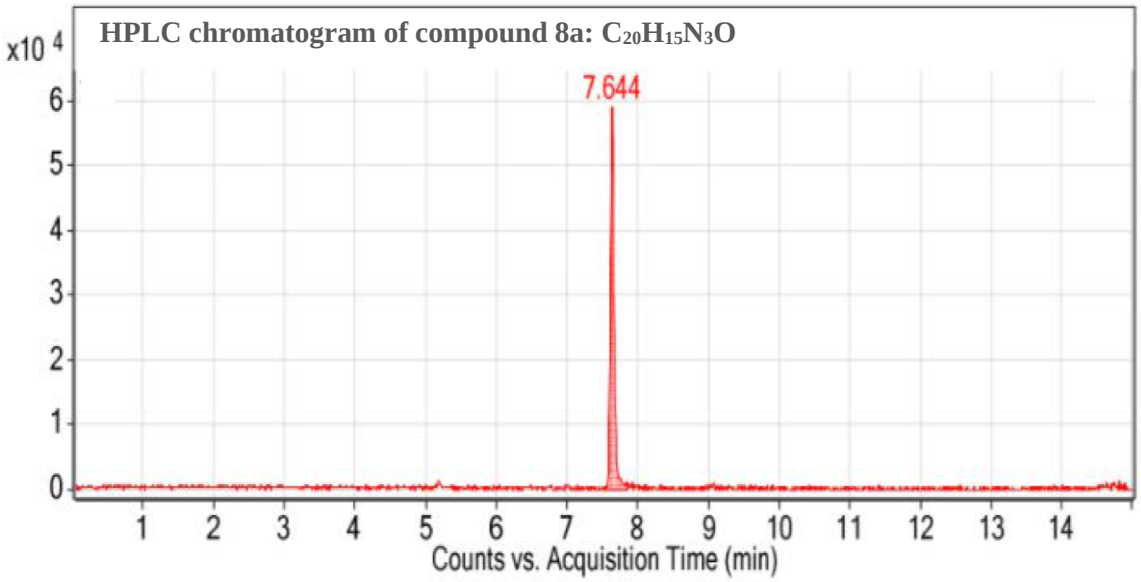


3-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenol (**8**). Beige solid. 83% yield, mp: 162 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.06 (t, J = 8.1 Hz, 1H, CH), 7.21 (ddd, J = 8.1,

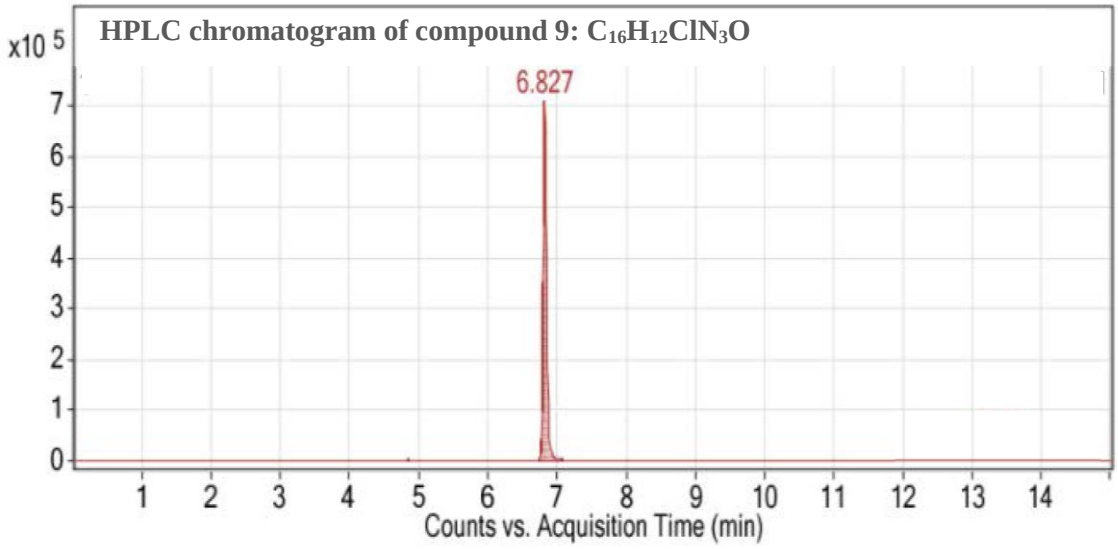
1.9, 0.8 Hz, 1H, CH), 7.36 (t, J = 2.2 Hz, 1H, CH), 7.38 (d, J = 5.2 Hz, 1H, CH), 7.60 (d, J = 8.7 Hz, 2H, H-Ar), 7.60 (d, J = 8.7 Hz, 2H, H-Ar), 8.54 (d, J = 5.2 Hz, 1H, CH), 9.25 (s, 1H, CH), 9.56 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{16}H_{12}ClN_3O$, 298.0747; found, 298.0743.



3-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenol (**8a**). Beige solid, mp: 158 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.41 (dd, J = 7.9, 1.3 Hz, 1H, CH), 7.10 (t, J = 8.0 Hz, 1H, CH), 7.28 (d, J = 7.9 Hz, 1H, CH), 7.45 (s, 1H, CH), 7.53 (d, J = 5.2 Hz, 1H, CH), 7.70 - 7.55 (m, 2H), 8.02 – 7.92 (m, 1H, CH), 8.06 (d, J = 8.3 Hz, 2H), 8.29 (d, J = 8.5 Hz, 1H, CH), 8.57 (d, J = 5.2 Hz, 1H, CH), 8.76 (s, 1H, CH), 9.29 (s, 1H, NH), 9.59 (s, 1H, OH). ESI-QTOF (positive ionization) M+H calcd. for $C_{20}H_{15}N_3O$, 314.1293; found, 314.1287.

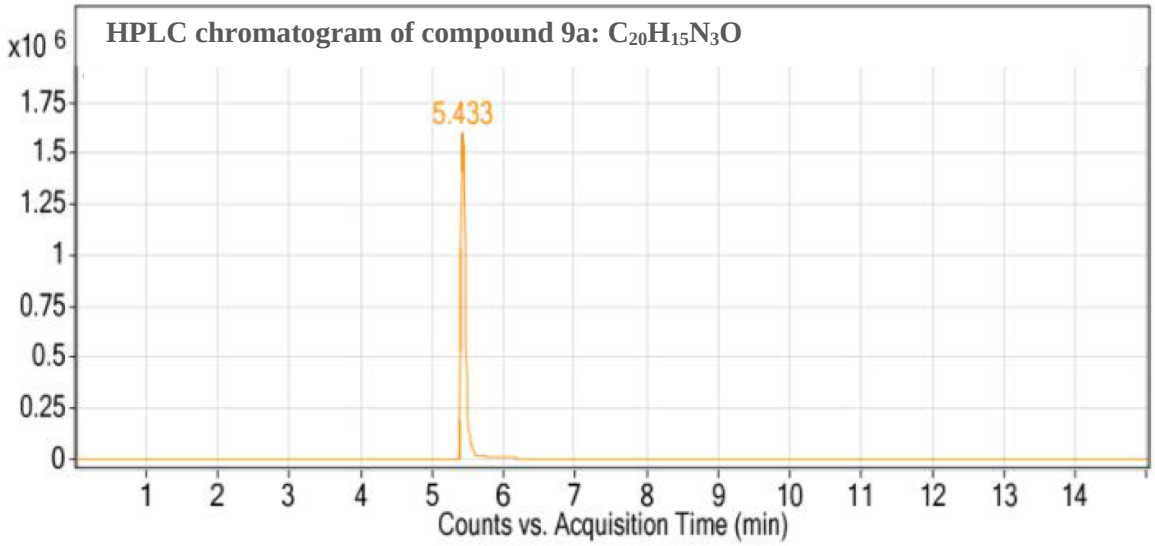


4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenol (**9**). Beige solid, mp: 152 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.02 (s, OH), 6.81 (d, J = 8.8 Hz, 2H, H-Ar), 7.06 (d, J = 5.3 Hz, 1H, CH), 7.15 (s, 1H, NH), 7.49 - 7.36 (m, 4H, H-Ar), 7.97 (d, J = 8.7 Hz, 2H, H-Ar), 8.41 (d, J = 5.3 Hz, 1H, CH). ESI-QTOF (positive ionization) M+H calcd. for $C_{16}H_{12}ClN_3O$, 298.0747; found, 298.0742.

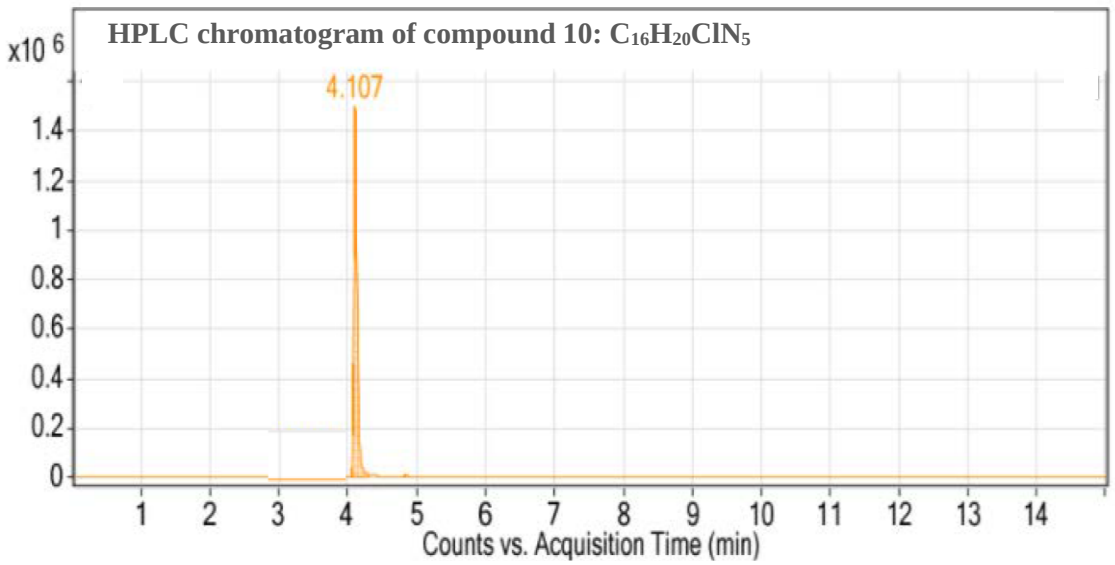


4-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenol (**9a**). Beige solid, mp: 187°C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.75 (d, J = 8.2 Hz, 2H, H-Ar), 7.45 (d, J = 5.2 Hz, 1H, CH), 7.67 - 7.52 (m, 4H), 8.01 - 7.91 (m, 1H, CH), 8.05 (d, J = 8.2 Hz, 2H, H-Ar), 8.25

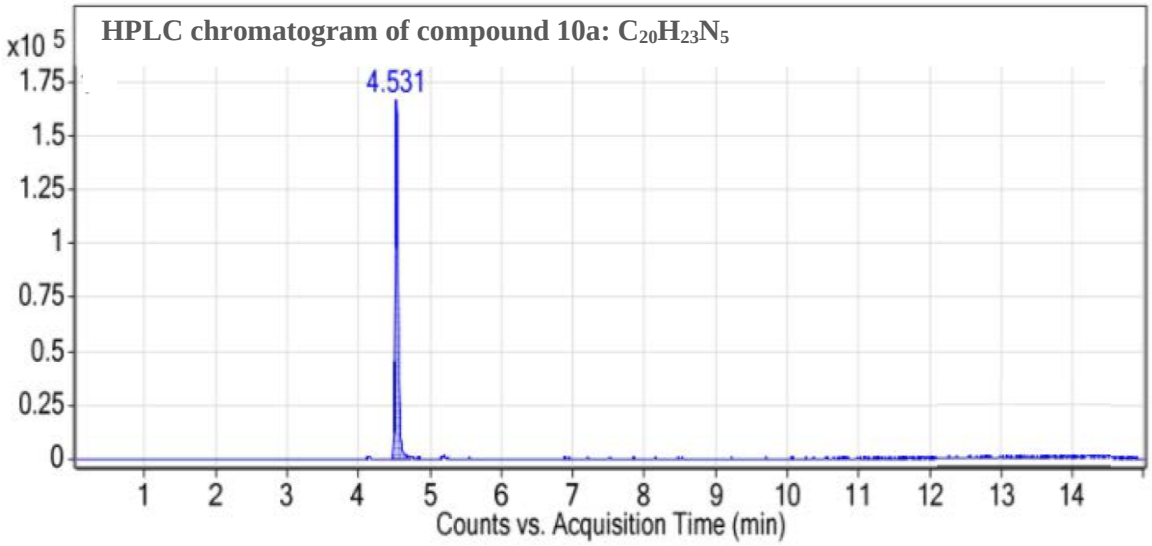
(dd, J = 8.7, 1.8 Hz, 1H, CH), 8.50 (d, J = 5.2 Hz, 1H, CH), 8.71 (s, 1H, CH), 9.04 (s, 1H, NH), 9.35 (s, 1H, OH). ESI-QTOF (positive ionization) M+H calcd. for $C_{20}H_{15}N_3O$, 314.1293; found, 314.1288.



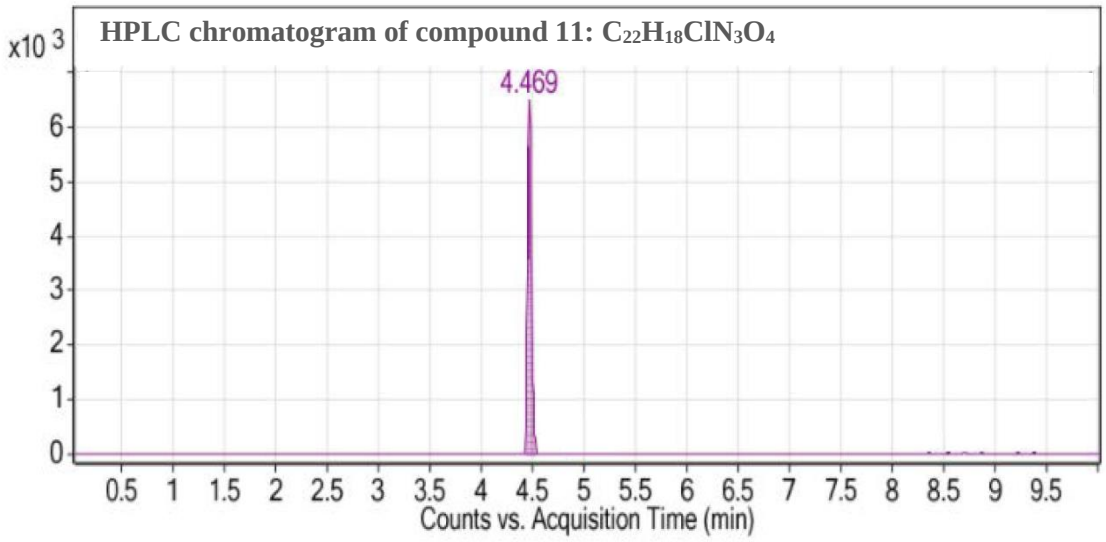
2-(4-(4-(4-chlorophenyl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-amine (**10**). White solid, mp: 93 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.68 (s, 2H, NH_2), 2.49 (t, J = 6.1 Hz, 2H, CH_2), 2.65 – 2.52 (m, 4H, CH_2), 2.86 (t, J = 6.1 Hz, 2H, CH_2), 4.09 - 3.82 (m, 4H, CH_2), 6.88 (d, J = 5.1 Hz, 1H, CH), 7.43 (d, J = 8.6 Hz, 2H, H-Ar), 7.98 (d, J = 8.6 Hz, 2H, H-Ar), 8.37 (d, J = 5.1 Hz, 1H, CH). ESI-QTOF (positive ionization) M+H calcd. for $C_{16}H_{20}ClN_5$, 318,1485; found, 318,1480.



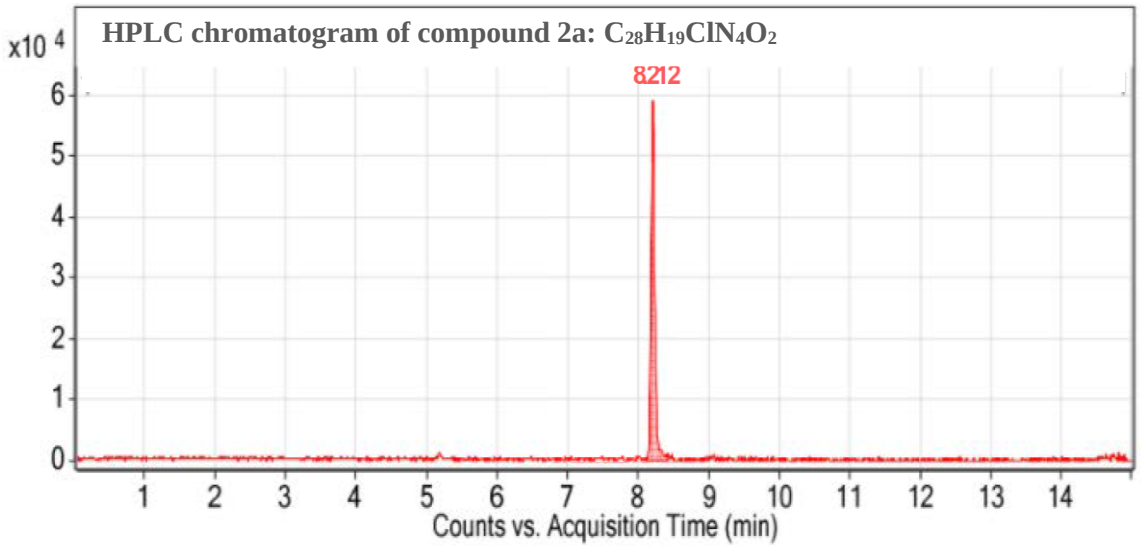
2-(4-(4-(naphthalen-2-yl)pyrimidin-2-yl)piperazin-1-yl)ethan-1-amine (**10a**). White solid, mp: 107 C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63 (s, 2H, NH_2), 2.50 (t, J = 6.1 Hz, 2H, CH_2), 2.64 - 2.53 (m, 4H, CH_2), 2.86 (t, J = 6.1 Hz, 2H, CH_2), 4.06 - 3.88 (m, 4H, CH_2), 7.07 (d, J = 5.2 Hz, 1H, CH), 7.57 - 7.47 (m, 2H), 7.89 - 7.84 (m, 1H, CH), 7.92 (d, J = 8.7 Hz, 1H, CH), 7.99 - 7.93 (m, 1H, CH), 8.16 (dd, J = 8.6, 1.8 Hz, 1H, CH), 8.41 (d, J = 5.2 Hz, 1H, CH), 8.52 (d, J = 1.2 Hz, 1H, CH). ESI-QTOF (positive ionization) M+H calcd. for $C_{20}H_{23}N_5$, 334.2032; found, 334.2025.



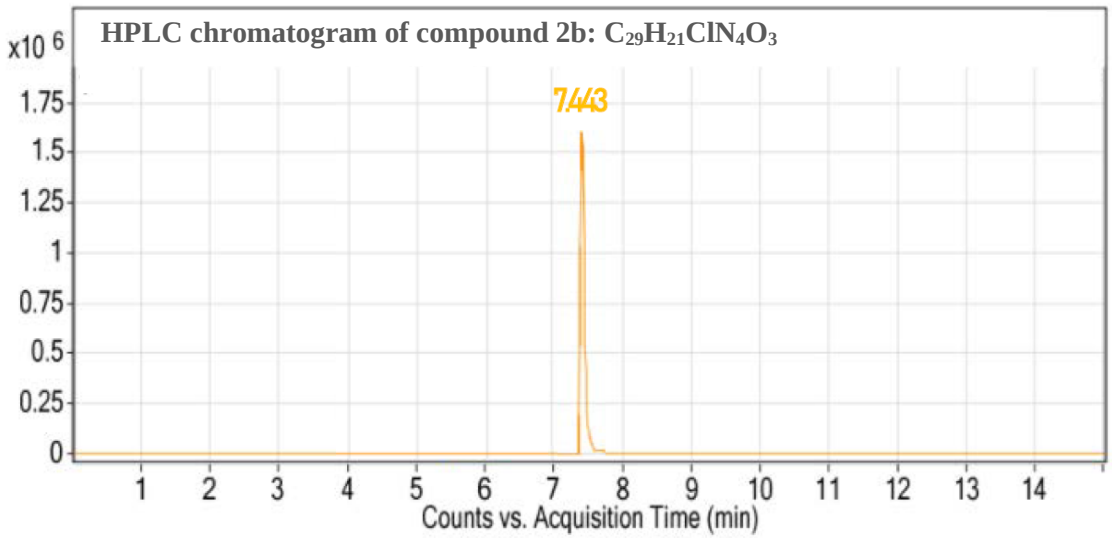
3-(((4-(4-chlorophenyl)pyrimidin-2-yl)oxy)methyl)-6,7-dimethoxyquinolin-2(1H)-one (**11**)**.** Beige solid, mp: 206 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.11 (s, 2H, OCH_2), 6.95 (s, 1H, Quin-H), 7.18 (s, 1H, Quin-H), 7.27 (d, J = 3.5 Hz, 1H, CH), 7.57 (d, J = 8.6 Hz, 2H, Ar-H), 8.07 (s, 1H, Quin-H), 8.16 (d, J = 8.5 Hz, 2H, Ar-H), 8.48 (d, J = 4.9 Hz, 1H, CH), 12.07 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{22}H_{18}ClN_3O_4$, 424,1064; found, 424.1058.



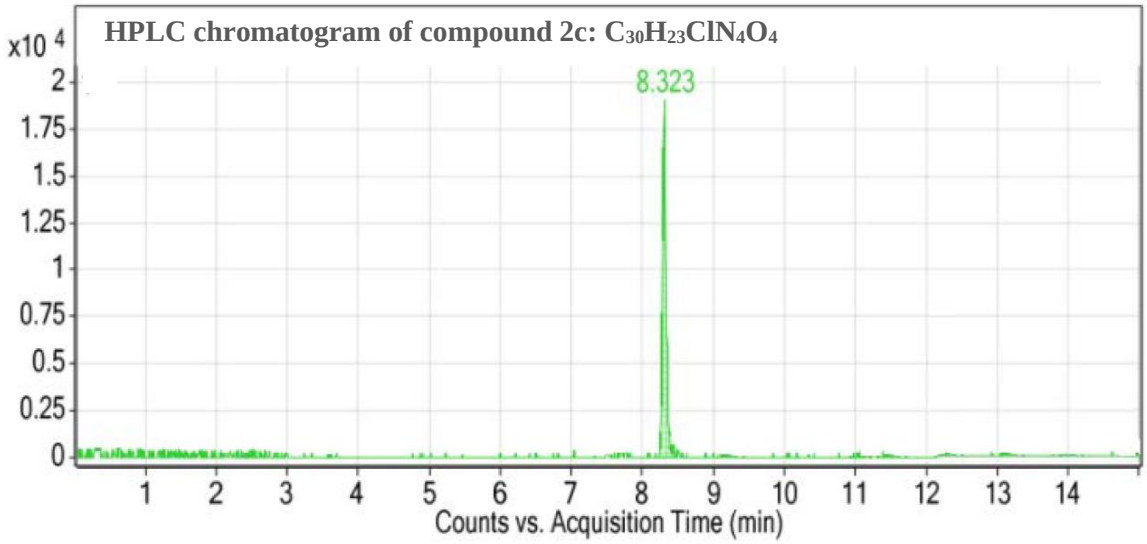
(E)-3-(3-(4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenyl)-3-oxoprop-1-en-1-yl)quinolin-2(1H)-one (**2a**). Yellow solid, mp: >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.21 (t, J = 7.5 Hz, 1H, Quin-H), 7.38 (d, J = 8.2 Hz, 1H, Quin-H), 7.43 (d, J = 5.2 Hz, 1H, CH), 7.53 (t, J = 7.7 Hz, 1H, Quin-H), 7.60 (d, J = 8.6 Hz, 2H, Ar-H), 7.71 (d, J = 7.9 Hz, 1H, Quin-H), 7.75 (d, J = 15.7 Hz, 1H, =CH), 8.09 - 7.97 (m, 4H, Ar-H), 8.17 (d, J = 8.6 Hz, 2H, Ar-H), 8.23 (d, J = 15.7 Hz, 1H, =CH), 8.40 (s, 1H, Quin-H), 8.62 (d, J = 5.2 Hz, 1H, CH), 9.66 (s, 1H, NH), 11.56 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{28}H_{19}ClN_4O_2$, 479.1275; found, 479.1262.



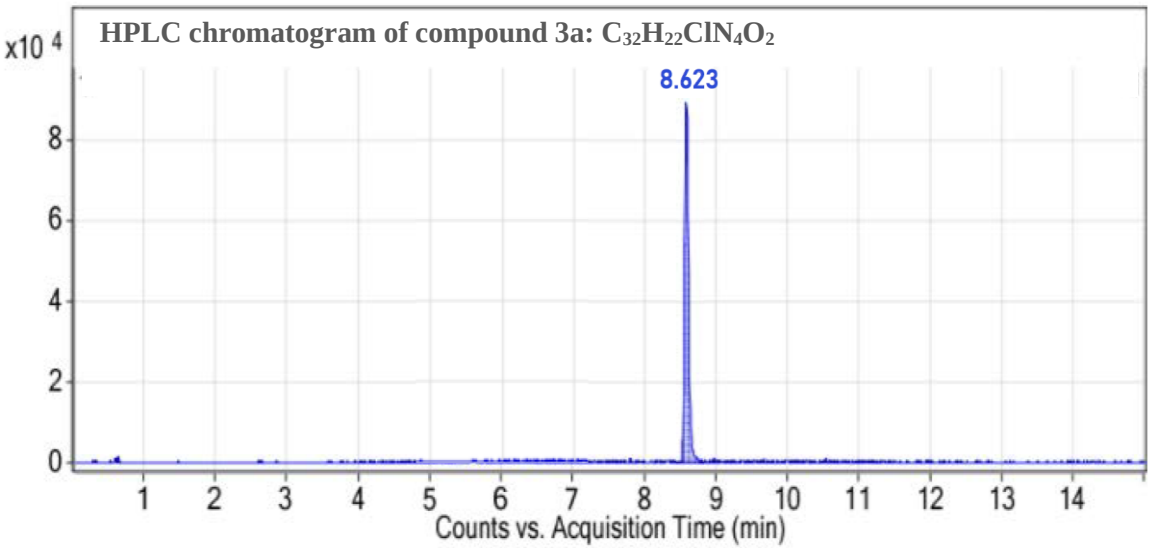
(E)-3-(3-(4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenyl)-3-oxoprop-1-en-1-yl)-6-methoxyquinolin-2(1H)-one (**2b**). Yellow solid, mp: >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (s, 3H, OCH_3). 7.26 - 7.16 (m, 2H, Quin-H), 7.33 (d, J = 8.8 Hz, 1H, Quin-H), 7.43 (d, J = 5.2 Hz, 1H, CH), 7.59 (d, J = 8.5 Hz, 2H, Ar-H), 7.74 (d, J = 15.6 Hz, 1H, =CH), 8.09 - 7.99 (m, 4H, Ar-H), 8.17 (d, J = 8.5 Hz, 1H, Ar-H), 8.23 (d, J = 15.6 Hz, 1H, =CH), 8.35 (s, 1H, Quin-H), 8.61 (d, J = 5.2 Hz, 1H, CH), 9.65 (s, 1H, NH), 11.45 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{29}H_{21}ClN_4O_3$, 509,1380; found, 509.1371.



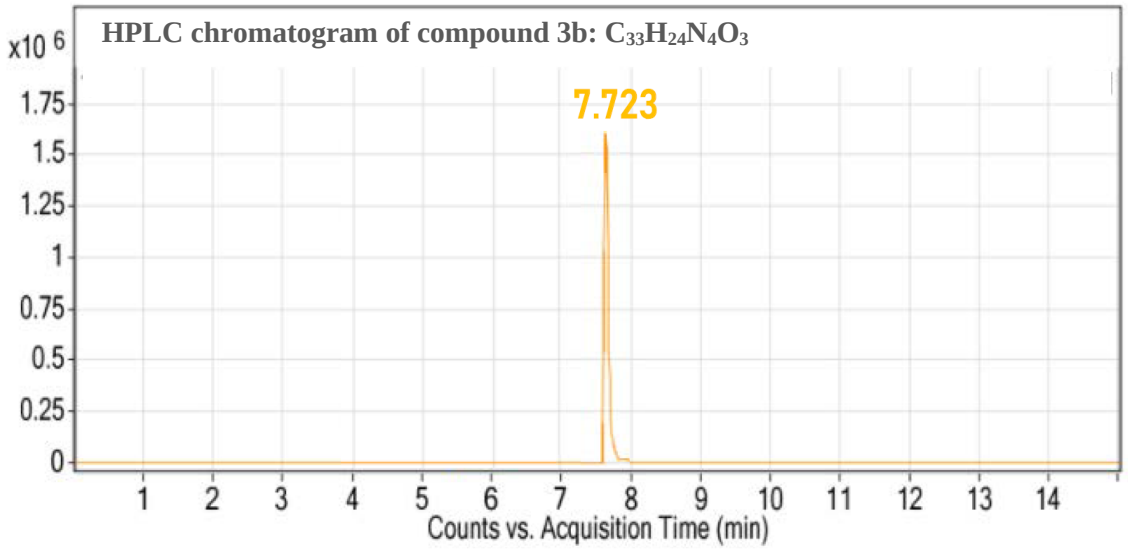
(E)-3-(3-(4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenyl)-3-oxoprop-1-en-1-yl)-6,7-dimethoxyquinolin-2(1H)-one (**2c**). Yellow solid, mp: >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.02 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.94 (s, 1H, Quin-H), 7.21 (s, 1H, Quin-H), 7.55 (d, J = 4.8 Hz, 1H, CH), 7.65 (d, J = 7.9 Hz, 2H, Ar-H), 7.76 (d, J = 14.6 Hz, 1H, =CH), 8.02 (d, J = 8.5 Hz, 2H, Ar-H), 8.05 (d, J = 8.5 Hz, 2H, Ar-H), 8.20 (d, J = 7.9 Hz, 2H, Ar-H), 8.27 (d, J = 14.6 Hz, 1H, =CH), 8.39 (s, 1H, Quin-H), 8.60 (d, J = 4.8 Hz, 1H, CH), 10.05 (s, 1H, NH), 11.65 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{30}H_{23}ClN_4O_4$, 539.1481; found, 539.1477.



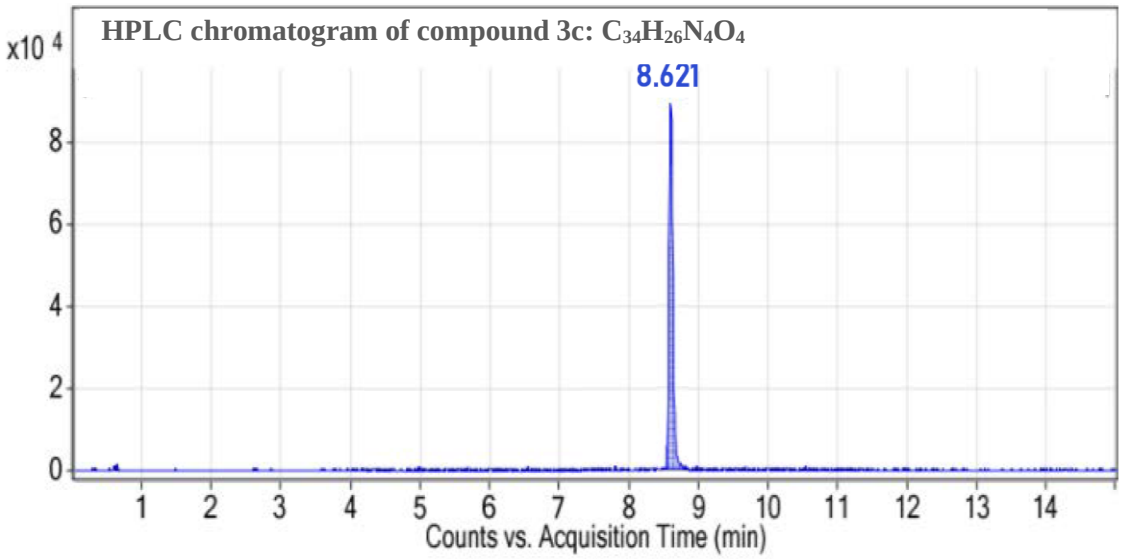
(E)-3-(3-(4-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenyl)-3-oxoprop-1-en-1-yl)quinolin-2(1H)-one (**3a**). Yellow solid, mp: >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.27 (t, J = 7.2 Hz, 1H, Quin-H). 7.39 (d, J = 8.1 Hz, 1H, Quin-H), 7.59 (t, J = 7.3 Hz, 1H, Quin-H), 7.68 - 7.62 (m, 2H), 7.72 (d, J = 5.1 Hz, 1H, CH), 7.75 (d, J = 7.8 Hz, 1H, Ar-H), 7.84 (d, J = 15.5 Hz, 1H, =CH), 8.03 (d, J = 4.9 Hz, 1H Ar-H), 8..08 - 8.21 (m, 6H, Ar-H), 8.36 (d, J = 7.1 Hz, 1H, Quin-H), 8.38 (d, J = 15.1 Hz, 1H, =CH), 8.64 (s, 1H, Quin-H), 8.72 (d, J = 4.9 Hz, 1H, NH), 8.83 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{32}H_{22}N_4O_2$, 495.1821; found, 495.1814.



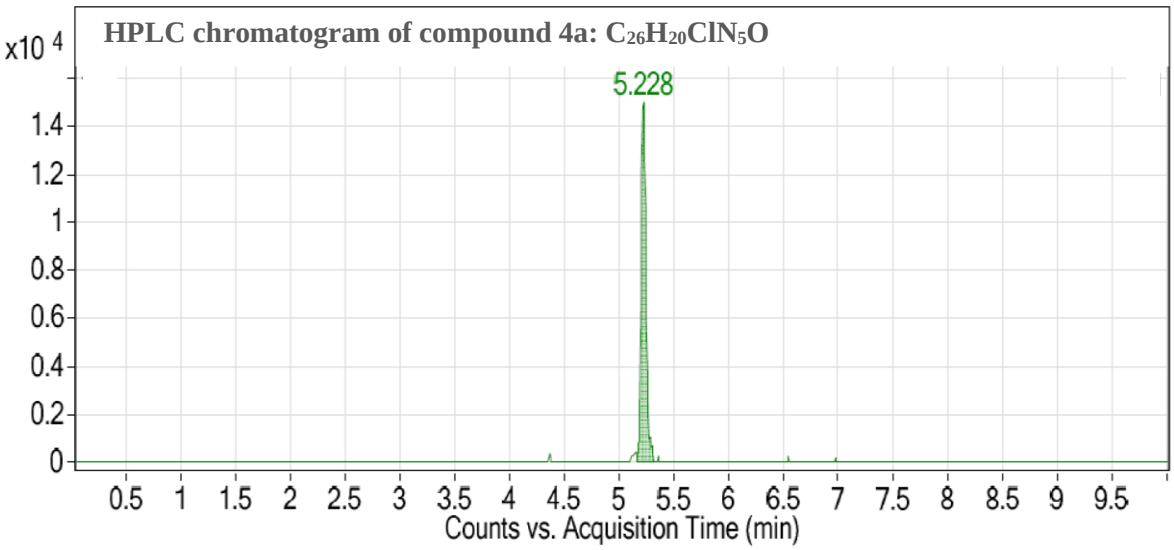
(E)-6-methoxy-3-(3-(4-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenyl)-3-oxoprop-1-en-1-yl)quinolin-2(1H)-one (**3b**). Yellow solid, mp: >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (s, 3H, OCH_3), 7.25 (s, 1H, Quin-H), 7.26 (d, J = 7.1Hz, 1H, Quin-H), 7.33 (d, J = 9.7 Hz, 1H, Ar-H), 7.70 - 7.59 (m, 2H, Ar-H), 7.73 (d, J = 5.2 Hz, 1H, CH), 7.83 (d, J = 15.5 Hz, 1H, =CH), 8.08 - 8.00 (m, 1H, Ar-H), 8.21 - 8.11 (m, 6H, Ar-H), 8.36 (d, J = 7.0 Hz, 1H, Quin-H) 8.39 (d, J = 15.4 Hz, 1H, =CH), 8.58 (s, 1H, Ar-H), 8.73 (d, J = 5.2 Hz, 1H, CH), 8.84 (s, 1H, Quin-H), 10.30 (s, 1H, NH), 12.0 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{33}H_{24}N_4O_3$, 525.1927; found, 525.1910.



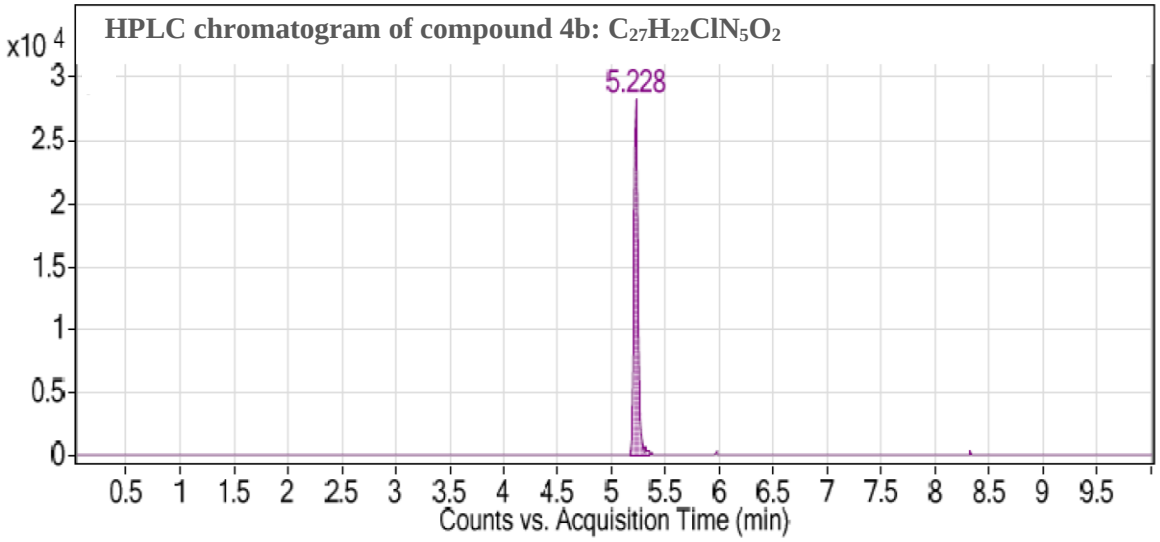
(E)-6,7-dimethoxy-3-(3-(4-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenyl)-3-oxoprop-1-en-1-yl)quinolin-2(1H)-one (**3c**). Yellow solid, mp: >300 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.84 (s, 3H, OCH_3). 3.87 (s, 3H, OCH_3), 6.92 (s, 1H, Quin-H), 7.20 (s, 1H, Quin-H),7.69 - 7.59 (m, 2H, Ar-H), 7.72 (d, J = 5.2 Hz, 1H, CH), 7.81 (d, J = 15.4 Hz, 1H, =CH), 8.04 (m, 2H, Ar-H), 8.18 - 8.10 (m, 6H, Ar-H, Ar-H), 8.35 (d, J = 15.5 Hz, 1H, =CH), 8.36 (d, J = 8.8 Hz, 1H, Ar-H), 8.45 (s, 1H, Quin-H), 8.72 (d, J = 5.2 Hz, 1H, CH), 8.83 (s, 1H, NH), 10.26 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{34}H_{26}N_4O_4$, 555.2032; found, 555.2018.



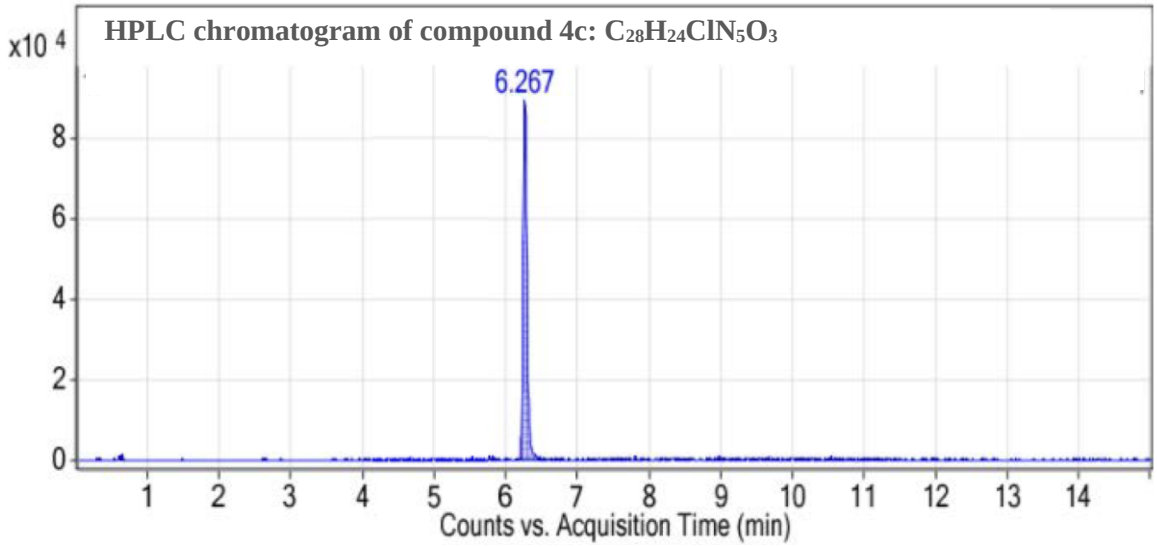
3-(((4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenyl)amino)methyl)quinolin-2(1H)-one (**4a**). Yellow solid. mp: 261-262 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.21 (s, 2H, CH_2), 5.37 (s, 1H, CH_2-NH), 6.66 (d, J = 8.8 Hz, 2H, Ar-H), 7.12 (t, J = 8.0 Hz, 1H, Quin-H), 7.16 (d, J = 5.1 Hz, 1H, CH), 7.3 (d, J = 7.7 Hz, 1H, Quin-H), 7.41 (t, J = 8.3 Hz, 1H, Quin-H), 7.44 (d, J = 8.8 Hz, 2H, Ar-H), 7.51 (d, J = 8.6 Hz, 2H, Ar-H), 7.54 (d, J = 8.1 Hz, 1H, Quin-H), 7.80 (s, 1H, Quin-H), 8.07 (d, J = 8.6 Hz, 2H, Ar-H), 8.41 (d, J = 5.1 Hz, 1H, CH), 8.59 (s, 1H, NH), 11.34 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{26}H_{20}ClN_5O$, 454.1429; found, 454.1412.



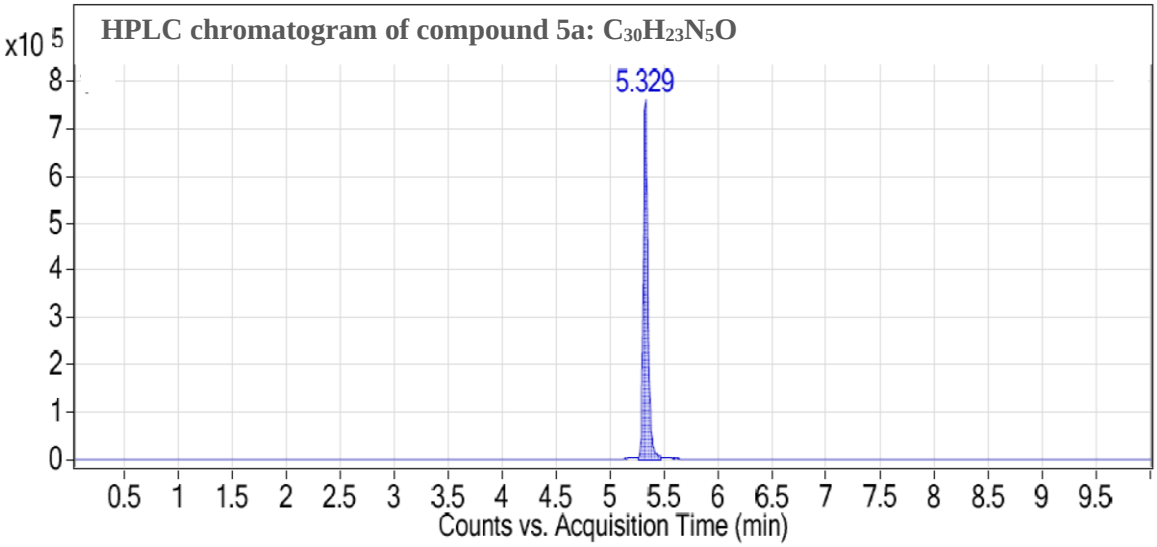
3-(((4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenyl)amino)methyl)-6-methoxyquinolin-2(1H)-one (**4b**). Yellow solid, mp: 255-256 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 3H, OCH_3). 4.17 (d, J = 5.5 Hz, 2H, CH_2), 5.92 (t, J = 5.5 Hz, 1H, CH_2-NH), 6.60 (d, J = 8.6 Hz, 2H, Ar-H), 7.11 (dd, J = 8.9, 2.5 Hz, 1H, Quin-H), 7.16 (s, 1H, Quin-H), 7.34 - 7.24 (m, 2H, Quin-H, CH), 7.47 (d, J = 8.6 Hz, 2H, Ar-H), 7.59 (d, J = 8.3 Hz, 2H, Ar-H), 7.74 (s, 1H, Quin-H), 8.14 (d, J = 8.3 Hz, 2H, Ar-H), 8.45 (d, J = 5.1 Hz, 1H, CH), 9.23 (s, 1H, NH), 11.78 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{27}H_{22}ClN_5O_2$, 484.1535; found, 484.1522.



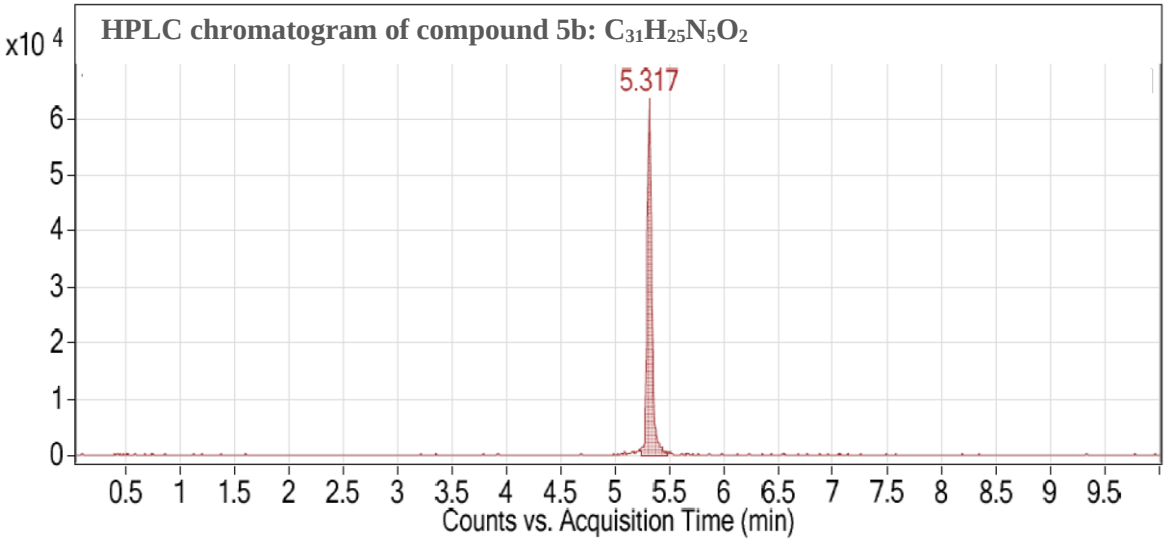
3-(((4-((4-(4-chlorophenyl)pyrimidin-2-yl)amino)phenyl)amino)methyl)-6,7-dimethoxyquinolin-2(1H)-one (**4c**). Yellow solid, mp: 261-262 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.73 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.10 (d, J = 5.8 Hz, 2H, CH_2), 5.84 (t, J = 5.8 Hz, 1H, CH_2-NH), 6.56 (d, J = 8.9 Hz, 2H, Ar-H), 6.86 (s, 1H, Quin-H), 7.12 (s, 1H, Quin-H), 7.24 (d, J = 5.2 Hz, 1H, CH), 7.43 (d, J = 8.8 Hz, 2H, Ar-H), 7.56 (d, J = 8.6 Hz, 2H, Ar-H), 7.66 (s, 1H, Quin-H), 8.11 (d, J = 8.6 Hz, 2H, Ar-H), 8.42 (d, J = 5.2 Hz, 1H, CH), 9.19 (s, 1H, NH), 11.63 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{28}H_{24}ClN_5O_3$, 514.1646; found, 514.1618.



3-(((4-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenyl)amino) methyl)quinolin-2(1H)-one (**5a**). Yellow solid, mp: 294-295 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.20 (d, J = 5.6 Hz, 2H, CH_2), 5.90 (t, J = 5.9 Hz, 1H, CH_2-NH), 6.65 (d, J = 8.6 Hz, 2H, Ar-H), 7.15 (t, J = 7.5 Hz, 1H, Quin-H), 7.35 (d, J = 8.2 Hz, 1H, Quin-H), 7.42 (d, J = 5.1 Hz, 1H, CH), 7.47 (t, J = 7.6 Hz, 1H, Quin-H), 7.53 (d, J = 8.6 Hz, 2H, Ar-H), 7.67 - 7.56 (m, 3H, Ar-H), 7.81 (s, 1H, Ar-H), 7.99 (d, J = 8.3 Hz, 1H, Ar-H), 8.05 (d, J = 8.7 Hz, 2H, Ar-H), 8.26 (d, J = 8.5 Hz, 1H, Quin-H), 8.49 (d, J = 5.1 Hz, 1H, CH), 8.72 (s, 1H, Quin-H), 9.25 (s, 1H, NH), 11.89 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{30}H_{23}N_5O$, 470.1975; found, 470.1968.

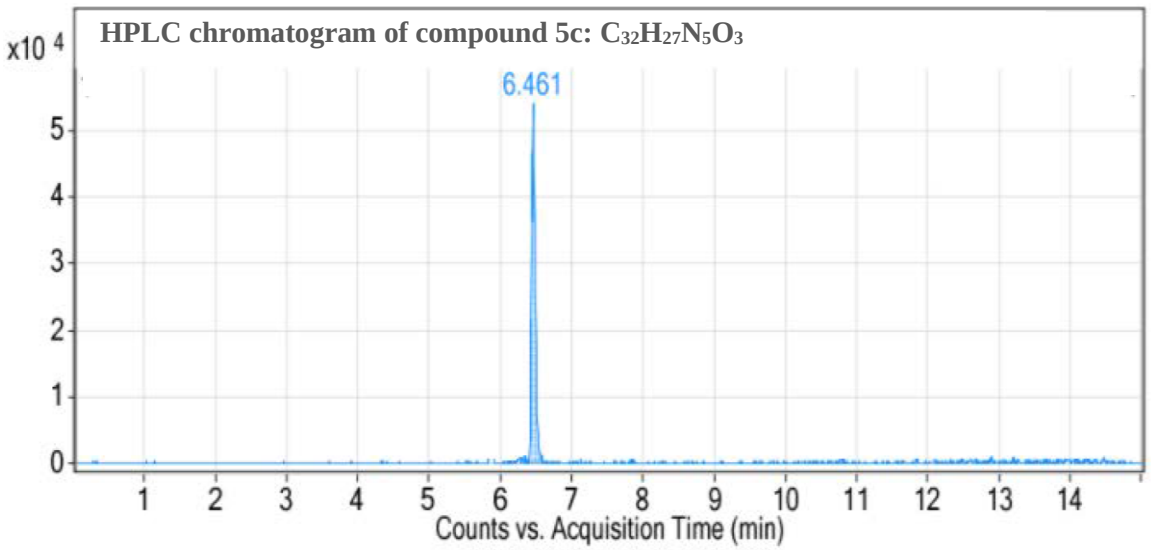


6-methoxy-3-(((4-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenyl)amino) methyl)quinolin-2(1H)-one (**5b**). Yellow solid, mp: 294-295 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.75 (s, 3H, OCH_3). 4.20 (d, J = 5.4 Hz, 2H, CH_2), 5.93 (t, J = 5.4 Hz, 1H, CH_2-NH), 6.64 (d, J = 8.7 Hz, 2H, Ar-H), 7.11 (dd, J = 8.9, 2.3 Hz, 1H, Quin-H), 7.17 (s, 1H, Quin-H), 7.29 (d, J = 8.9 Hz, 1H, Quin-H), 7.42 (d, J = 5.1 Hz, 1H, CH), 7.53 (d, J = 8.5 Hz, 2H, Ar-H), 7.67 - 7.56 (m, 2H, Ar-H), 7.77 (s, 1H, Ar-H), 7.98 (d, J = 7.4 Hz, 1H, Ar-H), 8.04 (d, J = 8.7 Hz, 2H, Ar-H), 8.25 (d, J = 8.5 Hz, 1H, Ar-H), 8.49 (d, J = 5.1 Hz, 1H, CH), 8.72 (s, 1H Quin-H), 9.26 (s, 1H, NH), 11.80 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{31}H_{25}N_5O_2$, 500.2081; found, 500.2065.

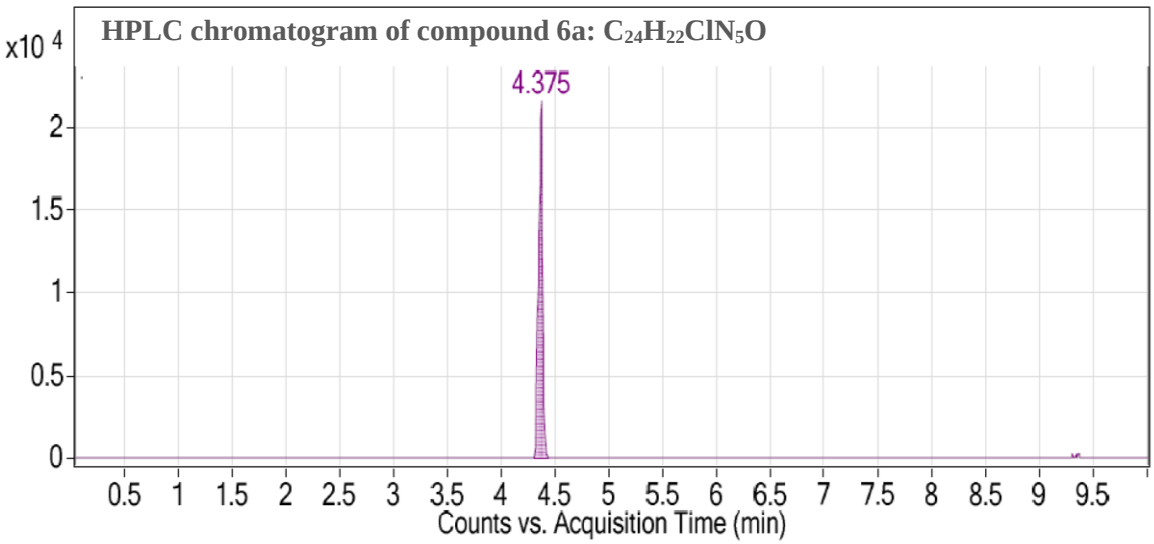


6,7-dimethoxy-3-(((4-((4-(naphthalen-2-yl)pyrimidin-2-yl)amino)phenyl)amino)methyl)quinolin-2(1H)-one (**5c**). Yellow solid, mp: 266-267 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.72 (s, 3H, OCH_3). 3.79 (s, 3H, OCH_3), 4.13 (d, J = 5.5 Hz, 2H, CH_2), 5.84 (t, J = 6.1 Hz, 1H, CH_2-NH), 6.59 (d, J = 8.8 Hz, 2H, Ar-H), 6.86 (s, 1H, Quin-H), 7.12 (s, 1H, Quin-H), 7.39 (d, J = 5.2 Hz, 1H, CH), 7.48 (d, J = 8.8 Hz, 2H, Ar-H), 7.62 - 7.52 (m, 2H, Ar-H), 7.68 (s, 1H, Quin-H), 8.10 - 7.88 (m, 3H, Ar-H), 8.23 (dd, J = 8.6, 1.7 Hz, 1H, Ar-H), 8.46 (d, J = 5.2 Hz, 1H, CH), 8.69 (s, 1H, CH, Ar-H), 9.21 (s, 1H,

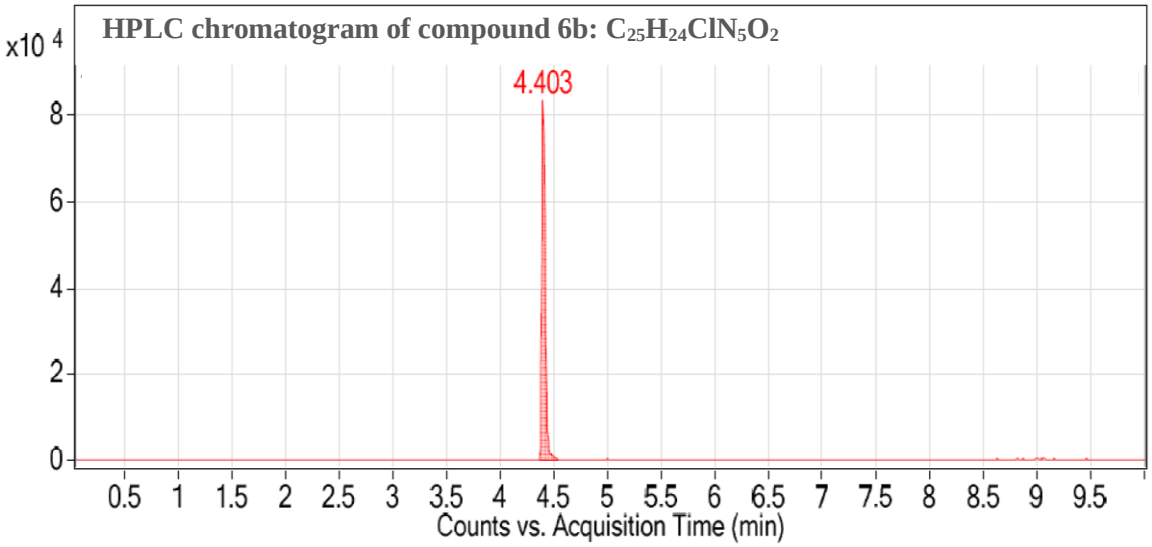
NH), 11.64 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{32}H_{27}N_5O_3$, 530.2187 found, 530.2169.



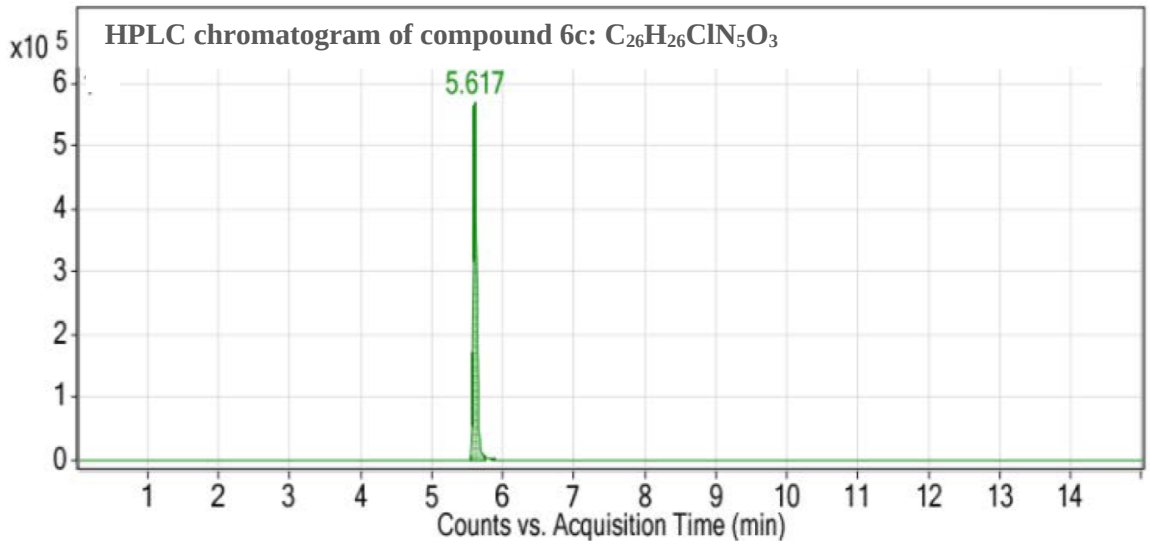
3-((4-(4-(4-chlorophenyl)pyrimidin-2-yl)piperazin-1-yl)methyl)quinolin-2(1H)-one (**6a**). White solid, mp: 251-252 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (br, 4H, CH_2 x 2). 3.49 (s, 2H, CH_2), 3.90 (br, 4H, CH_2 x 2), 7.19 (t, J = 8.1 Hz, 1H, Quin-H), 7.23 (d, J = 5.2 Hz, 1H, CH), 7.33 (d, J = 8.1 Hz, 1H, Quin-H), 7.48 (t, J = 8.5 Hz, 1H, Quin-H), 7.58 (d, J = 8.6 Hz, 2H, Ar-H), 7.71 (d, J = 7.1 Hz, 1H, CH), 7.94 (s, 1H, Quin-H), 8.17 (d, J = 8.6 Hz, 2H, Ar-H), 8.46 (d, J = 5.1 Hz, 1H, CH), 11.81 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{24}H_{22}ClN_5O$, 432.1586; found, 432.1585.



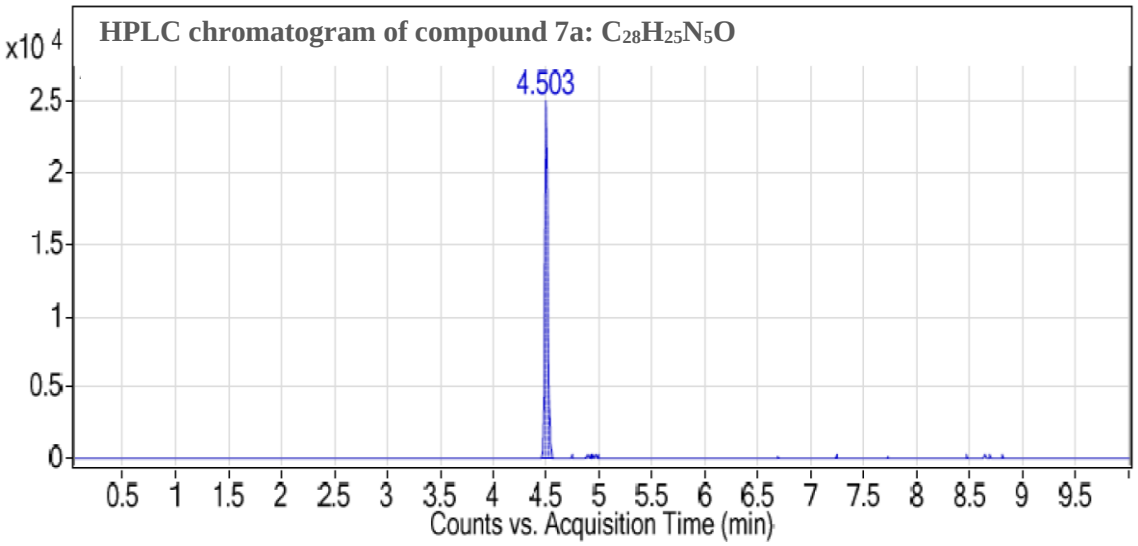
3-((4-(4-(4-chlorophenyl)pyrimidin-2-yl)piperazin-1-yl)methyl)-6-methoxyquinolin-2(1H)-one (**6b**). White solid, mp: 262-263 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.58 (br. 4H, CH_2 x 2), 3.48 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.90 (br, 4H, CH_2 x 2), 7.13 (d, J = 8.8 Hz, 1H, Quin-H), 7.22 (d, J = 5.0 Hz, 1H, CH), 7.26 (d, J = 8.8 Hz, 2H, Quin-H), 7.27 (s, 1H, Quin-H), 7.57 (d, J = 8.2 Hz, 2H, Ar-H), 7.90 (s, 1H Quin-H), 8.16 (d, J = 8.2 Hz, 2H, Ar-H), 8.46 (d, J = 5.0 Hz, 1H, CH), 11.70 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{25}H_{24}ClN_5O_2$, 462.1691; found, 462.1687.



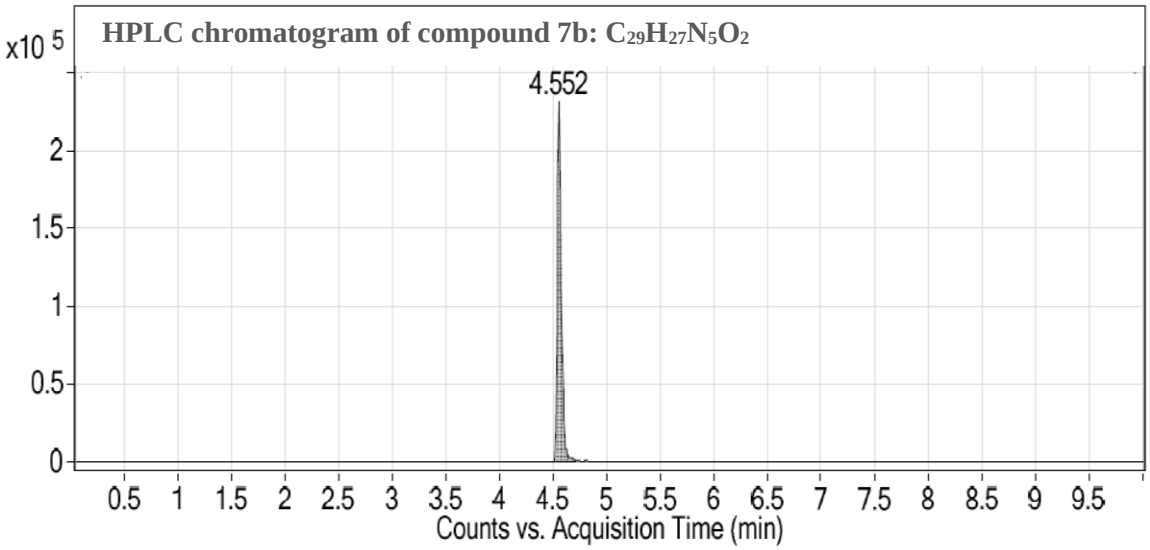
3-((4-(4-(4-chlorophenyl)pyrimidin-2-yl)piperazin-1-yl)methyl)-6,7-dimethoxyquinolin-2(1H)-one (**6c**). White solid, mp: 231-231°C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.71 (br, 4H, CH_2). 3.68 (s, 2H, CH_2), 6.87 (s, 1H), 394 (br, 4H, CH_2), 3.98 (s, 6H, OCH_3), 6.89 (d, J = 5.0 Hz, 1H, CH), 6.90 (s, 1H, CH, Quin-H), 6.98 (s, 1H, Quin-H), 7.42 (d, J = 8.2 Hz, 2H, H-Ar), 7.86 (s, 1H, Quin-H), 7.97 (d, J = 8.2 Hz, 2H, H-Ar), 8.37 (d, J = 5.0 Hz, 1H, CH), 12.00 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{26}H_{26}ClN_5O_3$, 492.1797; found, 492.1793.



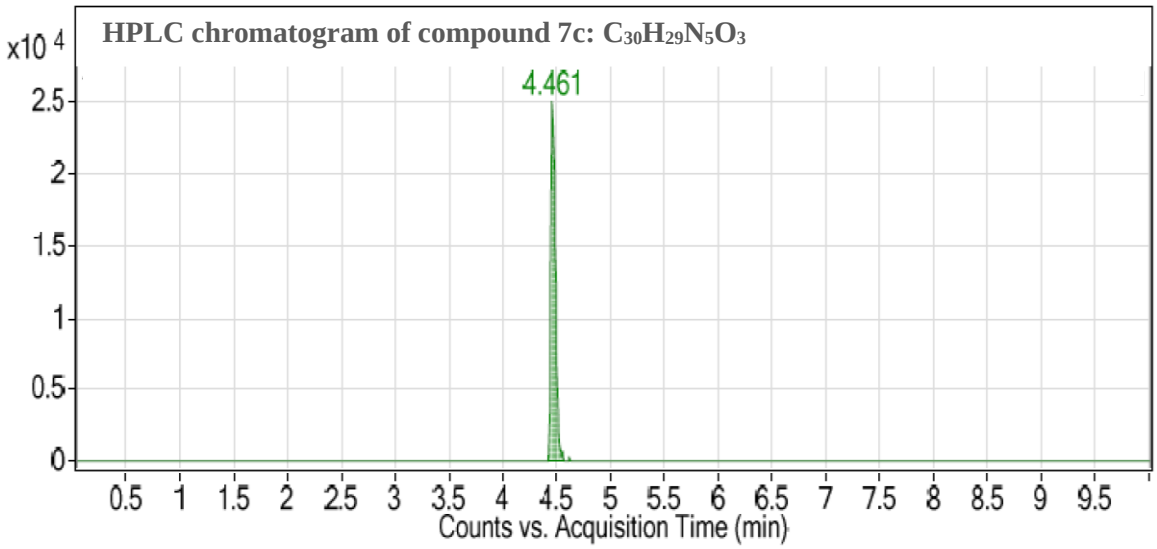
3-((4-(4-(naphthalen-2-yl)pyrimidin-2-yl)piperazin-1-yl)methyl)quinolin-2(1H)-one (**7a**). White solid, mp: 251-252 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.65 (t, J = 5.1 Hz, 4H, CH_2 x 2), 3.55 (s, 2H, CH_2), 3.94 (t, J = 5.1 Hz, 4H, CH_2 x 2), 7.14 (t, J = 8.5 Hz, 1H, Quin-H), 7.25 (d, J = 5.1 Hz, 1H, CH), 7.34 (d, J = 8.4 Hz, 1H, Quin-H), 7.43 (t, J = 8.4 Hz, 1H, Ar-H), 7.59 - 7.50 (m, 2H, Ar-H), 7.63 (dd, J = 7.8, 1.0 Hz, 1H, Ar-H), 7.89 (s, 1H, Ar-H), 7.96 - 7.90 (m, 1H, Ar-H), 7.99 (d, J = 8.6 Hz, 1H, Quin-H), 8.19 (dd, J = 8.5, 1.8 Hz, 1H, Quin-H), 8.45 (d, J = 5.1 Hz, 1H, CH), 8.63 (s, 1H, Quin-H), 11.24 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{28}H_{25}N_5O$, 448.2132; found, 448.2129.



6-methoxy-3-((4-(4-(naphthalen-2-yl)pyrimidin-2-yl)piperazin-1-yl)methyl)quinolin-2(1H)-one (**7b**). White solid, mp: 247-248 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.61 (br, 4H, CH_2 x 2). 3.50 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.96 (br, 4H, CH_2 x 2), 7.13 (d, J = 8.9 Hz, 1H, Ar-H), 7.27 (d, J = 9.2 Hz, 1H, Ar-H), 7.29 (s, 1H, Quin-H), 7.38 (d, J = 5.1 Hz, 1H, CH), 7.64 - 7.54 (m, 2H, Ar-H), 7.92 (s, 1H, Quin-H), 8.01 - 7.96 (m, 1H, Ar-H), 8.04 (d, J = 8.6 Hz, 1H, Quin-H), 8.09 (d, J = 5.9 Hz, 1H, Ar-H), 8.28 (d, J = 8.6 Hz, 1H, Quin-H), 8.50 (d, J = 5.1 Hz, 1H, Ar-H), 8.75 (s, 1H, Ar-H), 11.71 (s, 1H, NH). ESI-QTOF (positive ionization) M+H calcd. for $C_{29}H_{27}N_5O_2$, 478.2238; found, 478.2233.



6,7-dimethoxy-3-((4-(4-(naphthalen-2-yl)pyrimidin-2-yl)piperazin-1-yl)methyl)quinolin-2(1H)-one (**7c**). White solid, mp: 262-263 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.63 - 2.80 (m, 4H, CH_2 x 2), 3.70 (s, 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.12 - 4.01 (m, 4H, CH_2 x 2), 6.89 (s, 1H, CH), 6.98 (s, 1H, Quin-H), 7.08 (d, J = 5.2 Hz, 1H, Quin-H), 7.59 - 7.46 (m, 2H, CH), 8.00 - 7.81 (m, 4H), 8.15 (dd, J = 8.6, 1.7 Hz, 1H), 8.41 (d, J = 5.2 Hz, 1H, CH), 8.52 (s, 1H, Quin-H), 12.19 (s, 1H, NH). ESI-QTOF (positive ionization) M+H. calcd. for $C_{30}H_{29}N_5O_3$, 508.2343; found, 508.2339.



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