Supplementary Information

Synthesis and biological evaluation of novel quinazolin-4(3*H*)-one Schiff base derivatives as nitric oxide synthase inhibitors

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1. Analyses figuers

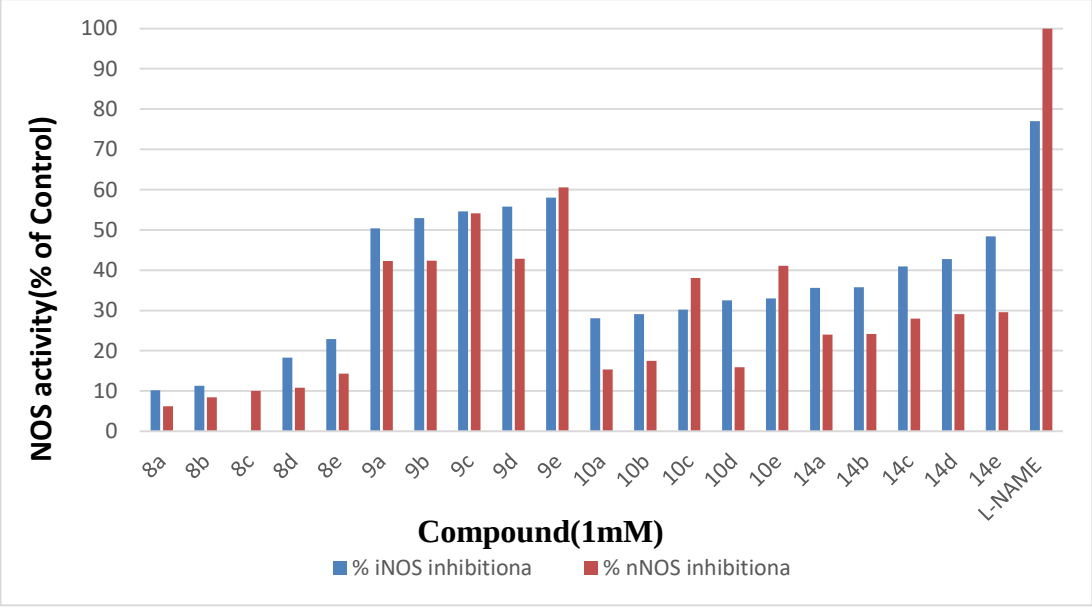


Fig. 2. Percentage of the nNOS and iNOS activities in the presence of 1 mM of the Quinazolinyl Schiff bases (compounds **8_e, 9_e, 10_e,** and **14_e**)) compared to control. Each value is the mean of three experiments performed in triplicate using recombinant iNOS or nNOS enzymes

2. Spectroscopic information

2-(2-(4-isobutylphenyl)propanamido)-5-nitrobenzoic acid (3)

During 10 minutes, portion-wise additions of 2-(4-isobutylphenyl)propanoyl chloride **(1)** (2.24 g; 10 mmol) was made to a stirred solution of commercially available 2-amino-5-nitrobenzoic acid **(2)** (1.80 g; 10 mmol) in dry pyridine (20 mL). The mixture was stirred for 2 hours at room temperature before being pour into ice-cold water (30 mL), acidified with hydrochloric acid (2N), and allowed to precipitate completely.

Recryst. Solvent: Methanol; Yellow solid; Yield: 70 %; mp: 120 C°; Anal. Calc. (%) for $C_{20}H_{22}N_2O_5$ (370): C, 64.85; H, 5.99; N, 7.56; found: C, 64.82; H, 5.95; N, 7.54; IR (KBr), ν (cm^{-1}): 3420 (O–H, N–H), 3080 (C–H_{arom}), 2960, 2940, 2880 (C–H_{aliph}), 1720 (C=$O_{carbonyl}$), 1644 (C=$O_{Carboxylic}$),1045 (C-N) ; ^{1}H NMR (DMSO-*d_6*) δ (ppm): 1.15 (d, 6H, $CH_2CH(CH_3)_2$), 1.50 (d, 3H, $CHCH_3$), 2.20 (m, 1H, $CH_2CH(CH_3)_2$), 2.45 (d, 2H, $CH_2CH(CH_3)_2$), 3.35 (q, H, $CHCH_3$), 7.00-8.20 (m, 7H, H_{arom}), 8.70 (bs, 1H exchangeable with D_2O, NH) , 12.66 (bs, 1H exchangeable with D_2O, OH) ; ^{13}C NMR (DMSO-*d6*) δ (ppm): 172.92, 169.60, 149.50, 143.84, 139.60, 133.53, 129.44, 129.40, 128.52, 128.71, 126.66, 125.75, 122.36, 116.86, 45.81, 42.62, 29.40, 22.35, 22.73,14.10; ESI-MS: *m/z* = 370 (M^+).

2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4H-benzo[d][1,3]oxazin-4-one(4)

Anilide **3** (3.70 g, 10 mmol) and acetic anhydride (50 mL) were heated under reflux for 2 hours before being concentrated. The benzoxazinone **4** was obtained by crystallizing of the residue from petroleum ether (60-80°C).

Recryst. Solvent: Ethanol; White solid; Yield 65 %; m. p. 180-182°C; Anal. Calc. (%) for $C_{20}H_{20}N_2O_4$(352): C, 68.17; H, 5.72; N, 7.95; found: C, 68.15; H, 5.70; N, 7.93; IR (KBr), ν (cm^{-1}): 3060 (C– H_{arom}), 2960, 2940, 2875 (C–H_{aliph}), 1685 (C=$O_{quinazolinone}$), 1617 (C=N), 1160 (C–O–C).^{1}H NMR (CDCl3) δ: 1.04 (d, 6H, $CH_2CH(CH_3)_2$), 1.45 (d, 3H, $CHCH_3$), 1.85 (m, 1H, $CH_2CH(CH_3)_2$), 2.50 (d, 2H, $CH_2CH(CH_3)_2$), 3.35 (q, H, $CHCH_3$), 7.10-8.20 (m, 7H, H_{arom}). ^{13}C NMR (DMSO-*d6*) δ (ppm): 169.80, 162.60, 148.60, 144.80, 139.75, 134.56, 129.40, 129.50, 128.70, 128,50, 125.60, 124.75, 120.35, 118.80, 44.85,43.60, 29.50, 23.65, 23.75,16.20; ESI-MS: m/z = 352 (M^+).

General procedures for the synthesis of compounds 5-7

To a cold solution of benzoxazinone (4) (3.53g, 10 mmol) in anhydrous pyridine (20ml) was drowsily added a solution of the appropriate amine (10 mmol); namely hydrazine hydrate (0.55 mL, 10 mmol), argnine (1.75g, 10 mmol), and lysine (1.46g, 10 mmol), in anhydrous pyridine (10ml) with continuous stirring. When the addition was complete, the reaction mixture was stirred vigorously for 30min at room temperature and subsequently heated under reflux for 4 h under anhydrous reaction conditions. The reaction mixture was allowed to cool to room temperature and poured into ice cold water containing diluted hydrochloric acid. The obtained crude precipitates were filtered off, washed repeatedly with water and dried, recrystallized.

3-amino-2-{1-[4-(2-methylpropyl)phenyl]ethyl}-6-nitroquinazolin-4(3*H*)-one (5)

Recryst. Solvent: Ethanol; Yellow solid; Yield: 80 %; m. p: 182-184ºC; Anal. Calc. (%) for $C_{20}H_{22}N_4O_3$ (366): C, 65.56; H: 6.05; N: 15.29; found: C: 65.54; H: 6.04; N: 15.27; FT-IR ν_{max} (cm^{-1}): 3230 (NH_2), 1672 ($C=O_{quinazolinone}$), 1628 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.40 (d, 3H, $CHCH_3$), 1.90 (m, 1H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 3.30 (q, H, $CHCH_3$), 6.20 (s, 2H, D_2O-exchangeable, NH_2), 7.10–8.00 (m, 7H, Ar—H)); ^{13}C NMR (DMSO-d_6) δ (ppm): 165.01, 161.22, 153.00, 147.25, 137.20, 137.50, 128.11, 128.70, 126.70, 126.50, 125.90, 124.90, 123.70, 121.5, 45.70, 41.50, 28.70, 22.80,22.30, 12.20; ESI-MS: m/z = 366 (M^+).

2-amino-5-(2-((R)-1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxo-3,4-dihydroquinazoline-3-carboxamidino)pentanoic acid (6)

Recryst. Solvent: Methanol; Yellow solid; Yield: 70%; mp 210–212°C; Anal. Calc. (%) for $C_{26}H_{32}N_4O_5$ (508): C, 61.40, H: 6.34, N: 16.52; found: C: 61.39, H: 6.30, N: 16.49; FT-IR ν_{max} (cm^{-1}): 3456-3578 (OH), 3250 (NH_2), 1676 ($C=O_{quinazolinone}$), 1648 ($C=O_{carboxyl}$), 1624 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.09 (d, 6H, $CH_2CH(CH_3)_2$), 1.44 (d, 3H, $CHCH_3$), 1.50 (m, 2H, NH $CH_2CH_2CH_2$), 1.70 (m, 2H, $NHCH_2CH_2CH_2$), 2.5 (m, 2H, NH $CH_2CH_2CH_2$), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.50(d, 2H, $CH_2CH(CH_3)_2$), 3.33 (q, H, $CHCH_3$), 4.00 (q, H, NCHCOOH), 5.50 (s, H, D_2O-exchangeable, C=*NH*), 6.30 (s, 2H, D_2O-exchangeable, NH_2), 7.20–8.10 (m, 7H, Ar—H)), 9.70 (m, H, D_2O-exchangeable, NH), 10.80 (s, H, COO*H*); ^{13}C NMR (DMSO-d_6) δ (ppm): 174.00,170.50, 164.20, 163.90, 153.00, 147.20, 137.30, 137.80 128.50, 128.70, 126.30, 126.70, 125.40, 123.40, 123.90, 121.50, 50.50, 45.70, 37.50, 33.50 ,30.20, 28.10, 22.80, 21.20, 22.80, 12.20; ESI-MS: m/z = 508 (M^+).

2-amino-6-(2-((R)-1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)hexanoic acid (7)

Recryst. Solvent: Ethanol; Bale yellow solid; Yield 65%; mp 198-200°C; Anal. Calc. (%) for $C_{26}H_{32}N_4O_5$ (480): C, 64.98; H, 6.71; N, 11.66; found: C, 64.95; H, 6.69; N, 11.64; FT-IR ν_{max} (cm^{-1}): 3456-3578 (OH), 3270 (NH_2), 1677 ($C=O_{quinazolinone}$), 1645 ($C=O_{carboxyl}$), 1624 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.30 (m, 2H, $NHCH_2CH_2CH_2CH_2$), 1.38 (d, 3H, $CHCH_3$), 1.60 (m, 2H, $NHCH_2CH_2CH_2CH_2$), 1.80 (m, 2H, $NHCH_2CH_2CH_2\ CH_2$), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.30(d, 2H, $CH_2CH(CH_3)_2$), 3.20 (m, 2H, $NHCH_2CH_2CH_2CH_2$), 3.50 (q, H, NCHCOOH), 3.35 (q, H, $CHCH_3$), 6.50 (s, 2H, D_2O-exchangeable, NH_2), 7.20 – 8.20 (m, 7H, Ar—H)), 10.80 (s, H, COO*H*); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.10, 164.10, 161.80, 153.90, 147.30, 137.40, 137.10 128.40, 128.20, 126.20, 126.10, 125.40, 123.60, 123.30, 121.20, 55.50, 45.10, 41.20, 34.20, 33.40, 30.00, 27.40, 22.60, 22.60, 21.10, 12.80; ESI-MS: m/z = 480 (M^+).

General procedures for the synthesis of Schiff bases 8-10

An equimolar mixture of compound **5-7** (10 mol) and substituted aldehydes namely; 4-hydroxybenzaldehyde (1.22g, 10 mmol), 4-chlorobenzaldehyde (1.40g, 10 mmol), 4- methoxybenzaldehyde (1.36g, 10 mmol), 4-*N*,*N*-dimethylbenzaldehyde (1.49g, 10 mmol) and 4-nitrobenzaldehyde (1.51g, 10 mmol) (10 mmol) in absolute ethanol (10 mL) was allowed to be refluxed for 8 h, H_2SO_4 (0.5 mL) was added slowly to the reaction mixture and the progress of the reaction was monitored through thin layer chromatography. The

reaction was allowed to cool to room temperature once it was finished. On standing, the solid crystalline product was filtered, dried, and crystallized.

3-(4-hydroxybenzylideneamino)-2-ibuprofenyl-6-nitro-4(3H)-quinazolin-4ones (8a)

Recryst. Solvent: Methanol; White solid; Yield 25%; mp 220-222 °C; Anal. Calc. (%) for $C_{27}H_{26}N_4O_4$ (470): C, 68.92; H, 5.57; N, 11.91; found: C, 68.90; H,5.53; N, 11.87; FT-IR ν_{max} (cm^{-1}): 3415- 3525 (OH), 1669 ($C{=}O_{quinazolinone}$), 1629 (C=N), 1591 ($N{=}CH_{streching}$); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.15 (d, 6H, $CH_2CH(CH_3)_2$), 1.40 (d, 3H, $CHCH_3$), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.45 (d, 2H, $CH_2CH(CH_3)_2$), 3.33 (q, H, $CHCH_3$),4.90 (s, H, OH), 7.10–8.10 (m, 11H, Ar—H), 8.90 (s, H, CH=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 168.20, 161.30, 161.00, 153.10, 147.35, 143.40, 137.20, 137.30, 130.10, 130.15, 128.50, 128,55, 126.15, 126.10, 126.00, 125.30, 123.20, 123.70, 121.10, 116.30, 116.35, 45.75, 31.50, 28.10, 22.80, 22.85, 12.20; ESI-MS: m/z = 470 (M^+).

3-(4-chlorobenzylideneamino)-2-ibuprofenyl-6-nitro-4(3H)-quinazolin-4ones (8b)

Recryst. Solvent: Ethanol; Yellow solid; Yield 30%; mp 330-332 °C; Anal. Calc. (%) for $C_{27}H_{25}N_4O_3Cl$ (488): C, 66.32; H,5.15; N, 11.46; found: C, 66.34; H, 5.18; N, 11.48; FT-IR ν_{max} (cm^{-1}): 1669 ($C{=}O_{quinazolinone}$), 1629 (C=N), 1595 ($N{=}CH_{streching}$), 820 (C-Cl); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.55 (d, 3H, $CHCH_3$), 1.90 (m, H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 3.35 (q, H, $CHCH_3$), 7.20–8.10 (m, 11H, Ar—H), 8.95 (s, H, CH=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 167.10, 162.30,162.00, 155.10, 148.30, 143.10, 137.1, 137.10, 131.15, 131.10, 127.45, 127,40, 125.15, 125.10, 125.00, 124.30, 123.10, 123.60, 120.15, 114.30, 114.35, 44.70, 30.40, 28.20, 22.70, 21.80, 12.10; ESI-MS: m/z = 488 (M^+).

3-(4-methoxybenzylideneamino)-2-ibuprofenyl-6-nitro-4(3H) -quinazolin-4ones (8c)

Recryst. Solvent: Ethanol; Yellow solid; Yield 35%; mp 230-233 °C; Anal. Calc. (%) for $C_{28}H_{28}N_4O_4$ (484): C, 69.41; H, 5.82; N, 11.56; found: C, 69.39; H, 5.80; N, 11.58; FT-IR ν_{max} (cm^{-1}): 1675 ($C{=}O_{quinazolinone}$), 1625 (C=N), 1590 ($N{=}CH_{streching}$); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.06 (d, 6H, $CH_2CH(CH_3)_2$), 1.50 (d, 3H, $CHCH_3$), 1.85 (m, H, $CH_2CH(CH_3)_2$), 2.45 (d, 2H, $CH_2CH(CH_3)_2$), 3.40 (s, 3H, OCH_3), 3.50 (q, H, $CHCH_3$), 7.10–8.20 (m, 11H, Ar—H), 9.05 (s, H, CH=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 164.10, 160.30,160.00, 154.20, 146.30, 141.10, 135.1, 135.10, 130.30, 130.20, 127.30, 127,10, 124.25, 124.20, 124.00, 123.30, 122.10, 122.60, 120.15, 116.20, 116.20, 55.95, 45.60, 31.60, 29.30, 22.40, 22.40, 12.40; ESI-MS: m/z = 484 (M^+).

3-(4-(dimethylamino)benzylideneamino)-2-ibuprofenyl-6-nitro-4(3H)-quinazolin-4ones (8d)

Recryst. Solvent: Methanol; pale yellow solid; Yield 30%; mp 185-187 °C; Anal. Calc. (%) for $C_{29}H_{31}N_5O_3$ (497): C: 70.00, H: 6.28, N: 14.07; found: C: 69.98, H: 6.80, N: 14.10; FT-IR ν_{max} (cm^{-1}): 1670 ($C{=}O_{quinazolinone}$), 1630 (C=N), 1595 ($C{=}N_{streching}$); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.44 (d, 3H, $CHCH_3$), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.50 (d, 2H, $CH_2CH(CH_3)_2$), 2.95 (d, 6H, $N(CH_3)_2$), 3.36 (q, H, $CHCH_3$), 7.15–8.20 (m, 11H, Ar—H), 9.00 (s, H, CH=N),; ^{13}C NMR (DMSO-d_6) δ (ppm): 164.20,

160.30, 153.00, 150.10, 147.20, 143.10, 136.35, 136.30, 130.10, 129.15, 128.40, 128,45, 127.15, 126.15, 126.00, 125.30, 123.20, 123.70, 121.15, 114.30, 114.35, 45.75,40.15, 40.10, 30.50, 29.10, 21.70, 21.75, 12.50; ESI-MS: m/z = 497 (M^+).

3-(4-nitrobenzylideneamino)-2-ibuprofenyl-6-nitro-4(3H) -quinazolin-4ones (8e)

Recryst. Solvent: Ethanol; Yellow solid; Yield 20 %; mp 225-227 °C; Anal. Calc. (%) for $C_{27}H_{25}N_5O_5$ (499): C, 64.92; H, 5.04; N, 14.02, found: C, 64.95; H, 5.09; N, 14.05; FT-IR ν_{max} (cm^{-1}): 1670 ($C=O_{quinazolinone}$), 1630 (C=N), 1595 ($C=N_{\ streching}$); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.15(d, 6H, $CH_2CH(CH_3)_2$),1.49 (d, 3H, $CHCH_3$), 2.00 (m, H, $CH_2CH(CH_3)_2$), 2.45 (d, 2H, $CH_2CH(CH_3)_2$), 3.30 (q, H, $CHCH_3$),7.10–8.10 (m, 11H, Ar—H)), 8.88 (s, H, *CH*=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 166.10, 160.30,153.00, 150.10, 147.30, 143.10, 139.10, 136.10, 136.15, 130.45, 130.40, 128.15, 128.10, 125.75, 125.70, 124.20, 123.25, 122.20, 121.35, 121.30, 120.10, 44.50, 32.50, 27.20, 22.75, 22.70, 12.10; ESI-MS: m/z = 499 (M^+).

2-(4-hydroxybenzylideneamino)-5-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxo-3,4-dihydroquinazoline-3-carboxamidino)pentanoic acid (9a)

Recryst. Solvent: Methanol; browen solid; Yield 20%; mp 245-247 °C; Anal. Calc. (%) for $C_{33}H_{36}N_6O_6$ (612): C, 64.69; H, 5.92; N, 13.72, found: C, 64.70; H, 5.95; N, 13.75; FT-IR ν_{max} (cm^{-1}): 3456-3578 (OH), 3315 (NH), 1676 ($C=O_{quinazolinone}$), 1648 ($C=O_{carboxyl}$), 1618 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.12 (d, 6H, $CH_2CH(CH_3)_2$), 1.42 (d, 3H, $CHCH_3$), 1.5 (m, 2H, NH $CH_2CH_2CH_2$) 1.7 (m, 2H, NH $CH_2CH_2CH_2$),1.90 (m, H, $CH_2CH(CH_3)_2$), 2.43 (d, 2H, $CH_2CH(CH_3)_2$), 2.50 (m, 2H, NH $CH_2CH_2CH_2$) , 3.35 (q, H, $CHCH_3$), 4.00 (q, H, N*CH*COOH), 4.40, (s, H, OH), 5.55 (s, H, D_2O-exchangeable, C=*NH*), 7.20–8.20 (m, 11H, Ar—H)), 9.00 (s, H, *CH*=N), 9.50 (s, H, D_2O-exchangeable, *NH*), 10.50 (s, H, COO*H*); ^{13}C NMR (DMSO-d_6) δ (ppm): ^{13}C NMR (DMSO-d_6) δ (ppm): 166.00, 165.40, 164.10, 163.50,160.8, 160.9, 152.40, 146.20, 137.40, 137.70,132.50, 130.40, 130.20, 127.70, 127.50, 125.70, 125.30, 124.40, 123.20, 123.10, 120.50,116.15, 116.10, 51.50, 47.70, 38.50, 32.50, 31.20, 27.10, 22.50, 22.30, 21.00, 12.10; ESI-MS: m/z = 612 (M^+).

2-(4-chlorobenzylideneamino)-5-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxo-3,4-dihydroquinazoline-3-carboxamidino)pentanoic acid(9b)

Recryst. Solvent: Ethanol; yellow solid; Yield 30%; mp 218-220 °C; Anal. Calc. (%) for $C_{33}H_{35}N_6O_5Cl$ (631): C: 62.80, H: 5.59, N: 13.32; found: C: 62.83, H: 5.61, N: 13.34 ; FT-IR ν_{max} (cm^{-1}): 3456-3578 (OH), 3315 (NH), 1676 ($C=O_{quinazolinone}$), 1648 ($C=O_{carboxyl}$), 1624 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.40 (d, 3H, $CHCH_3$), 1.60 (m, 2H, NH $CH_2CH_2CH_2$), 1.75 (m, 2H, NH $CH_2CH_2CH_2$), 1.92 (m, H, $CH_2CH(CH_3)_2$), 2.45 (d, 2H, $CH_2CH(CH_3)_2$), 2.62 (m, 2H, NH $CH_2CH_2CH_2$) , 3.35 (q, H, $CHCH_3$), 4.20 (q, H, N*CH*COOH), 5.50 (s, H, D_2O-exchangeable,, , C=*NH*), 7.20–8.10 (m, 11H, Ar—H)), 9.05 (s, H, *CH*=N), 9.30 (s, H, D_2O-exchangeable, *NH*), 11.50 (s, H, COO*H*); ^{13}C NMR (DMSO-d_6) δ (ppm): 166.50, 165.10, 164.40, 163.10,160.50, 160.90, 151.10, 144.20, 136.10, 136.00,132.10, 130.80, 130.90, 127.30, 127.10, 125.20, 125.10, 124.20, 123.20, 123.00, 120.00, 116.20, 116.10, 50.50, 45.70, 37.50, 31.50, 30.20, 27.20, 22.10, 22.00, 21.00, 12.50; ESI-MS: m/z = 631 (M^+).

2-(4-methoxybenzylideneamino)-5-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxo-3,4-dihydroquinazoline-3-carboxamidino)pentanoic acid (9c)

Recryst. Solvent: Ethanol; yellow solid; Yield 40%; mp 205-207 °C; Anal. Calc. (%) for $C_{34}H_{38}N_6O_6$ (626): C: 65.16, H: 6.11, N: 13.41; found: C: 65.12, H: 6.08, N: 13.39; FT-IR ν_{max} (cm^{-1}): 3450-3570 (OH), 3320 (NH), 1670 ($C=O_{quinazolinone}$), 1640 ($C=O_{carboxyl}$), 1615 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.05 (d, 6H, $CH_2CH(CH_3)_2$), 1.42 (d, 3H, $CHCH_3$), 1.65 (m, 2H, NH $CH_2CH_2CH_2$), 1.70 (m, 2H, NH $CH_2CH_2CH_2$), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.35 (d, 2H, $CH_2CH(CH_3)_2$), 2.55 (m, 2H, NH $CH_2CH_2CH_2$) , 3.33 (q, H, $CHCH_3$), 3.40 (s, 3H, OCH_3), 4.10 (q, H, $NCHCOOH$), 5.30 (s, H, D_2O-exchangeable, , (C=*NH*), 7.10–8.20 (m, 11H, Ar—H)) , 8.95 (s, H, *CH*=N), 9.20 (s, H, D_2O-exchangeable, N*H*),11.10 (s, H, COO*H*); ^{13}C NMR (DMSO-d_6) δ (ppm): 167.50, 166.10, 165.35, 163.40,160.90, 16010, 150.10, 145.20, 136.20, 136.10,132.70, 130.30, 130.60, 127.20, 127.10, 125.30, 125.20, 124.10, 123.10, 123.00, 120.50, 115.20, 115.10,55.90, 50.00, 45.30, 37.20, 31.10, 30.00, 27.00, 22.50, 22.20, 21.10, 12.80; ESI-MS: m/z = 626 (M^+).

2-(4-(dimethylamino)benzylideneamino)-5-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxo-3,4-dihydroquinazoline-3-carboxamidino)pentanoic acid (9d)

Recryst. Solvent: Ethanol; Pale yellow solid; Yield 40%; mp 250-252 °C; Anal. Calc. (%) for $C_{35}H_{41}N_7O_5$ (639): C: 65.71, H: 6.46, N: 15.33; found: C: 65.69, H: 6.43, N: 15.31; FT-IR ν_{max} (cm^{-1}): 3440-3570 (OH), 3300(NH), 1665 ($C=O_{quinazolinone}$), 1650 ($C=O_{carboxyl}$), 1615 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.40 (d, 3H, $CHCH_3$), 1.60 (m, 2H, NH $CH_2CH_2CH_2$), 1.75 (m, 2H, NH $CH_2CH_2CH_2$), 1.92 (m, H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 2.50 (m, 2H, NH $CH_2CH_2CH_2$) , 2.75 (d, 6H, $N(CH_3)_2$), 3.39 (q, H, $CHCH_3$), 4.20 (q, H, $NCHCOOH$), 5.60 (s, H, D_2O-exchangeable, C=*NH*), 7.10–8.20 (m, 11H, Ar—H)), 9.00 (s, H, *CH*=N), 9.40 (s, H, D_2O-exchangeable, *NH*), 11.20 (s, H, COO*H*); ^{13}C NMR (DMSO-d_6) δ (ppm): 167.00, 166.50, 164.25, 162.40,160.40, 160.10, 149.10, 144.20, 136.00, 136.00,131.70, 130.20, 130.10, 127.10, 127.00, 125.10, 125.00, 124.50, 123.30, 123.10, 120.10, 115.15, 115.10, 50.00, 44.30, 40.35, 40.30, 37.10, 31.50, 30.10, 27.20, 22.20, 22.10, 21.00, 12.30; ESI-MS: m/z = 639 (M^+).

2-(4-nitrobenzylideneamino)-5-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxo-3,4-dihydroquinazoline-3-carboxamidino)pentanoic acid (9e)

Recryst. Solvent: Methanol; browen solid; Yield 45%; mp 240-242 °C; Anal. Calc. (%) for $C_{33}H_{35}N_7O_7$ (641): C: 61.77, H: 5.50, N: 15.28; found: C: 61.75, H: 5.48, N: 15.25; FT-IR ν_{max} (cm^{-1}): 3445-3590 (OH), 3310 (NH), 1665 ($C=O_{quinazolinone}$), 1650 ($C=O_{carboxyl}$), 1628 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.11(d, 6H, $CH_2CH(CH_3)_2$), 1.50 (d, 3H, $CHCH_3$), 1.55 (m, 2H, NH $CH_2CH_2CH_2$), 1.75 (m, 2H, NH $CH_2CH_2CH_2$), 1.92 (m, H, $CH_2CH(CH_3)_2$), 2.45(d, 2H, $CH_2CH(CH_3)_2$), 2.50 (m, 2H, NH $CH_2CH_2CH_2$) , 3.32 (q, H, $CHCH_3$), 4.10 (q, H, $NCHCOOH$), 5.50 (s, H, D_2O-exchangeable, , C=*NH*), 7.20–8.10 (m, 11H, Ar—H)), 8.95 (s, H, *CH*=N), 9.30 (s, H, D_2O-exchangeable, , *NH*), 10.80 (s, H, COO*H*); ^{13}C NMR (DMSO-d_6) δ (ppm): ^{13}C NMR (DMSO-d_6) δ (ppm): 168.00, 167.40, 165.20, 164.50,162.80, 161.95, 150.40, 144.30, 137.20, 137.10,132.40, 130.50, 130.30, 127.40, 127.10, 125.40, 125.20, 124.00, 123.10, 123.00, 120.10,116.20, 116.10, 50.50, 47.30, 37.50, 33.50, 30.20, 28.10, 24.50, 24.30, 21.50, 12.00; ESI-MS: m/z = 641 (M^+).

2-(4-hydroxybenzylideneamino)-6-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)hexanoic acid (10a)

Recryst. Solvent: Methanol; Bale yellow solid; Yield 35%; mp 200-202°C; Anal. Calc. (%) for $C_{33}H_{36}N_4O_6$ (584): C: 67.79, H: 6.21, N: 9.58; found: C: 67.77, H: 6.18 N: 9.55; FT-IR ν_{max} (cm^{-1}):): 3400-3600 (OH), 1670 ($C=O_{quinazolinone}$), 1640 ($C=O_{carboxyl}$), 1610 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.09 (d, 6H, $CH_2CH(CH_3)_2$), 1.35 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.42 (d, 3H, $CHCH_3$), 1.50 (m, 2H, $NHCH_2CH_2CH_2CH_2$), 1.90 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 2.10 (m, H, $CH_2CH(CH_3)_2$), 2.50(d, 2H, $CH_2CH(CH_3)_2$), 3.20 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 3.30 (q, H, $CHCH_3$), 4.00 (q, H, $NCHCOOH$), 5.5 (s, H, OH), 7.10 – 8.00 (m, 11H, Ar—H)), 8.85 (s, 1H, CH=N), 11.40 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.20, 165.10, 162.80,160.50, 160.20 155.80, 145.50, 137.30, 137.00, 132.50, 130.80, 130.10, 127.50, 127.20, 125.30, 125.20, 125.00, 122.60, 122.30, 121.00,116.20, 116.00, 53.00, 43.10, 40.20, 32.20, 31.40, 30.00, 28.50, 24.50, 23.80, 22.40, 12.80; ESI-MS: m/z = 584 (M^+).

2-(4-chlorobenzylideneamino)-6-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)hexanoic acid (10b)

Recryst. Solvent: Methanol; Bale yellow solid; Yield 30%; mp 195-197°C; Anal. Calc. (%) for $C_{33}H_{35}N_4O_5Cl$ (602): C: 65.72, H: 5.85, N: 9.29; found: C: 65.69, H: 5.82, N: 9.26; FT-IR ν_{max} (cm^{-1}):): 3430-3620 (OH), 1674 ($C=O_{quinazolinone}$), 1645 ($C=O_{carboxyl}$), 1612 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.30 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.45 (d, 3H, $CHCH_3$), 1.60 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.85 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 2.00 (m, H, $CH_2CH(CH_3)_2$), 2.44 (d, 2H, $CH_2CH(CH_3)_2$), 3.10 (m, 2H, $NHCH_2CH_2CH_2CH_2$), 3.35 (q, H, $CHCH_3$), 3.90 (q, H, $NCHCOOH$), 7.30 – 8.10 (m, 11H, Ar—H)), 8.95 (s, 1H, CH=N), 11.60 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.10, 166.25, 164.70,162.50, 162.20 160.10, 147.30, 136.30, 136.10, 133.40, 132.60, 132.10, 129.50, 129.20, 126.30, 126.20, 125.00, 122.10, 122.00, 121.60,120.30, 120.10, 50.00, 45.10, 42.20, 35.20, 32.40, 31.00, 29.50, 25.55, 24.85, 21.45, 13.50; ESI-MS: m/z = 602 (M^+).

2-(4-methoxybenzylideneamino)-6-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)hexanoic acid (10c)

Recryst. Solvent: Methanol; Bale yellow solid; Yield 40%; mp 200-202°C; Anal. Calc. (%) for $C_{34}H_{38}N_4O_6$ (598): C: 68.21, H: 6.40, N: 9.36; found: C: 68.19, H: 6.38, N: 9.33; FT-IR ν_{max} (cm^{-1}):): 3410-3650 (OH), 1675 ($C=O_{quinazolinone}$), 1640 ($C=O_{carboxyl}$), 1610 (C=N). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.35 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.40 (d, 3H, $CHCH_3$), 1.50 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.75 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.90 (m, H, $CH_2CH(CH_3)_2$), 2.42 (d, 2H, $CH_2CH(CH_3)_2$), 3.30 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 3.35 (q, H, $CHCH_3$), 3.60 (q, H, $NCHCOOH$), 3.75 (s, 3H, OCH_3), 7.20 – 8.10 (m, 11H, Ar—H)), 9.10 (s, 1H, CH=N), 11.60 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.10, 165.20, 163.30,162.80, 162.50, 161.50, 148.30, 137.30, 135.30, 134.45, 133.40, 133.30, 129.10, 129.00, 126.10, 126.00, 124.50, 121.80, 121.50, 121.10,120.10, 120.00, 55.50, 48.00, 44.10, 43.20, 34.20, 31.40, 30.00, 29.20, 26.50, 25.80, 22.40, 12.50; ESI-MS: m/z = 598 (M^+).

2-(4-(dimethylamino)benzylideneamino)-6-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)hexanoic acid (10d)

Recryst. Solvent: Methanol; yellow solid; Yield 35%; mp 252-254 °C; Anal. Calc. (%) for $C_{35}H_{41}N_5O_5$ (611): C: 68.72, H: 6.76, N: 11.45; found: C: 68.70, H: 6.73, N: 11.41; FT-IR ν_{max} (cm^{-1}): 3400-3510 (OH), 1675 ($C{=}O_{quinazolinone}$), 1655 ($C{=}O_{carboxyl}$), 1610 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.15 (d, 6H, $CH_2CH(CH_3)_2$), 1.30 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.45 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.50 (d, 3H, $CHCH_3$), 1.65 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 2.00 (m, H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 2.55 (d, 6H, $N(CH_3)_2$), 3.10 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 3.40 (q, H, $CHCH_3$), 3.65 (q, H, $NCHCOOH$), 7.10 – 8.10 (m, 11H, Ar—H)), 9.10 (s, 1H, CH=N), 11.60 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 176.50, 166.20, 164.50,162.50, 162.10, 160.50, 147.30, 138.10, 135.50, 134.00, 132.10, 132.00, 129.50, 129.20, 125.10, 125.00, 123.50, 122.70, 122.50, 122.10,118.30, 118.10, 47.00, 46.10, 44.20, 40.20, 40.70, 36.20, 33.40, 30.10, 29.50, 27.50, 23.40, 23.40, 12.50; ESI-MS: m/z = 611(M^+).

2-(4-nitrobenzylideneamino)-6-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)hexanoic acid (10e)

Recryst. Solvent: Methanol; yellow solid; Yield 30%; mp 248-250 °C; Anal. Calc. (%) for $C_{33}H_{35}N_5O_7$ (613): C: 64.59, H: 5.75, N: 11.41; found: C: 64.62, H: 5.77, N: 11.44; FT-IR ν_{max} (cm^{-1}): 3430-3520 (OH), 1670 ($C{=}O_{quinazolinone}$), 1660 ($C{=}O_{carboxyl}$), 1612 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): 1.11 (d, 6H, $CH_2CH(CH_3)_2$), 1.25 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.40 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.45 (d, 3H, $CHCH_3$), 1.60 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.43 (d, 2H, $CH_2CH(CH_3)_2$), 3.20 (m, 2H, $NHCH_2CH_2CH_2$ CH_2), 3.40 (q, H, $CHCH_3$), 3.70 (q, H, $NCHCOOH$), 7.15 – 8.10 (m, 11H, Ar—H)), 9.00 (s, 1H, CH=N), 11.30 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 177.50, 165.20, 164.10,163.30, 163.10, 161.50, 145.30, 137.20, 134.50, 134.00, 132.40, 132.20, 129.20, 129.10, 125.50, 125.20, 123.40, 122.20, 122.10, 122.00,114.30, 114.10, 47.30, 46.20, 43.20, 35.20, 32.40, 31.10, 28.30, 25.50, 22.20, 22.10, 13.00; ESI-MS: m/z = 613(M^+).

General procedures for the synthesis of compounds $\mathbf{11_{a-d}}$

A mixture of benzoxazinone 4 (3.53g, 10 mmol) and the appropriate amino acids; glycine (0.75g, 10 mmol), alanine (0.89 g, 10 mmol), cysteine (1.21g, 10 mmol) and leucine (1.31 g, 10 mmol), in DMF (20 ml) was refluxed for 2 h. After the completion of reaction, the reaction mixture was poured into ice water to give a precipitate that was filtered off and recrystallized from ethanol to give $\mathbf{11_{a,d}}$.

2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)acetic acid(11a)

Recryst. Solvent: Methanol; white solid; Yield: 70 %; m. p: 270-272ºC; Anal. Calc. (%) for $C_{22}H_{23}N_3O_5$ (409): C: 64.54, H: 5.62, N: 10.26; found: C: 64.50, H: 5.60, N: 10.24; FT-IRν_{max} (cm^{-1}): 3450-3570 (OH), 1675 ($C{=}O_{quinazolinone}$), 1665 ($C{=}O_{carboxyl}$),1612 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm):): δ 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.30 (d, 3H, $CHCH_3$), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.50 (d, 2H, $CH_2CH(CH_3)_2$), 3.40 (q, H, CHCH3), 4.71 (s, 2H, CH_2COOH), 7.10–8.20 (m, 7H, Ar—H)), 11.20 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 173.20, 165.10, 161.30, 154.00, 146.25, 137.60, 137.30, 128.601, 128.40, 126.50, 126.10, 125.70, 124.30, 123.10, 122.50, 45.50, 44.50, 31.10, 28.50, 22.60, 22.10, 12.50; ESI-MS: m/z = 409 (M^+).

2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)propanoic acid (11b)

Recryst. Solvent: Ethanol; white solid; Yield: 65 %; m. p: 280-282ºC; Anal. Calc. (%) for $C_{23}H_{25}N_3O_5$ (423): C, 65.24; H, 5.92; N, 9.92; found: C, 65.20; H, 5.95; N, 9.89; FT-IRν_{max} (cm^{-1}): 3440-3560 (OH), 1670 (C=$O_{quinazolinone}$), 1660 (C=$O_{carboxyl}$), 1610(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm):): δ 1.15 (d, 6H, $CH_2CH(CH_3)_2$), 1.44 (d, 3H, $CHCH_3$), 1.55 (d, 3H, $CHCH_3COOH$), 1.90 (m, H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 3.45 (q, H, *CH*CH3), 4.75 (q, 1H, *CH*CH_3COOH), 7.20–8.10 (m, 7H, Ar—H)), 11.45 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 174.10, 164.10, 163.30, 156.00, 147.25, 138.60, 138.30, 129.601, 129.40, 126.30, 126.20, 124.70, 124.30, 123.70, 122.30, 50.50, 45.50, 30.10, 28.10, 22.40, 22.10, 13.20, 12.50; ESI-MS: m/z = 423 (M^+).

2 2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanoic acid (11c)

Recryst. Solvent: DMF; Yellowish crystals; Yield: 70%; m. p: 240-242ºC; Anal. Calc. (%) for $C_{23}H_{25}N_3O_5S$ (455): C: 60.64, H: 5.53, N: 9.22; found: C: 60.60, H: 5.50, N: 9.20; FT-IRν_{max} (cm^{-1}): 3400-3500 (OH), 2540 (SH), 1675 (C=$O_{quinazolinone}$), 1665 (C=$O_{carboxyl}$), 1612(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm):): δ 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.40 (d, 3H, $CHCH_3$), 1.55 (s, 1H, SH), 1.90 (m, H, $CH_2CH(CH_3)_2$), 2.45 (d, 2H, $CH_2CH(CH_3)_2$), 3.35 (q, H, *CH*CH3), 4.70 (q, 1H, *CH*CH_2SH), 3.15 (d, 2H, $CHCH_2SH$),7.10–8.20 (m, 7H, Ar—H)), 11.50 (s, H, COOH); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.10, 165.10, 165.30, 153.00, 145.25, 138.50, 138.20, 129.50, 129.20, 126.50, 126.30, 123.50, 123.40, 123.50, 122.70, 55.60, 47.50, 30.40, 28.10, 24.50, 22.10, 22.00, 12.60; ESI-MS: m/z = 455 (M^+).

2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-4-methylpentanoic acid (11d)

Recryst. Solvent: Ethanol; white solid; Yield: 75 %; m. p: 277-280ºC; Anal. Calc. (%) for $C_{26}H_{31}N_3O_5$ (465): C, 67.08;H, 6.71, N: 9.03; found: C: 67.10, H: 6.69, N: 9.00; FT-IRν_{max} (cm^{-1}): 3430-3550 (OH), 1675 (C=$O_{quinazolinone}$), 1660 (C=$O_{carboxyl}$), 1612(C=N); δ 1.15 (d, 6H, $CH_2CH(CH_3)_2$), 1.20 (d, 6H, $CHCH_2CH(CH_3)_2$), 1.45 (d, 3H, $CHCH_3$), 1.83 (m, H, $HCHCH_2CH(CH_3)_2$), 1.70 (t, 2H, $HCHCH_2CH(CH_3)_2$), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.50 (d, 2H, $CH_2CH(CH_3)_2$), 3.30 (q, H, *CH*CH3), 3.95 (t, H, *CH*$CH_2CH(CH_3)_2$), 7.20–8.20 (m, 7H, Ar—H), 11.50 (s, H, COOH). ^{13}C NMR (DMSO-d_6) δ (ppm): 174.50, 164.40, 163.60, 158.00, 145.25, 137.60, 137.30, 129.30, 129.10, 126.50, 126.00, 124.50, 124.30, 123.50, 122.20, 50.00, 45.10, 38.20, 31.10, 27.10, 22.90, 22.85, 22.50, 22.40, 22.35, 13.20; ESI-MS: m/z = 465 (M^+).

2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanoyl chloride (12)

Mixture of both compounds **11c** (4.55 g, 10 mmol) and $SOCl_2$ (% 95, 5 mL) was heated for 4 hours in an oil bath (80°C). At 50°C, the solvents were removed using a rotary evaporator. Ethyl ether (3x15 mL) was used to wash the residue. The product was crystallized from toluene and dried in vacuo at 70°C.

Recryst. Solvent: Toluene; pale Yellow crystals; Yield: 60 %; m. p: 205-207ºC; Anal. Calc. (%) for $C_{23}H_{24}N_3O_4SCl$ (473): C, 58.28; H, 5.10; N, 8.87; found: C: 58.25, H: 5.08, N: 8.85; FT-IRν_{max} (cm^{-1}): 2550 (SH), 1678 (C=$O_{quinazolinone}$), 1664 (C=$O_{carboxyl}$), 1610(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.12 (d, 6H, $CHCH_2CH(CH_3)_2$), 1.38 (d, 3H, $CHCH_3$), 1.50 (s, 1H, SH), 1.90 (m, H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 3.10 (d, 2H, $CHCH_2SH$), 3.35 (q, H, *CH*CH3), 4.75 (q, 1H, *CH*CH_2SH), 7.10–8.20 (m, 7H, Ar—H); ^{13}C

NMR (DMSO-d_6) δ (ppm): 176.10, 165.30, 164.10, 153.00, 147.20, 138.70, 138.10, 129.30, 129.10, 124.40, 124.30, 123.70, 123.20, 123.10, 121.60, 58.90, 46.40, 33.10, 27.10, 24.20, 22.15, 22.10, 13.00; ESI-MS: m/z = 473 (M^+).

2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanamide (13)

Method A.

A solution of compound 12 (4.73g, 10 mmol) in THF (20 mL) were cooled to 0°C. The obtained solution was slowly added to ammonium hydroxide solution (1.5 mL, 20 mmol) at 0°C, stirred, and kept at this temperature for 4 hours. The obtained solution was filtered. The solvents were removed on a rotary evaporator at 40°C. The residue was washed with ether (3 × 15 mL). The product was crystallized from ethyl acetate and dried in vacuo at 70° C.

Method B.

In dichloromethane, compound 11_c (4.55g, 10 mmol) was mixed with ammonium hydroxide (1.5 mL, 20 mmol) and 3 mmol triethylamine (Et_3N), then $SOCl_2$ (% 95, 5 mL) was added at room temperature under continuous stirring for 20 minutes. Evaporating the solvent under lower pressure was used to recover the reaction product. The resultant residue was dissolved in dichloromethane and washed with 1 N HCl and 1 N NaOH. The organic phase was dried (Na_2SO_4), and evaporated to dryness to afford the corresponding carboxylic amide.

Recryst. Solvent: Ethyl acetate; White crystals; Yield: Method A, (35 %); method B, (80%); m. p: 185-187°C; Anal. Calc. (%) for $C_{23}H_{26}N_4O_4S$ (454): C, 60.77; H, 5.77; N, 12.33; found: C: 60.75, H: 5.74, N: 12.30; FT-IRν_{max} (cm^{-1}): 3360-3339 (NH_2), 2500 (SH), 1675 ($C{=}O_{quinazolinone}$), 1645 ($C{=}O_{amide}$), 1612(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.12 (d, 6H, $CH_2CH(CH_3)_2$),1.40 (d, 3H, $CHCH_3$), 1.50 (s, 1H, SH), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 3.15 (d, 2H, $CHCH_2SH$), 3.35 (q, H, *CH*CH3), 4.74 (q, 1H, $CHCH_2SH$), 5.85 (br, s, 2H, NH_2), 7.10–8.20 (m, 7H, Ar—H); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.90, 165.60, 164.30, 154.10, 146.20, 137.70, 137.10, 128.30, 128.10, 124.50, 124.40, 123.60, 123.50, 123.20, 122.60, 57.590, 45.40, 32.10, 26.10, 25.20, 21.15, 21.10, 12.50; ESI-MS: m/z = 454 (M^+).

General procedure synthesis compound (14a-e)

The quinazolinyl amide derivative **13** (4.54, 10 mmol) was stirred at room temperature with the appropriate aldehyde namely; 4-hydroxybenzaldehyde (1.22g, 10 mmol), 4-chlorobenzaldehyde (1.40g, 10 mmol), 4- methoxybenzaldehyde (1.36g, 10 mmol), 4-*N*,*N*-dimethylbenzaldehyde (1.49g, 10 mmol) and 4-nitrobenzaldehyde (1.51g, 10 mmol) in absolute ethanol (20 mL), for 10 minutes before adding 0.1 mol/L aqueous solution of iodine/potassium iodide (10 mL). The completion of the reaction was monitored by TLC using chloroform:methanol (7:4) as eluent. The resulting crystalline products were filtered and rinsed with sodium thiosulphate solution 5 % and hot water, respectively. The crystalline products was dried and further crystallized from the proper solvent.

(28Z)-N-(4-hydroxybenzylidene)-2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanamide (14a)

Recryst. Solvent: Mehanol; Browen crystals; Yield: 80 %; m. p: 285-287ºC; Anal. Calc. (%) for $C_{30}H_{30}N_4O_5S$ (558): C, 64.50; H, 5.41; N, 10.03; found: C: 64.47, H: 5.39, N: 10.00; FT-IRν_{max} (cm^{-1}): 3375-3455 (OH), 2530 (SH), 1670 (C=$O_{quinazolinone}$), 1640 (C=O_{amide}), 1612(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.02 (d, 6H, $CH_2CH(CH_3)_2$), 1.40 (d, 3H, $CHCH_3$), 1.55 (s, 1H, SH), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.50 (d, 2H, $CH_2CH(CH_3)_2$), 3.35 (q, H, *CH*CH3), 3.43 (d, 2H, $CHCH_2$SH), 4.70 (q, 1H, *CH*CH_2SH), 5.10 (s, H, O*H*), 7.10–8.20 (m, 11H, Ar—H), 8.80 (s, H, *CH*=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 175.90, 165.60, 164.30, 163.70, 160.80, 154.10, 146.20, 137.70, 137.10, 130.70, 130.60 128.30, 128.10, 126.40, 124.50, 124.40, 123.60, 123.50, 123.20, 122.60, 116.50, 116.55, 57.50, 45.40, 32.10, 26.10, 25.20, 21.15, 21.10, 12.50; ESI-MS: m/z = 558 (M^+).

((28Z)-N-(4-chlorobenzylidene)-2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanamide (14b)

Recryst. Solvent: Mehanol; yellow crystals; Yield: 85 %; m. p: 265-267ºC; Anal. Calc. (%) for $C_{30}H_{29}ClN_4O_4S$ (577): C, 62.44; H, 5.07; N, 9.71; found: C: 62.40, H: 5.10, N: 9.68; FT-IRν_{max} (cm^{-1}): 2510 (SH), 1675 (C=$O_{quinazolinone}$), 1642 (C=O_{amide}), 1610(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.43 (d, 3H, $CHCH_3$), 1.50 (s, 1H, SH), 1.90 (m, H, $CH_2CH(CH_3)_2$), 2.40 (d, 2H, $CH_2CH(CH_3)_2$), 3.35 (q, H, *CH*CH3), 3.42 (d, 2H, $CHCH_2$SH), 4.70 (q, 1H, *CH*CH_2SH), 7.20–8.10 (m, 11H, Ar—H), 8.82 (s, H, *CH*=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 170.80, 165.10, 164.50, 163.8, 155.10, 147.20, 138.20, 138.10,136.10, 132.75, 132.70, 130.20, 130.10, 129.70, 128.60, 128.10, 125.50, 124.50, 123.40, 123.30, 123.20, 122.50, 58.10, 44.40, 33.10, 27.10, 26.20, 22.15, 22.10, 13.00; ESI-MS: m/z = 577 (M^+).

((28Z)-N-(4-methoxybenzylidene)-2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanamide (14c)

Recryst. Solvent: Mehanol; yellow crystals; Yield: 75 %; m. p: 265-266ºC; Anal. Calc. (%) for $C_{31}H_{32}N_4O_5S$ (572): C, 65.02; H, 5.63; N, 9.78; found: C: 65.05, H: 5.66, N: 9.81; FT-IRν_{max} (cm^{-1}): 2510 (SH), 1675 (C=$O_{quinazolinone}$), 1642 (C=O_{amide}), 1610(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.12 (d, 6H, $CH_2CH(CH_3)_2$), 1.45 (d, 3H, $CHCH_3$), 1.50 (s, 1H, SH), 2.00 (m, H, $CH_2CH(CH_3)_2$), 2.45 (d, 2H, $CH_2CH(CH_3)_2$), 3.30 (q, H, *CH*CH3), 3.45 (d, 2H, $CHCH_2$SH), 3.50 (s, 3H, OCH_3), 4.60 (q, 1H, *CH*CH_2SH), 7.20–8.10 (m, 11H, Ar—H), 8.83 (s, H, *CH*=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 171.50, 165.30, 164.20, 163.70, 162.50, 156.10, 146.20, 137.10,136.30, 132.70, 132.60, 130.50, 130.40, 129.50, 125.60, 124.60, 123.60, 123.50, 123.10, 122.40,114.50, 114.45, 57.10, 54.50 43.40, 31.10, 27.80, 26.70, 22.30, 22.20, 12.80; ESI-MS: m/z = 572 (M^+).

((28Z)-N-(4-(dimethylamino)benzylidene)-2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanamide (14d)

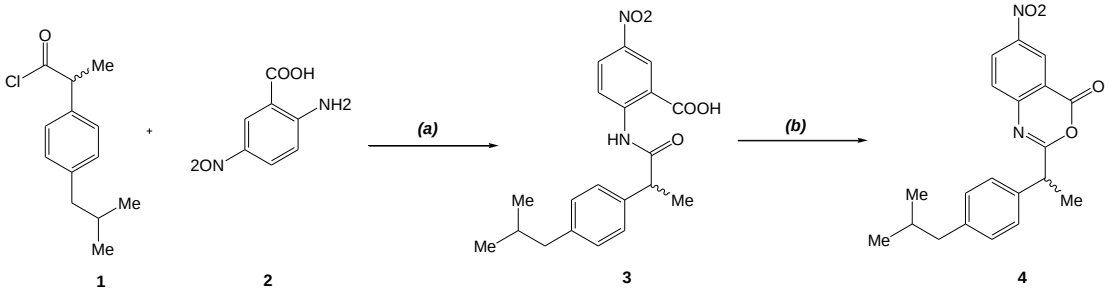
Recryst. Solvent: AcOH; Brown crystals; Yield: 84 %; m. p: 250-252ºC; Anal. Calc. (%) for $C_{32}H_{35}N_5O_4S$ (585): C, 65.62; H, 6.02; N, 11.96; found: C: 65.60, H: 5.98, N: 11.94; FT-IRν_{max} (cm^{-1}): 2515 (SH), 1673 (C=$O_{quinazolinone}$), 1644 (C=O_{amide}), 1615(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.10 (d, 6H, $CH_2CH(CH_3)_2$), 1.38 (d, 3H, $CHCH_3$), 1.53 (s, 1H, SH), 1.95 (m, H, $CH_2CH(CH_3)_2$), 2.40

(d, 2H, C*H*$_2$CH(CH$_3$)$_2$), 2.75 (d, 6H, N(C*H*$_3$)$_2$), 3.30 (q, H, *CH*CH3), 3.40 (d, 2H, CH*CH*$_2$SH), 4.50 (q, 1H, *CH*CH$_2$SH), 7.20–8.10 (m, 11H, Ar—H), 8.80 (s, H, *CH*=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 170.50, 166.25, 164.70, 163.20, 163.00, 158.30, 147.20, 138.10,137.30, 132.50, 132.40, 130.55, 130.45, 129.80, 125.50, 124.30, 123.50, 123.40, 123.00, 122.50,115.50, 115.45, 56.50, 46.50, 41.50, 41.45, 35.10, 29.30, 25.50, 23.30, 23.20, 12.40; ESI-MS: m/z = 585 (M^+, 100).

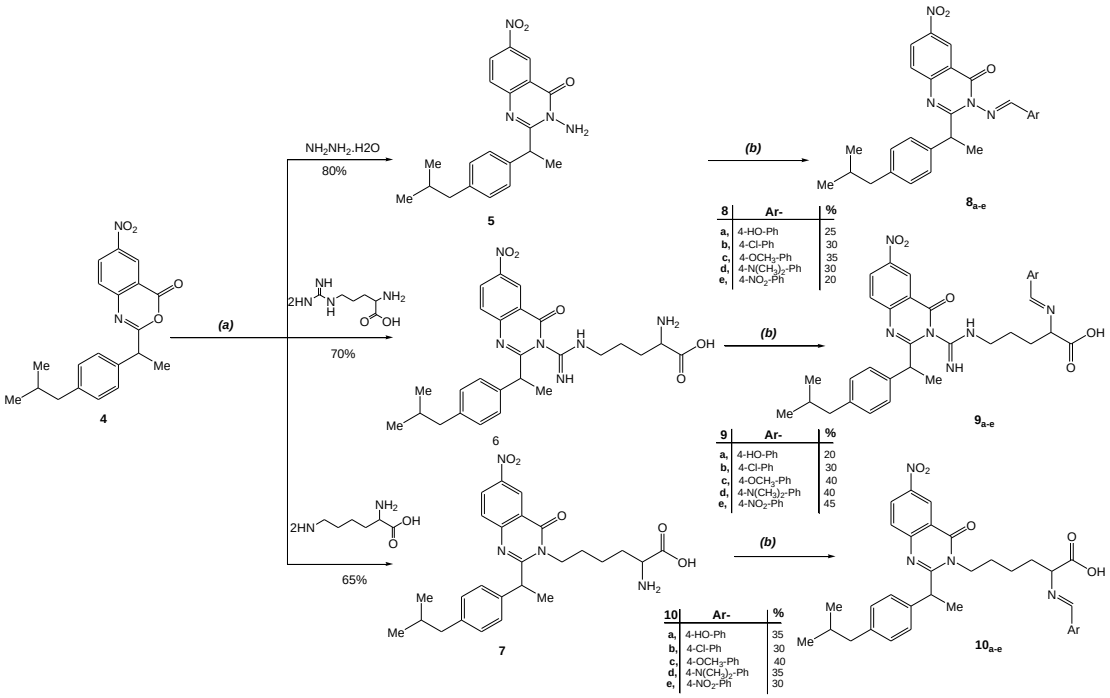
(28Z)-N-(4-nitrobenzylidene)-2-(2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4-oxoquinazolin-3(4H)-yl)-3-mercaptopropanamide (14e)

Recryst. Solvent: Ethanol; Browen crystals; Yield: 88 %; m. p: 280-282^{o}C; Anal. Calc. (%) for $C_{30}H_{29}N_5O_6S$ (587): C, 61.32; H, 4.97; N, 11.92; found: C: 61.30, H: 4.95, N: 11.96; FT-IRν_{max} (cm^{-1}): 2550 (SH), 1677 (C=O$_{quinazolinone}$), 1648 (C=O$_{amide}$), 1610(C=N); ^{1}H NMR (DMSO-d_6) δ (ppm): δ 1.05 (d, 6H, CH$_2$CH(C*H*$_3$)$_2$), 1.40 (d, 3H, CH*CH*$_3$), 1.50 (s, 1H, SH), 1.95 (m, H, CH$_2$*CH*(CH$_3$)$_2$), 2.44 (d, 2H, C*H*$_2$CH(CH$_3$)$_2$), 3.35 (q, H, *CH*CH3), 3.43 (d, 2H, CH*CH*$_2$SH),4.75 (q, 1H, *CH*CH$_2$SH), 7.10–8.20 (m, 11H, Ar—H), 8.82 (s, H, *CH*=N); ^{13}C NMR (DMSO-d_6) δ (ppm): 170.50, 165.10, 164.10, 163.30, 161.50, 156.10, 147.20, 138.70, 138.10, 131.50, 131.40 130.10, 130.00, 128.10, 128.00, 126.50, 124.20, 124.10, 123.40, 123.30, 123.00, 122.00, 55.50, 45.10, 32.50, 26.40, 25.10, 21.30, 21.20, 12.80; ESI-MS: m/z = 587 (M^+).

3.Schemes and tables



Scheme 1. Syntheses of 2-(1-(4-isobutylphenyl)ethyl)-6-nitro-4*H*-benzo[*d*][1,3]oxazin-4-one **(4),** Reagent and conditions: (*a*) Dry Pyridine, rt, 2hr, 70% ; (*b*) Ac_2O, reflux, 2hr, 65%.

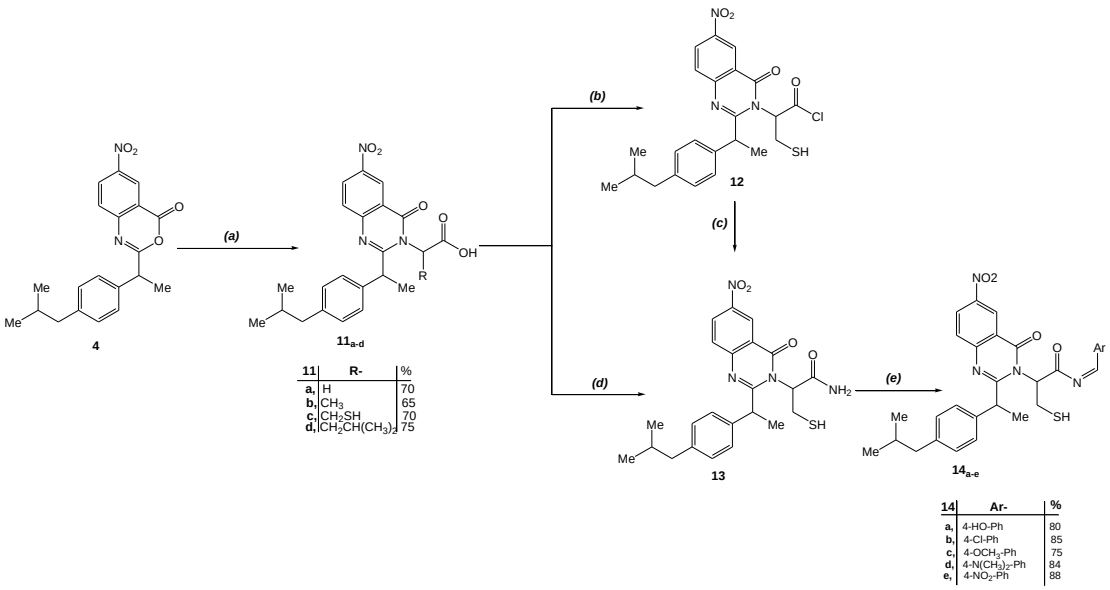


8	Ar-	%
a,	4-HO-Ph	25
b,	4-Cl-Ph	30
c,	4-OCH_3-Ph	35
d,	4-$N(CH_3)_2$-Ph	30
e,	4-NO_2-Ph	20

9	Ar-	%
a,	4-HO-Ph	20
b,	4-Cl-Ph	30
c,	4-OCH_3-Ph	40
d,	4-$N(CH_3)_2$-Ph	40
e,	4-NO_2-Ph	45

10	Ar-	%
a,	4-HO-Ph	35
b,	4-Cl-Ph	30
c,	4-OCH_3-Ph	40
d,	4-$N(CH_3)_2$-Ph	35
e,	4-NO_2-Ph	30

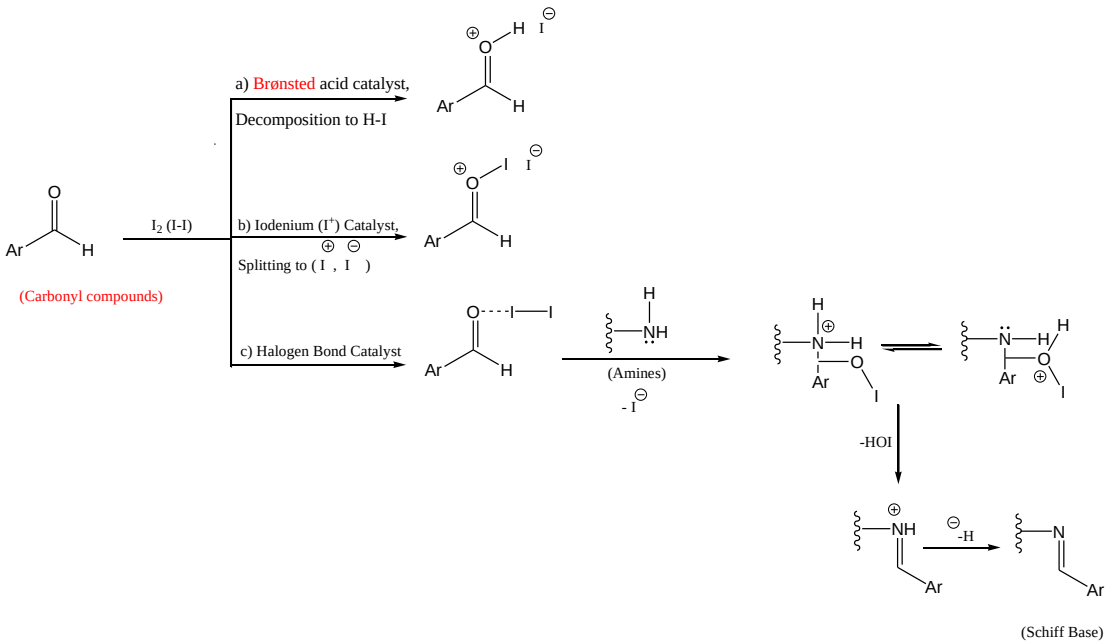
Scheme 2. Syntheses of compounds 5-10, Reagent and conditions: (*a*) Pyridine, Reflux, 4hr, (*b*) ArCHO, EtOH, Reflux, 8hr.



11	R-	%
a,	H	70
b,	CH_3	65
c,	CH_2SH	70
d,	$CH_2CH(CH_3)_2$	75

14	Ar-	%
a,	4-HO-Ph	80
b,	4-Cl-Ph	85
c,	4-OCH_3-Ph	75
d,	4-$N(CH_3)_2$-Ph	84
e,	4-NO_2-Ph	88

Scheme 3. (*a*) $NH_2CH(R)COOH$, DMF, Reflux, 2hr, (*b*)$SOCl_2$, Reflux, 4 hr *(c)* NH_4OH, THF, Stirring, ^0C, 4hr *(d)* NH_4OH, $SOCl_2$, ET_3N, DCM, Stirring, r.t, 20 min. *(e)* ArCHO, I2/KI, EtOH, Stirring, r.t.



Scheme 4. Proposed mechanism for an iodine-catalyzed Prins reaction. (Silva Jr, et al., 2009)

Table 1 In vitro nNOS and iNOS inhibition (%) observed in the presence of 1 mM concentration of final Schiff base compounds (8, 9, 10, and 14). **L-NAME** is included as control.

Compound	% iNOS inhibition[a]	% nNOS inhibition[a]
8_a	10.20 ± 1.21	6.20 ± 0.35
8_b	11.33 ± 0.98	8.45 ± 0.62
8_c	17.16 ± 1.04	10.04 ± 1.21
8_d	18.30 ± 0.52	10.82 ± 1.02
8_e	22.90 ± 1.40	14.32 ± 3.65
9_a	50.40 ± 1.52	42.31 ± 0.13
9_b	52.94 ± 0.41	42.33 ± 0.76
9_c	54.62 ± 1.43	54.11 ± 1.05
9_d	55.80 ± 1.84	42.80 ± 0.06
9_e	58.03 ± 1.50	60.55 ± 0.69
10_a	28.08 ± 0.40	15.32 ± 1.58
10_b	29.13 ± 1.06	17.46 ± 1.94
10_c	30.20 ± 0.58	38.05 ± 0.73
10_d	32.53 ± 1.23	15.89 ± 1.23
10_e	33.01 ± 0.35	41.08 ± 3.12
14_a	35.58 ± 2.33	24.03 ± 0.71
14_b	35.76 ± 2.32	24.20 ± 0.71
14_c	40.95 ± 2.03	28.01 ± 0.32
14_d	42.78 ± 1.10	29.06 ± 2.82
14_e	48.41 ± 1.76	29.58 ± 0.87
L-NAME [b]	77.01 ± 0.96	100.0 ± 1.03

[a] Data represent the mean ± SEM of the percentage of iNOS and nNOS inhibition produced by 1 mM concentration of each compound. Each value is the mean of three experiments performed by triplicate using recombinant iNOS and nNOS enzymes

[b] See Ref. Kilbourn and Griffith (1992)

Table 2 IC_{50} values (μM) for the iNOS and nNOS inhibition by the four most potent derivatives **8_e, 9_e, 10_e** and **14_e**

Compound	IC_{50} iNOS (μM)*	IC_{50} nNOS (μM) *
8_e	43.03±0.90	30.52±0.84
9_e	22.05±0.72	18.50±0.49
10_e	32.66±0.82	28.22±0.62
14_e	22.85±0.73	33.81±0.94

* Data were obtained by measuring percentage of inhibition with at least five concentrations of inhibitor

4. Spectra

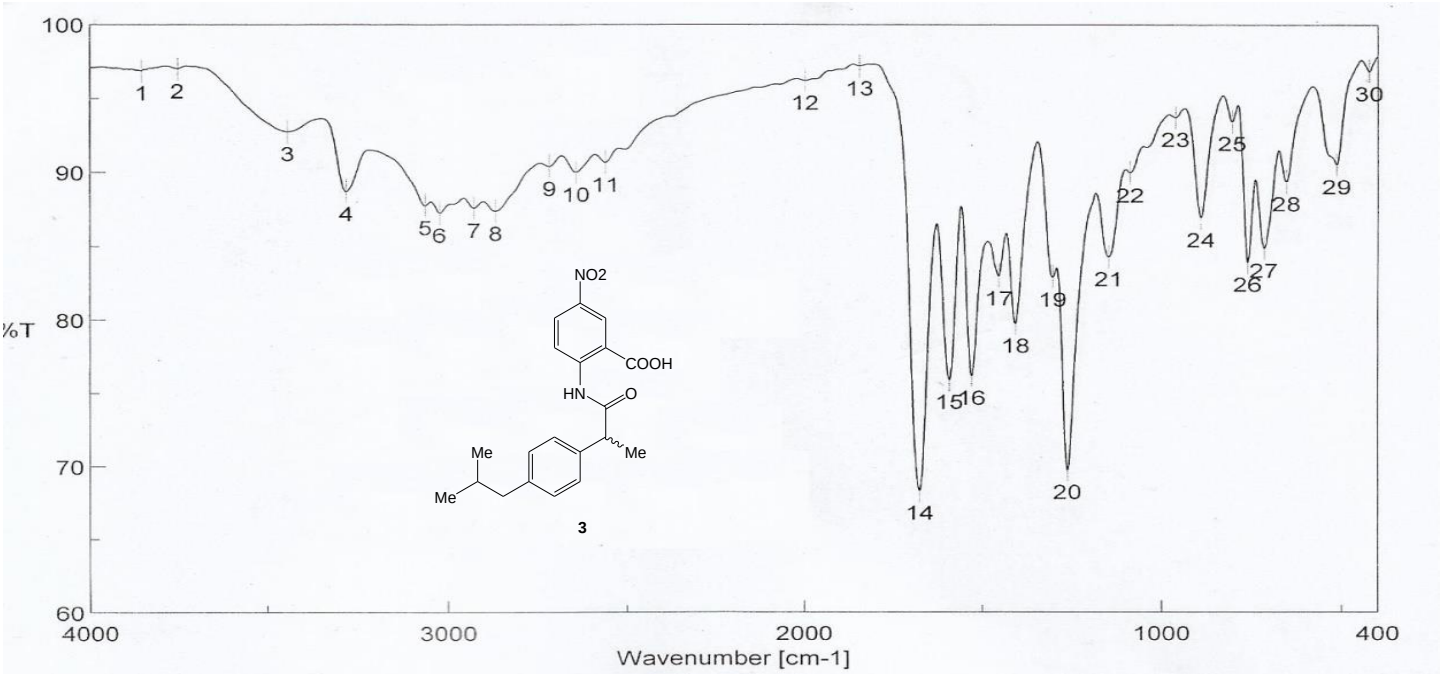


Figure S1. IR spectrum of compound **3**

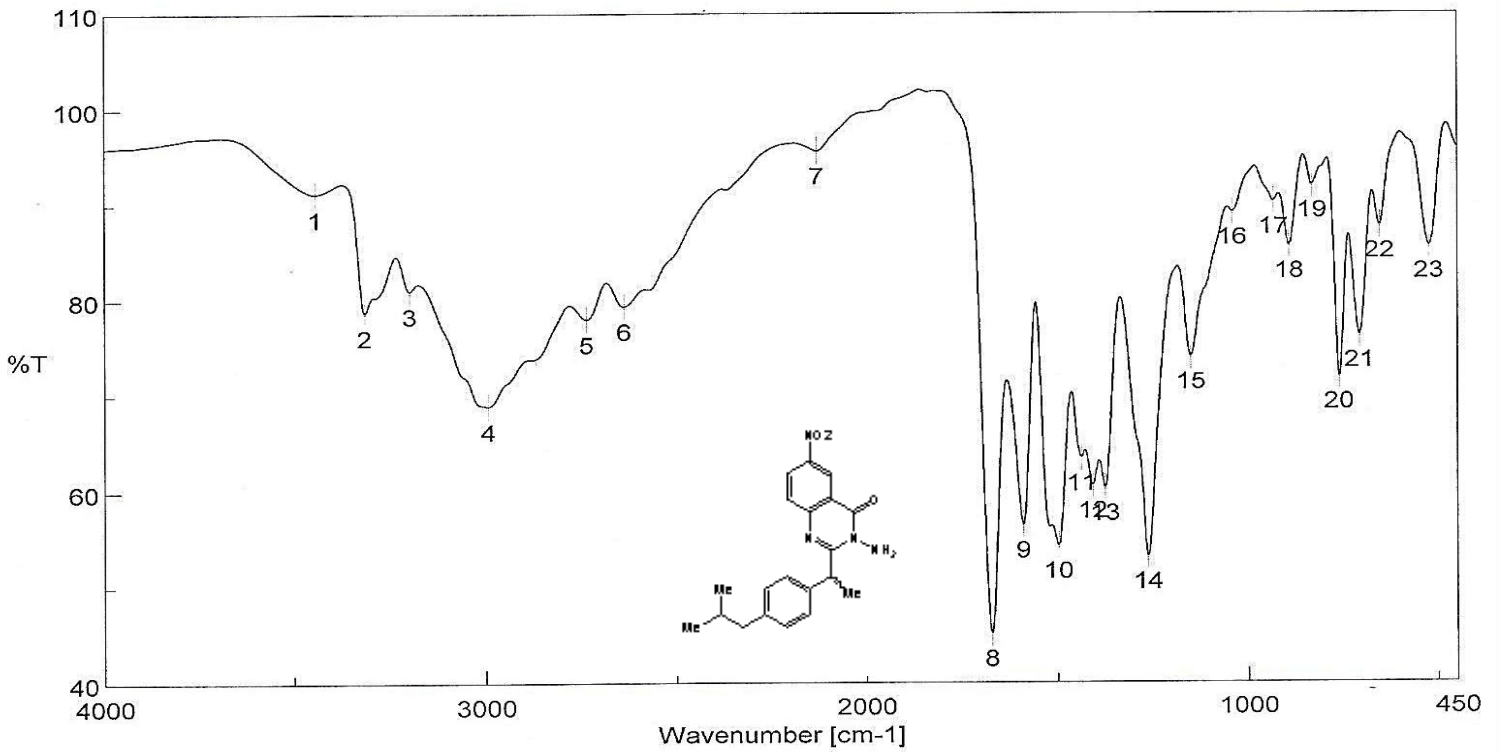


Figure S2. IR spectrum of compound **5**

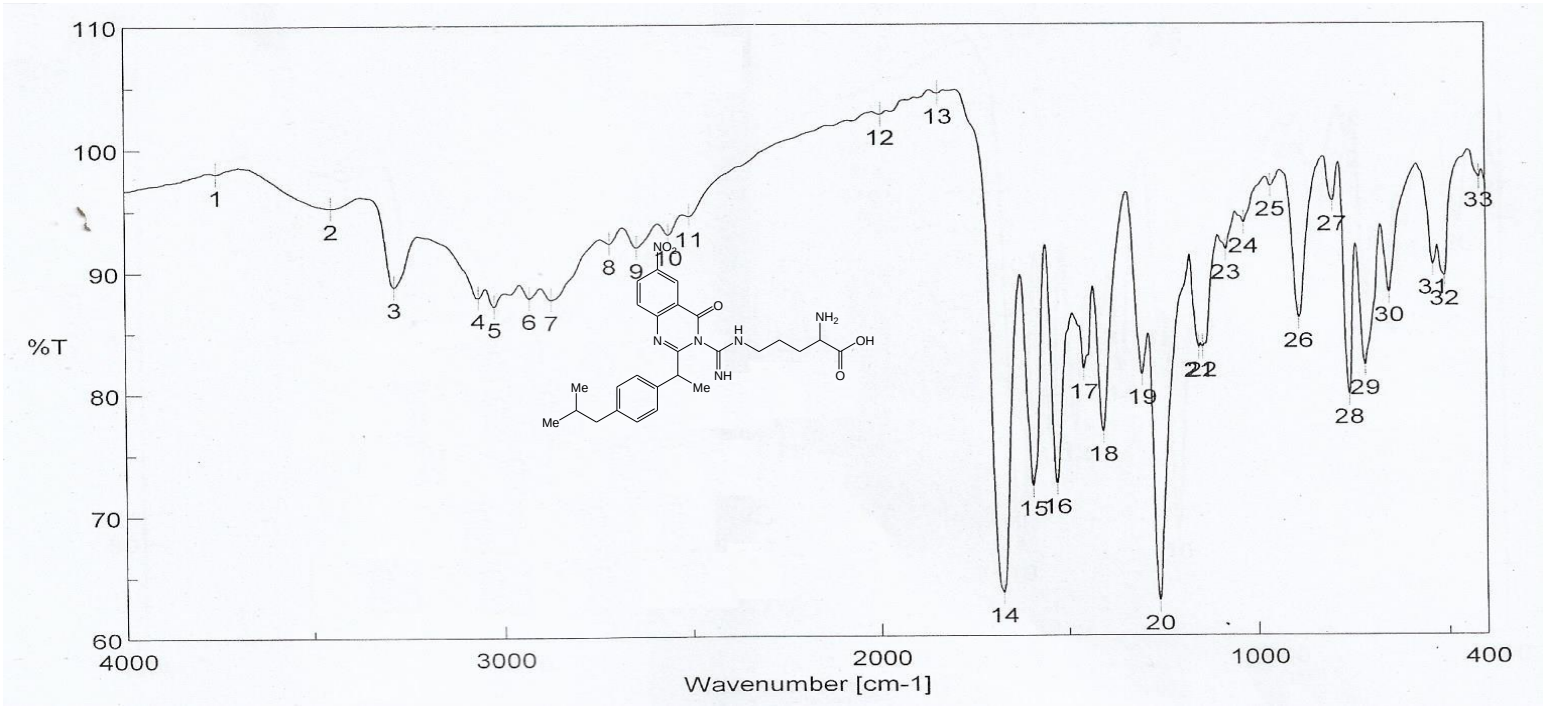


Figure S3. IR spectrum of compound **6**

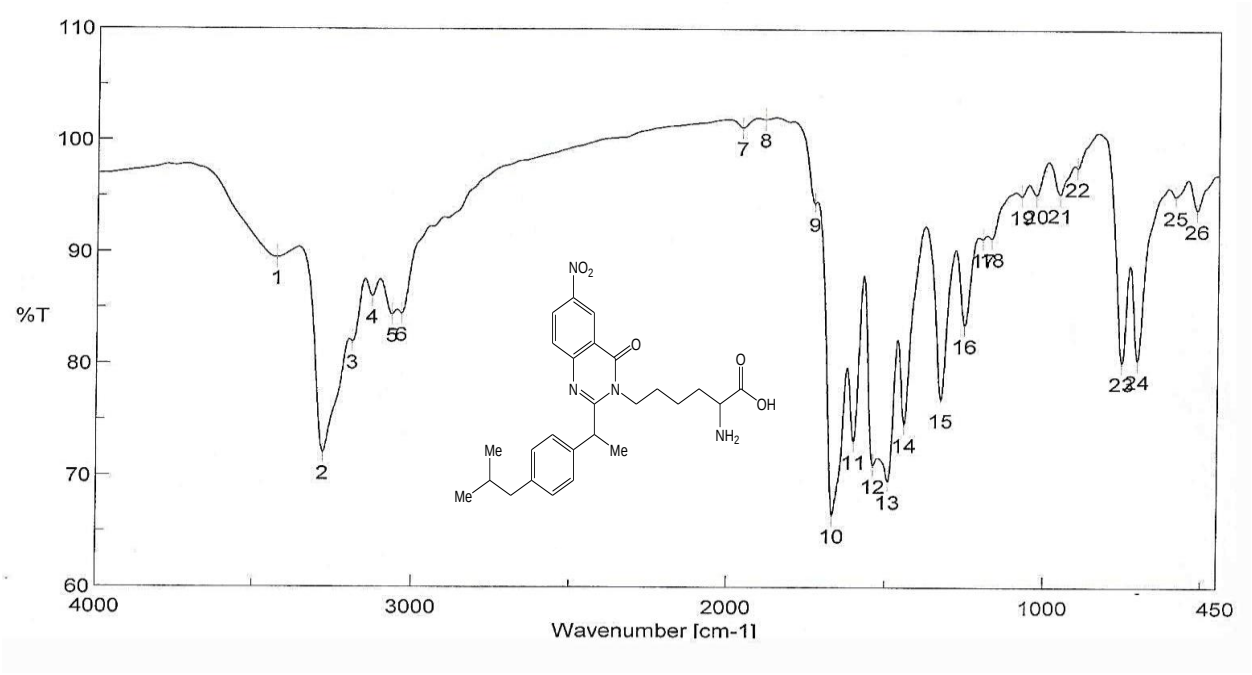


Figure S4. IR spectrum of compound **7**

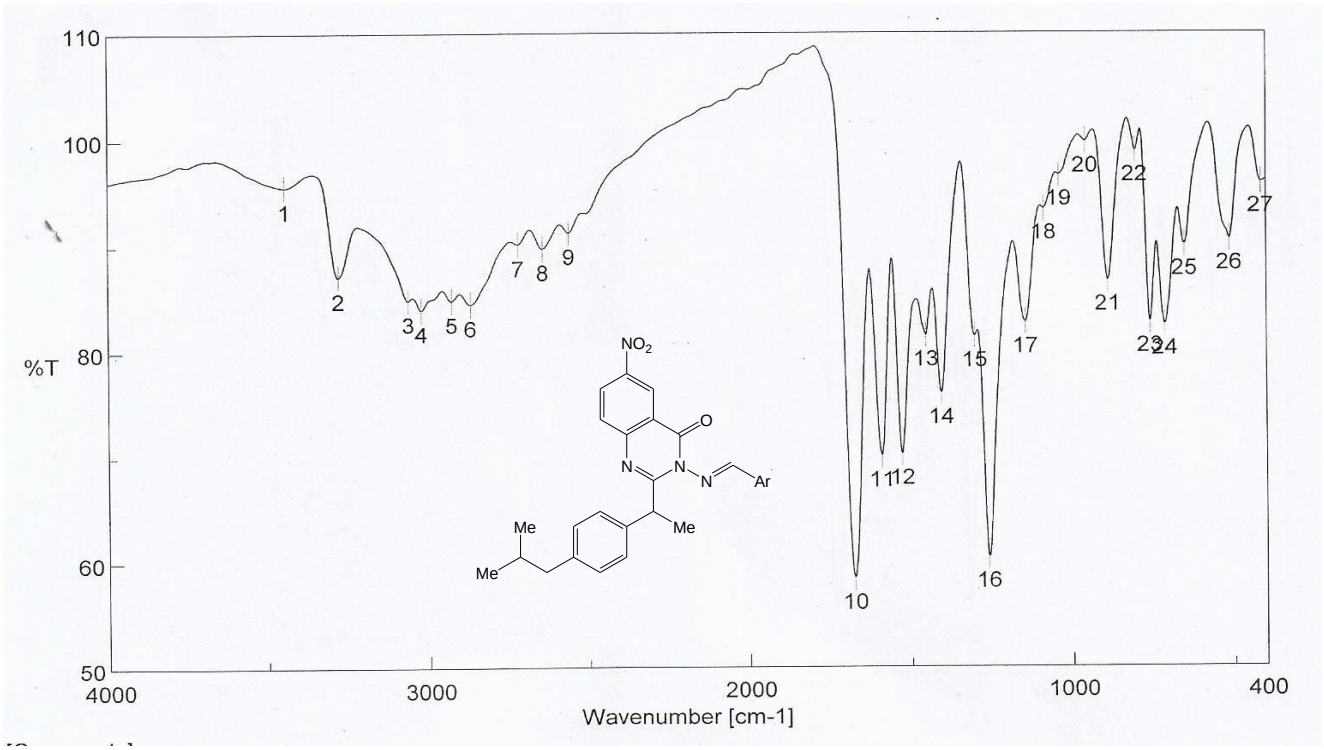


Figure S5. IR spectrum of compound **8a**

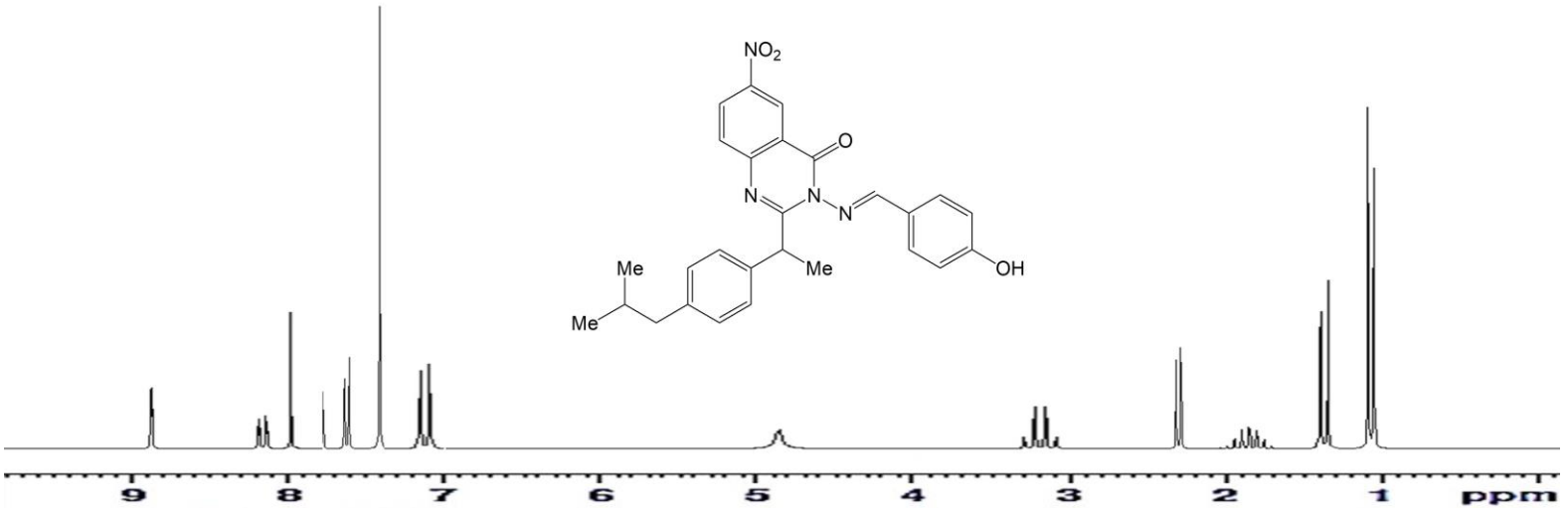


Figure S6. ^{1}H NMR (DMSO) spectrum of compound **8a**

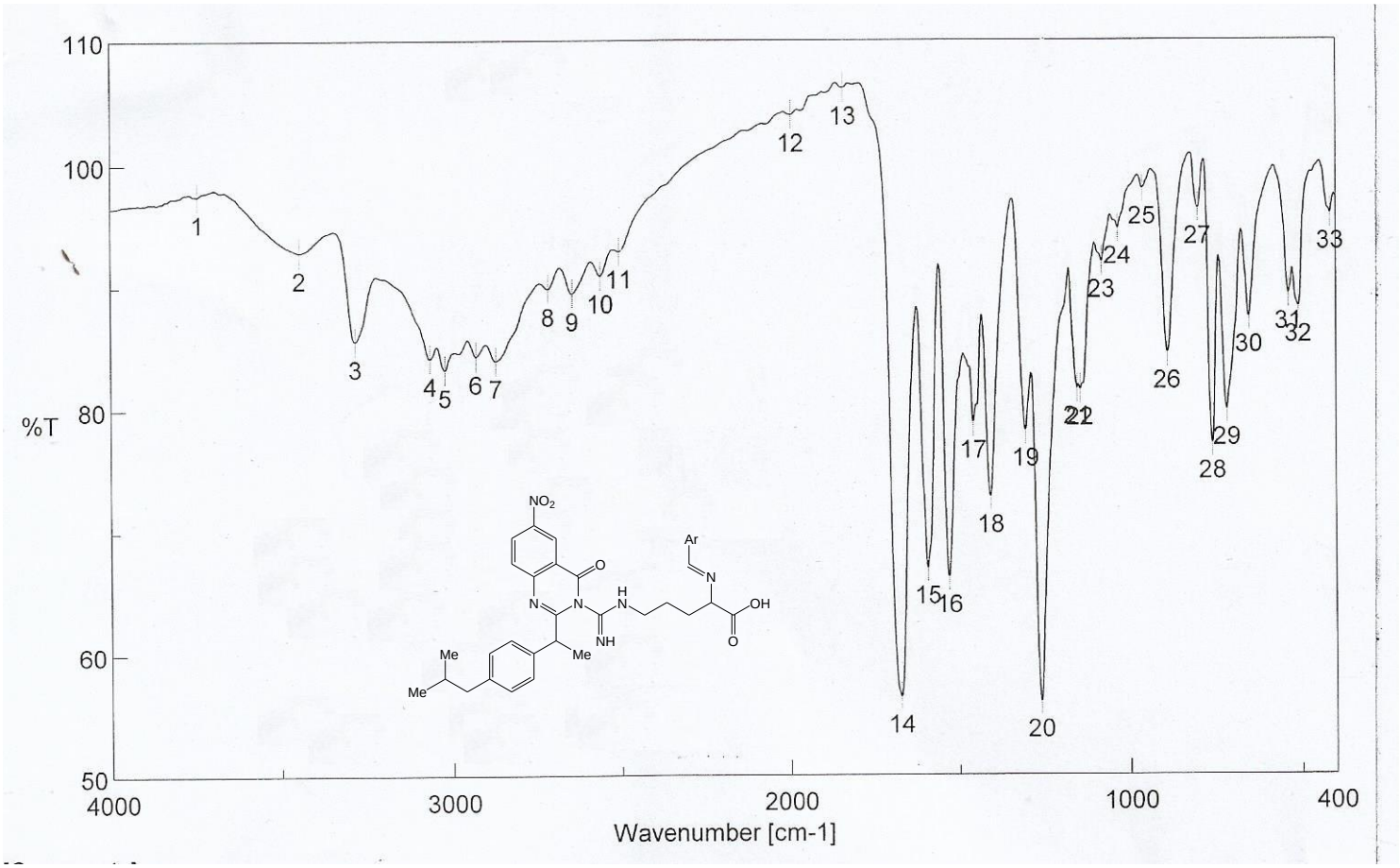


Figure S7. IR spectrum of compound **9a**

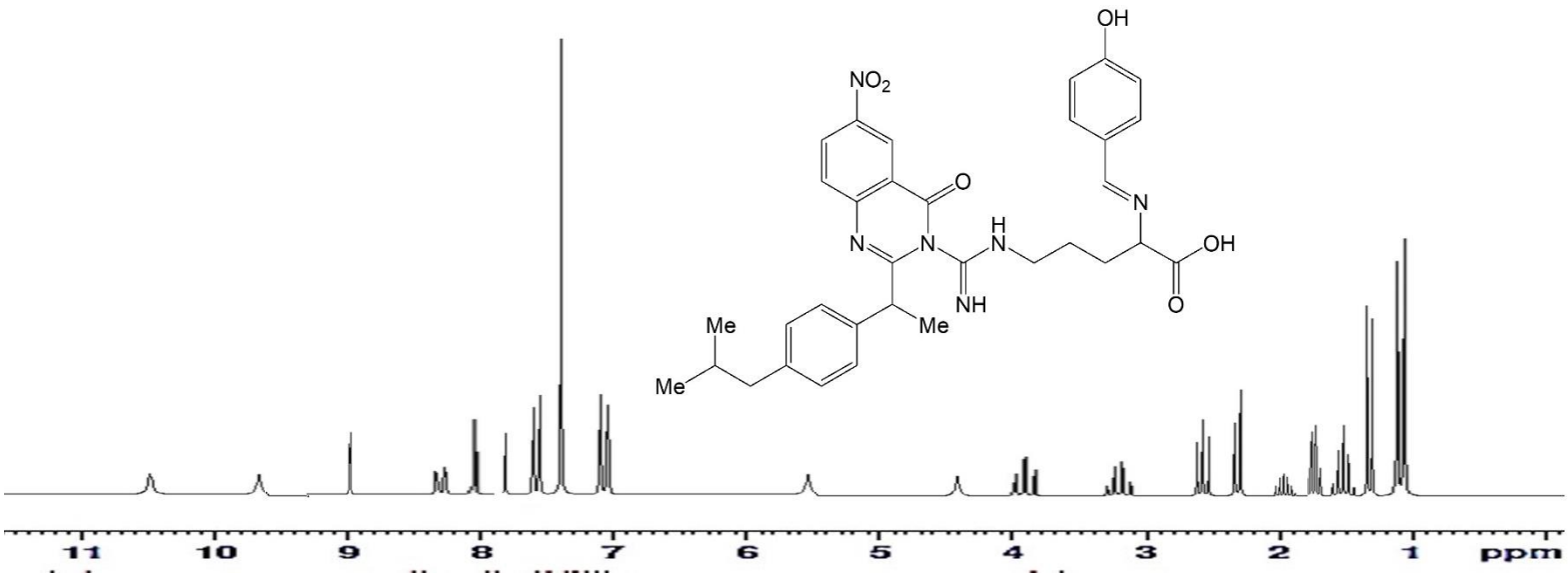


Figure S8. ^{1}H NMR (DMSO) spectrum of compound **9a**

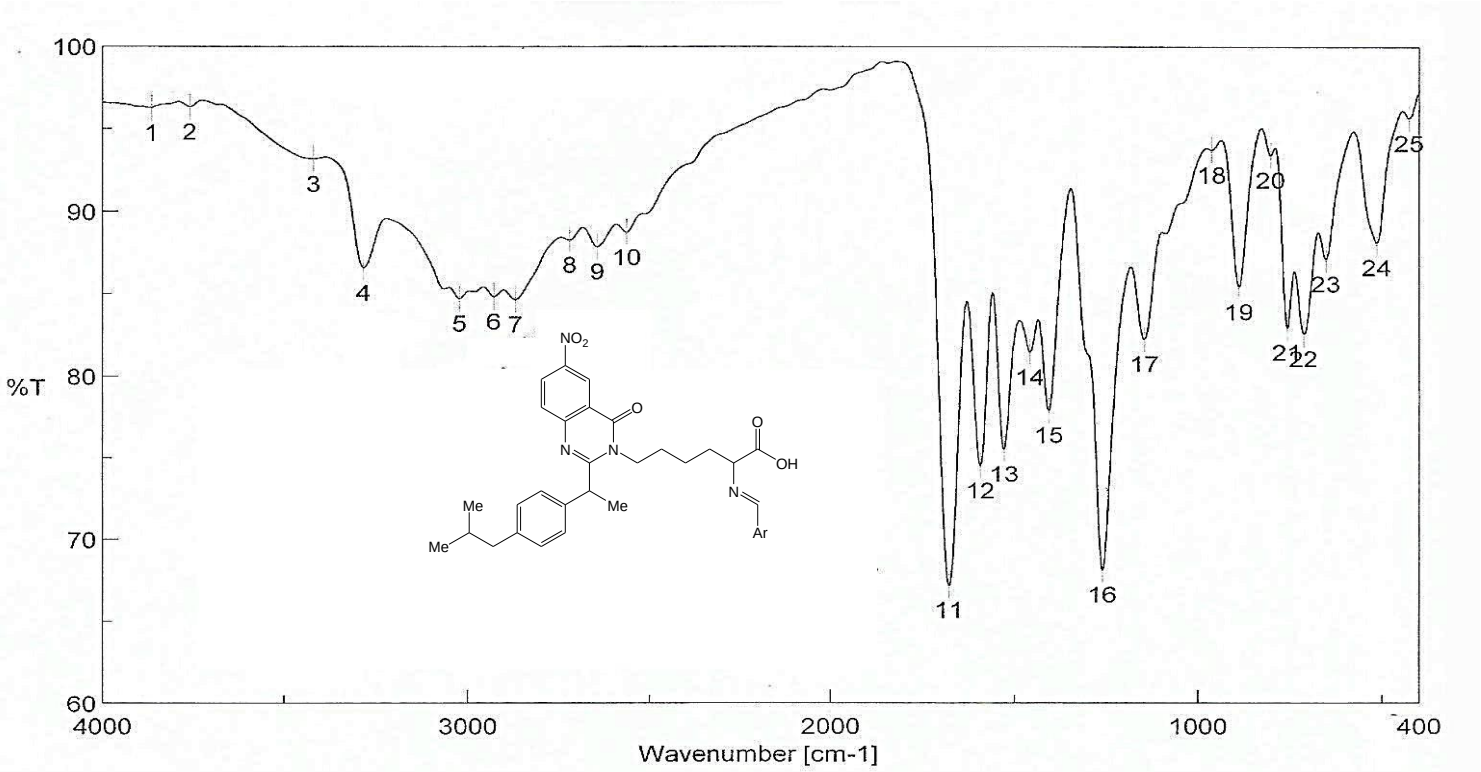


Figure S9. IR spectrum of compound **10a**

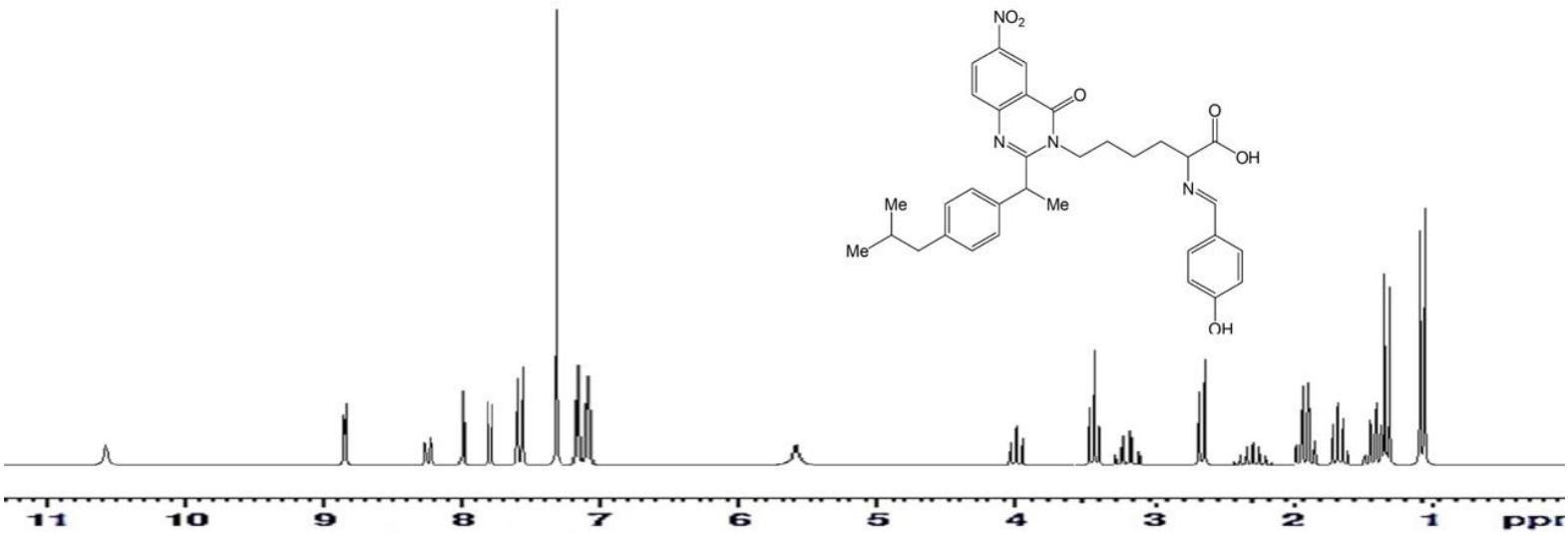


Figure S10. ^{1}H NMR (DMSO) spectrum of compound **10a**

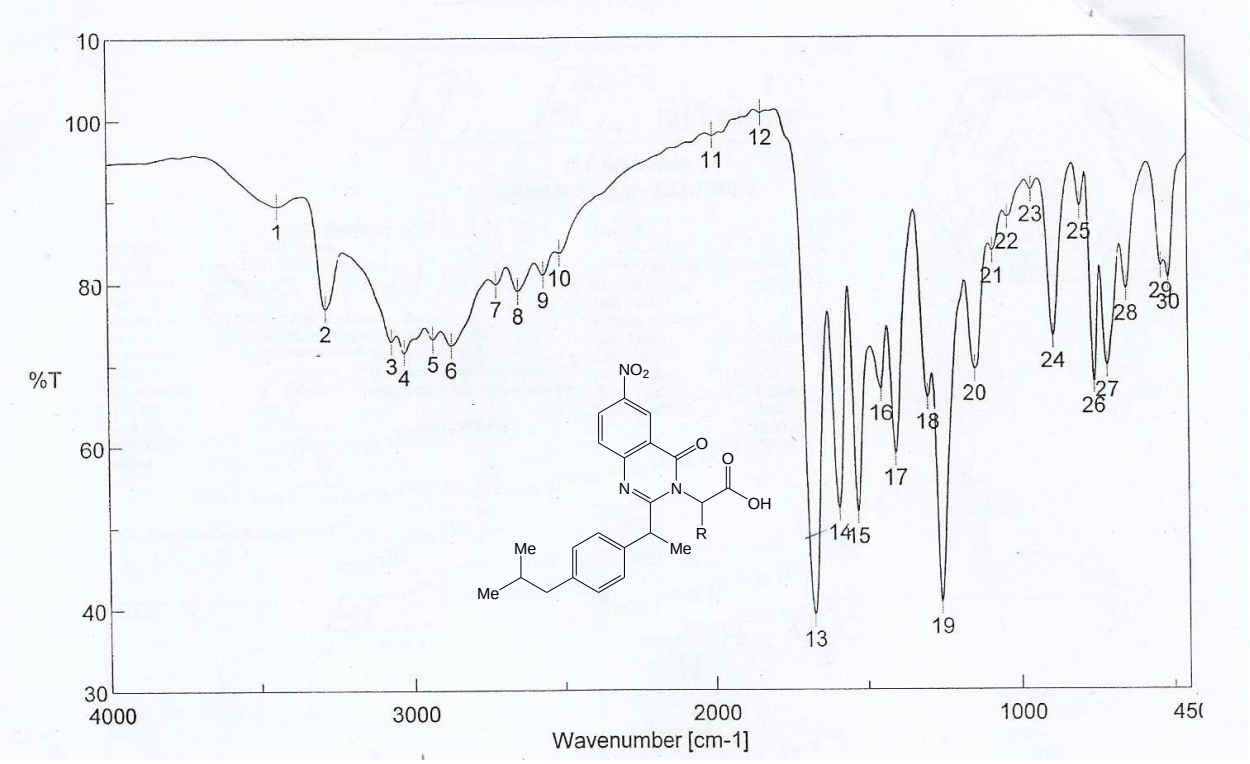


Figure S11. IR spectrum of compound 11**a**

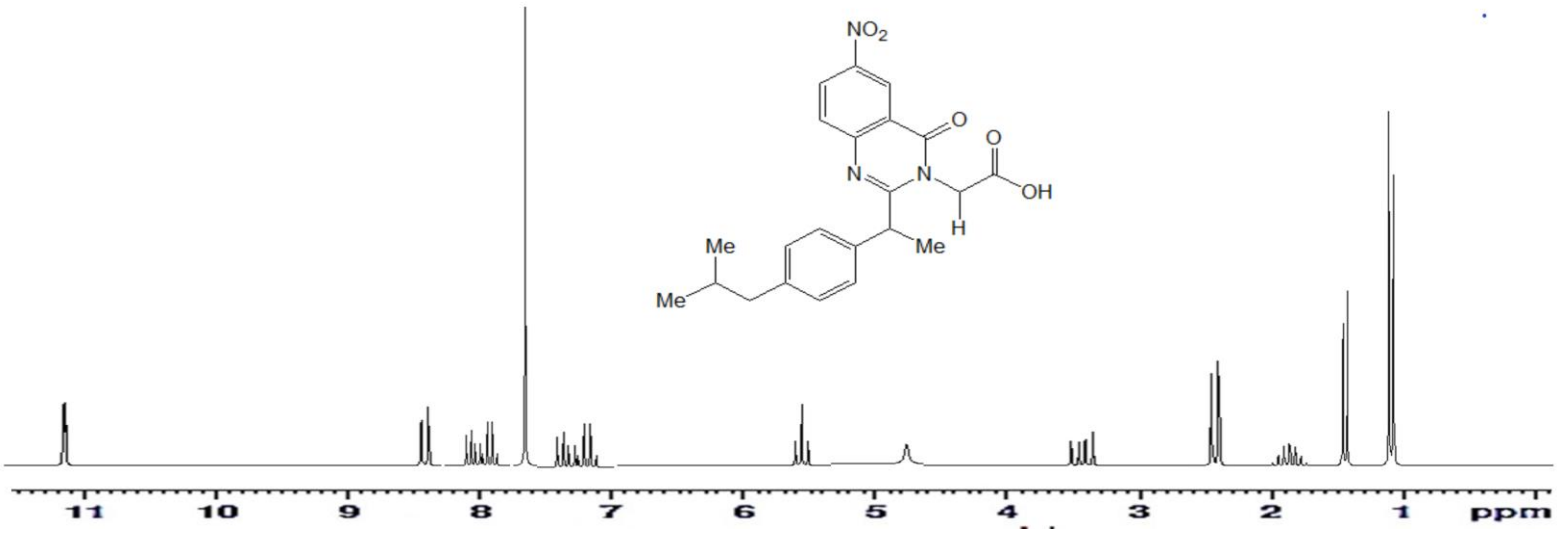


Figure S12. ^{1}H NMR (DMSO) spectrum of compound **11a**

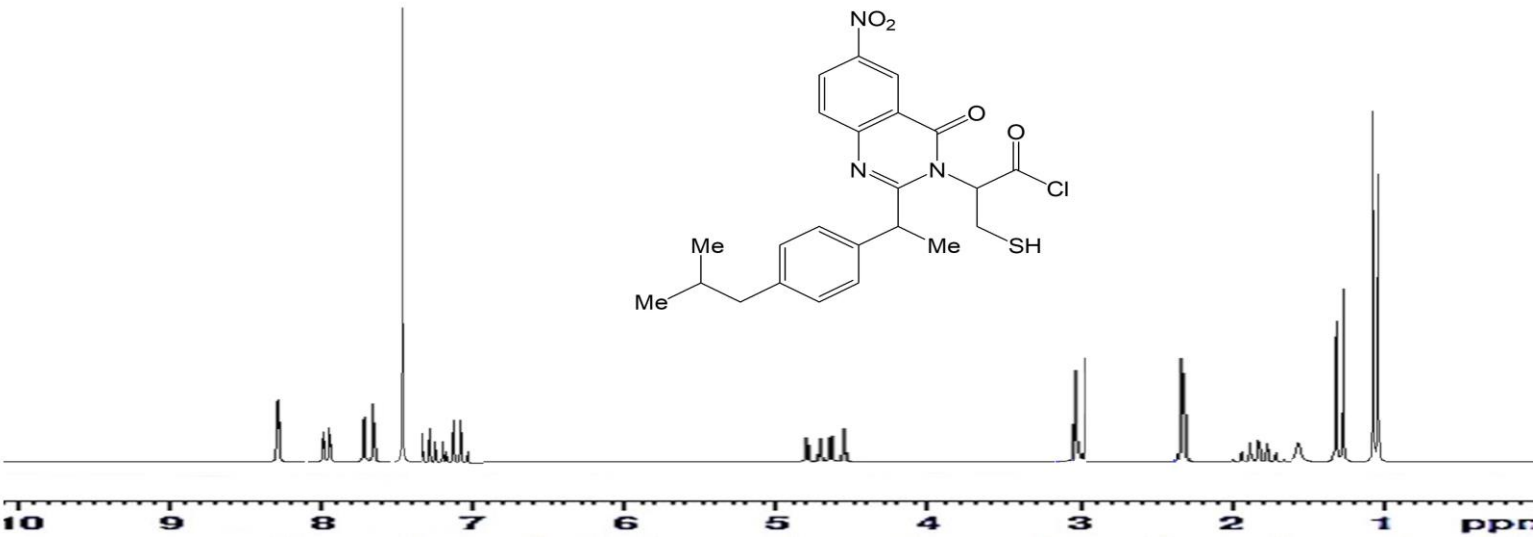


Figure S13. ^{1}H NMR (DMSO) spectrum of compound **12**

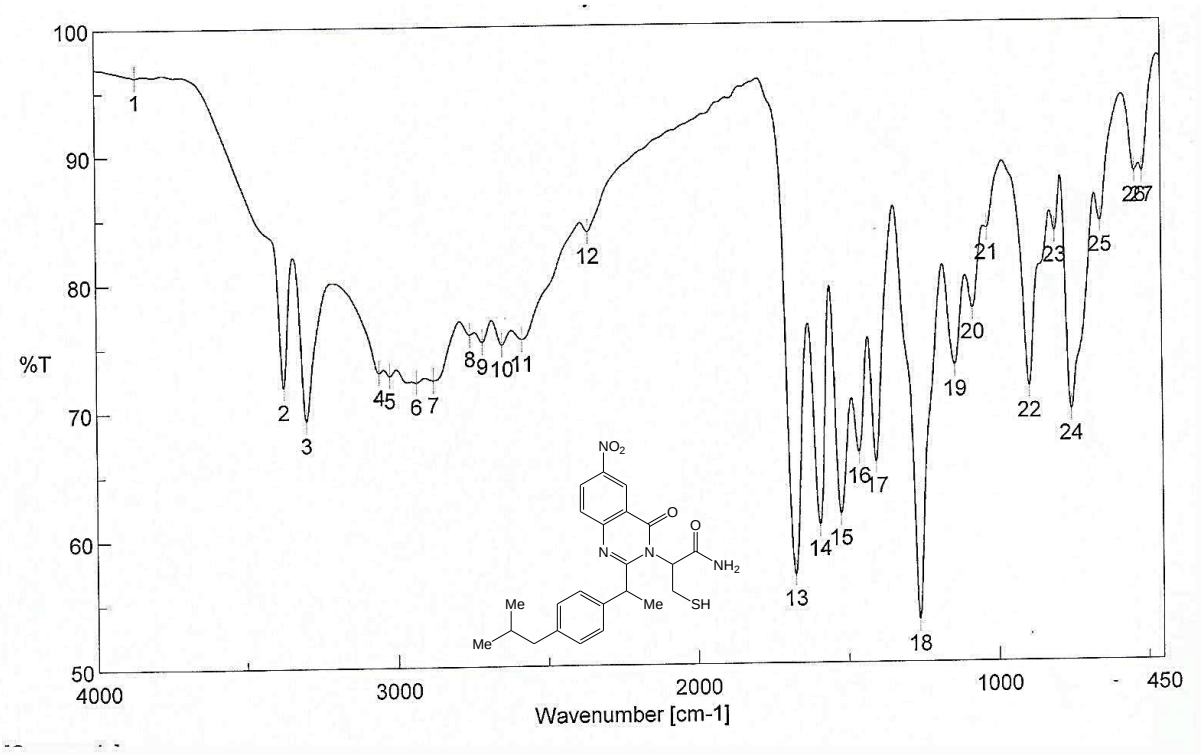


Figure S14. IR spectrum of compound **13**

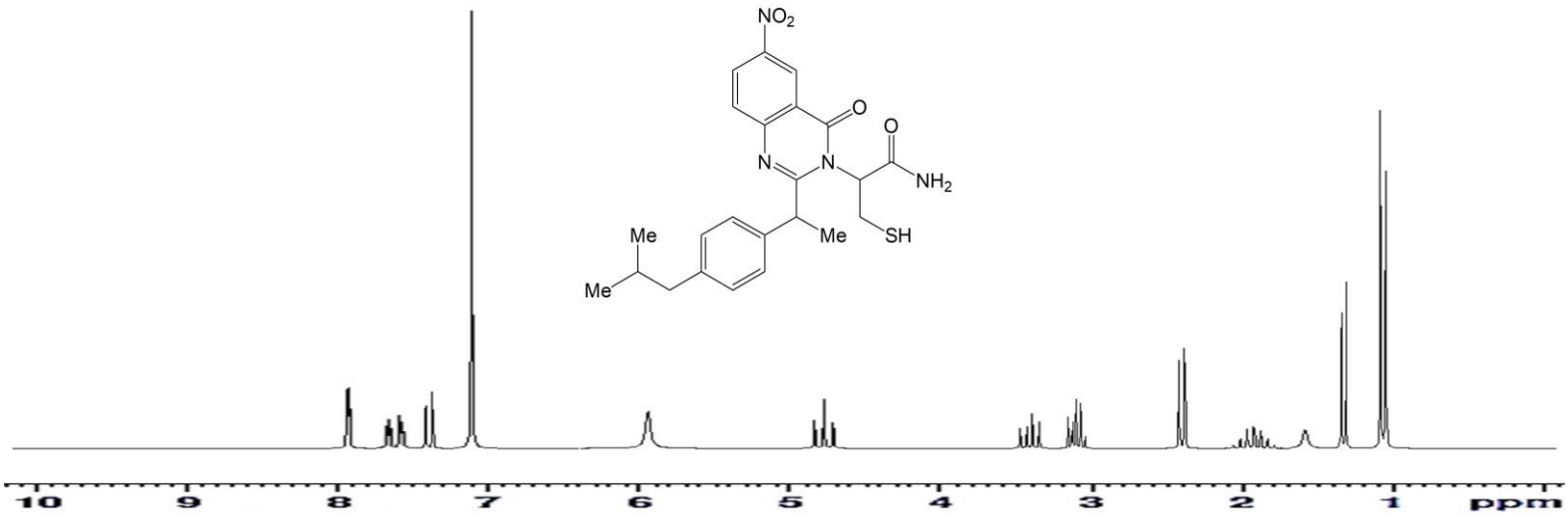


Figure S15. ^{1}H NMR (DMSO+D_2O) spectrum of compound **13**

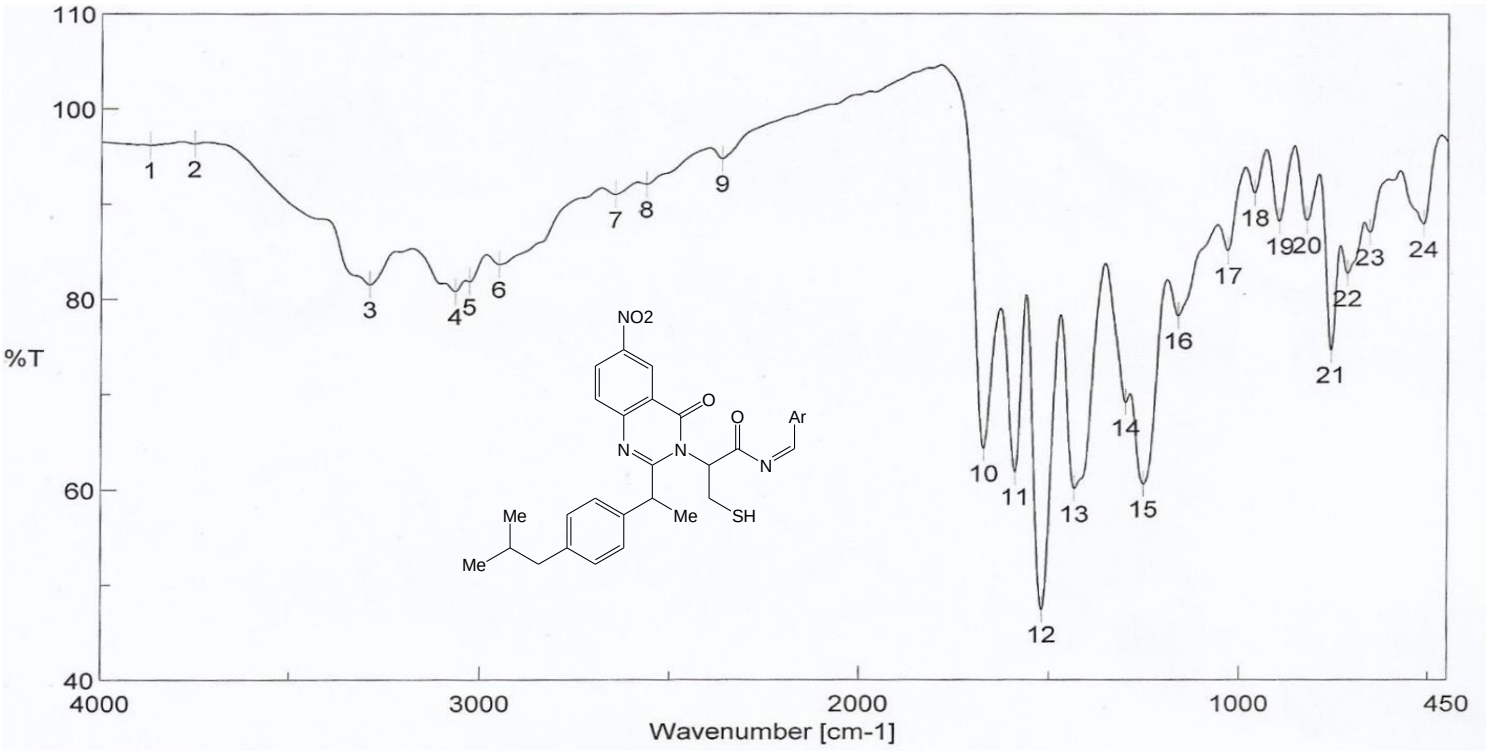


Figure S16. IR spectrum of compound **14a**

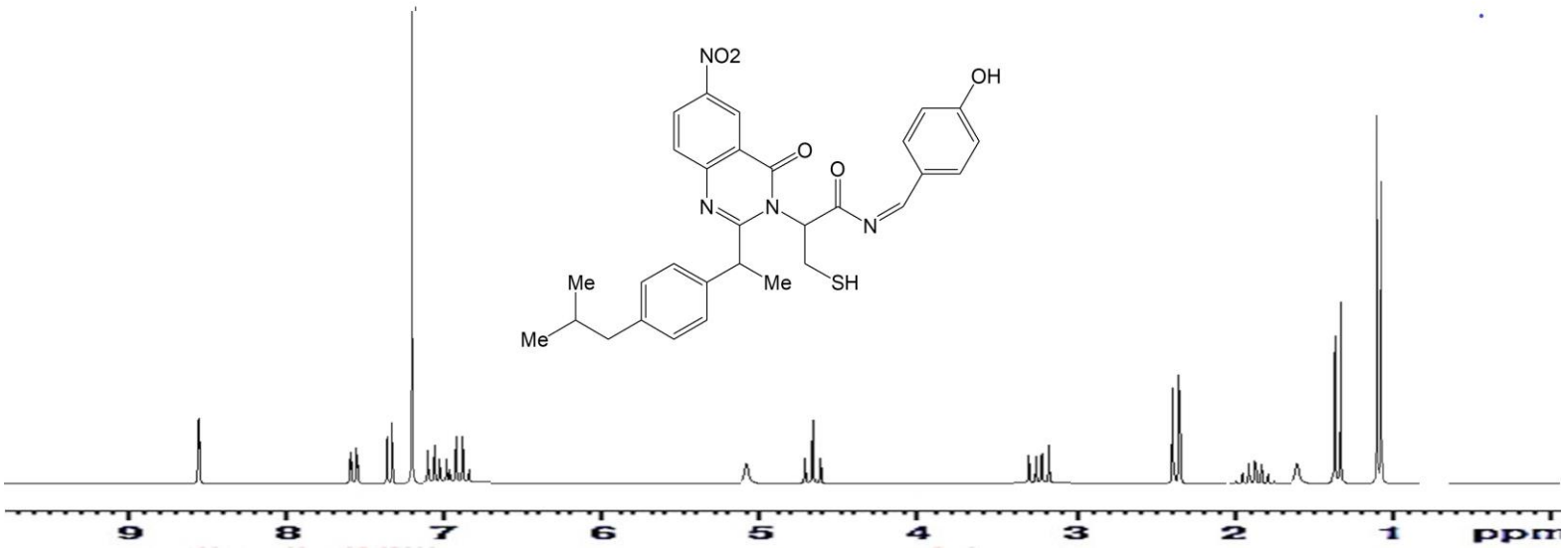


Figure S17. ^{1}H NMR (DMSO+D_2O) spectrum of compound **14a**