Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting information

Linifanib – A Multi-Targeted Receptor Tyrosine Kinase Inhibitor and a Low Molecular Weight Gelator

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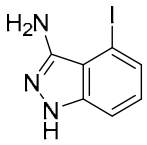
S1. Synthesis and analysis of linifanib (1) and its analogues (2-9)

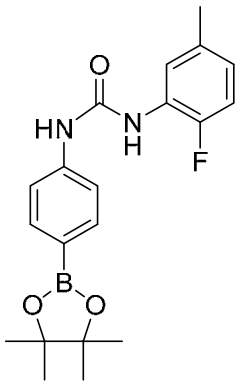
Synthesis of 4-iodo-1H-indazol-3-amine



1a

Dai, Yujia, et al. "Discovery of N-(4-(3-Amino-1 H-indazol-4-yl) phenyl)-N'-(2-fluoro-5-methylphenyl) urea (ABT-869), a 3-Aminoindazole-Based Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitor." *Journal of medicinal chemistry* 50.7 (2007): 1584-1597.

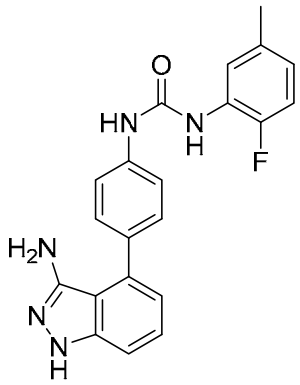
Synthesis of 1-(2-fluoro-5-methylphenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



1b

Dai, Yujia, et al. "Discovery of N-(4-(3-Amino-1 H-indazol-4-yl) phenyl)-N'-(2-fluoro-5-methylphenyl) urea (ABT-869), a 3-Aminoindazole-Based Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitor." *Journal of medicinal chemistry* 50.7 (2007): 1584-1597.

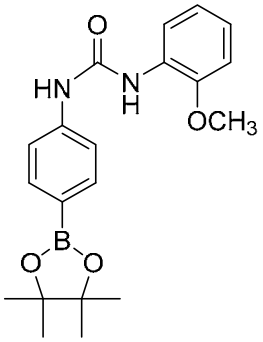
Synthesis of linifanib (1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2-fluoro-5-methylphenyl)urea)



1

Dai, Yujia, et al. "Discovery of N-(4-(3-Amino-1 H-indazol-4-yl) phenyl)-N'-(2-fluoro-5-methylphenyl) urea (ABT-869), a 3-Aminoindazole-Based Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitor." *Journal of medicinal chemistry* 50.7 (2007): 1584-1597.

Synthesis of 1-(2-methoxyphenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



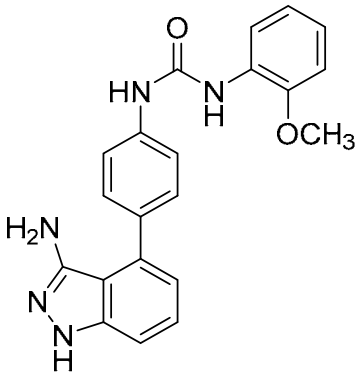
2a

To a stirred solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (238 mg, 2 mmol) in DCM (10 ml) at 0 °C (water ice bath) 2-methoxyphenyl isocyanate (298 mg, 2 mmol) was added. The whole solution was allowed to stir overnight. Then, the solution was evaporated under vacuum and purified by flush column chromatography (20-80 % EtAOc/hexane) to get the title compound as a white solid (600 mg, 80 %). MS: m/z (HRMS) calculated for $C_{20}H_{25}BN_2O_4$ 369.1907 [M+H], MS found: 369.1861 [M+H]. ^{1}H NMR (400

MHz, $CHCl_3$-d_1): δ 1.30 (s, 12H), 3.85 (s, 3H), 7.20 (m, 1H), 7.26 (s, 1H), 7.40 (d, J = 8.40 Hz, 2H), 7.77 (d, J = 8.29 Hz, 2H), 8.07 (s, 1H), 8.09 (s, 1H).

The same procedure was used in preparation of urea boronates of the linifanib analogues by the addition of the corresponding isocyanate to the aniline.

Synthesis of 1-(4-(3-a mino-1H-indazol-4-yl)phenyl)-3-(2-methoxyphenyl)urea

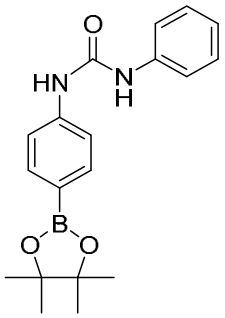


2

The 4-iodo-1H-indazol-3-amine **1a** (259 mg, 1 mmol), urea boronate **2a** (441 mg, 1.2 mmol) and Na_2CO_3 (260 mg, 2.4 mmol) were suspended in dimethoxyethane/water (36:12) mixture. The Palladium was then added and the reaction was allowed to stir at 85 °C overnight. TLC indicated complete conversion of the starting material. The solution was cooled, evaporated, extracted twice with ethyl acetate/water twice and dried over $MgSO_4$. The EtOAc extract was concentrated down and purified by flash column chromatography (20-80 % MeOH/DCM) to get the product (150 mg, 40 %). MS: m/z (HRMS) calculated for $C_{21}H_{19}N_5O_2$ 374.1538 [M+H], MS found: 374.1601 [M+H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.9 (s, 3H), 4.34 (s, 2H), 6.79 (dd, J = 1.95 Hz, J = 5.91 Hz, 1H), δ 6.95 (m, 2H), δ 7.04 (d, J = 7.95 Hz, 2H), δ 7.26 (s, 1H), δ 7.4 (d, J = 8.56 Hz, 2H), δ 7.61 (d, J = 8.60 Hz, 2H), δ 8.18 (d, 1H), δ 8.32 (s, 1H), δ 9.50 (s, 1H), δ 11.71 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 31.24, 33.56, 35.40, 56.25, 79.10, 79.45, 79.76, 111.20, 118.18, 118.76, 119.52, 120.50, 121.02, 122.30, 126.75, 128.88, 129.10, 129.79, 132.99, 139.88, 148.12, 152.85.

The same method was used in preparation of linifanib analogues by the reaction between the 4-iodo-1H-indazol-3-amine with its corresponding urea boronate.

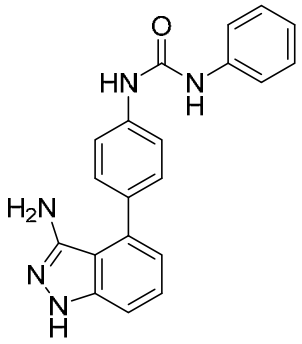
Synthesis of 1-phenyl-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



3a

Dai, Yujia, et al. "Discovery of N-(4-(3-Amino-1 H-indazol-4-yl) phenyl)-N'-(2-fluoro-5-methylphenyl) urea (ABT-869), a 3-Aminoindazole-Based Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitor." *Journal of medicinal chemistry* 50.7 (2007): 1584-1597.

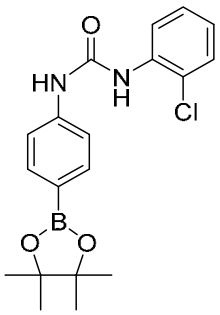
Synthesis of 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-phenylurea



3

Dai, Yujia, et al. "Discovery of N-(4-(3-Amino-1 H-indazol-4-yl) phenyl)-N'-(2-fluoro-5-methylphenyl) urea (ABT-869), a 3-Aminoindazole-Based Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitor." *Journal of medicinal chemistry* 50.7 (2007): 1584-1597.

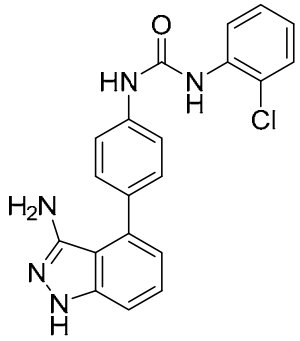
Synthesis of 1-(2-chlorophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



4a

By the method outlined for compound **2a**, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (238 mg, 2 mmol) and 2-chlorophenyl isocyanate (307 mg, 2 mmol) were reacted together in DCM to give 1-(2-chlorophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea (692 mg, 93 %). MS: m/z (HRMS) calculated for $C_{19}H_{22}BClN_2O_3$ 373.1412 [M+H], MS found: 372.9811 [M+H]. ^{1}H NMR (400 MHz, $CHCl_3$-d_1): δ 1.37 (s, 12H), 6.93 (s, 1H), 7.02 (t, J = 7.72 Hz, 1H), 7.22 (s, 1H), 7.28 (s, 1H), 7.36 (dd, J = 1.48 Hz, J = 7.95 Hz, 1H), 7.41 (s, 1H), 7.23 (s, 1H), 7.80 (s, 1H),7.82 (s, 1H), 8.17 (s, 1H), 8.20 (s, 1H).

Synthesis of 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2-chlorophenyl)urea

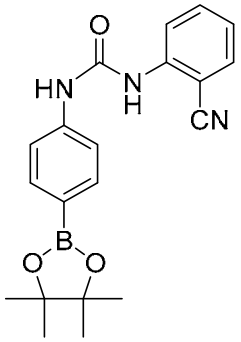


4

By the method outlined for compound **2,** the 4-iodo-1H-indazol-3-amine **1a** (259 mg, 1 mmol), urea boronate **8** (453 mg, 1.2 mmol) were reacted to give 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2-chlorophenyl)urea (139 mg, 37 %). MS: m/z (HRMS) calculated for $C_{20}H_{16}ClN_5O$ 378.1043 [M+H], MS found: 378.1006 [M+H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.34 (s, 2H), 6.80 (dd, J = 2.28 Hz, J = 5.52, 1H), 7.05 (t, J = 7.74 Hz, 1H), 7.26 (m, 2H), 7.32 (t, 1H), δ 7.42 (d, J = 8.53 Hz, 2H), 7.48 (d, J = 1.51 Hz, J = 8.00 Hz, 1H), 7.62 (d, J = 8.65 Hz, 2H), 8.20 (dd, 1H), 8.40 (s, 1H), 9.59 (s, 1H), 11.73 (s, 1H). ^{13}C NMR (125

MHz, DMSO-d_6): δ 60.20, 109.10, 118.50, 119.60, 121.84, 122.49, 123.83, 126.72, 128.10, 129.70, 129.85, 133.40, 135.91, 136.41, 139.48, 152.29.

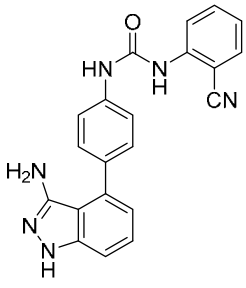
Synthesis of 1-(2-cyanophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



5a

By the method outlined for compound **2a**, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (238 mg, 2 mmol) and 2-cyanophenyl isocyanate (288 mg, 2 mmol) were reacted together in DCM to give 1-(2-cyanophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea (654 mg, 90 %). MS: m/z (HRMS) calculated for $C_{20}H_{22}BN_3O_3$ 364.1754 [M+H], MS found: 363.9423 [M+H]. ^{1}H NMR (400 MHz, $CHCl_3$-d_1): δ 1.37 (s, 12H), 7.13 (t, J = 7.65 Hz, 1H), 7.49 (d, J = 8.48 Hz, 2H), 7.59 (d, J = 7.60 Hz, 2H), 7.80 (d, J = 8.42 Hz, 2H), 7.81 (s, 1H), 8.39 (s, 1H), 8.42 (s, 1H).

Synthesis of 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2-cyanophenyl)urea

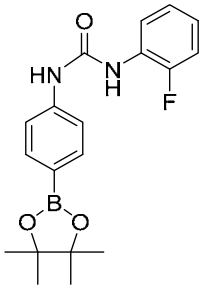


5

By the method outlined for compound **2,** the 4-iodo-1H-indazol-3-amine **1a** (259 mg, 1 mmol), urea boronate **10** (442 mg, 1.2 mmol) were reacted to give 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2-cyanophenyl)urea (158 mg, 43 %). MS: m/z (HRMS) calculated for

$C_{21}H_{16}N_6O$ 369.1385 [M+H], MS found: 369.1461 [M+H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.34 (s, 2H), 6.79 (dd, J = 1.98 Hz, J = 5.69 Hz, 1H), 7.05 (t, J = 7.62 Hz, 1H), δ 7.27 (m, 2H), 7.32 (m, 1H), 7.42 (d, J = 8.66 Hz, 2H), 7.48 (dd, J = 1.51 Hz, J = 8.08 Hz, 1H), 7.62 (d, J = 8.64 Hz, 2H), 8.20 (dd, J = 1.39 Hz, J = 8.27 Hz, 1H), 8.38 (s, 1H), 9.58 (s, 1H), 11.72 (s, 1H, NH). ^{13}C NMR (125 MHz, DMSO-d_6): δ 60.22, 114.76, 115.76, 119.85, 122.88, 123.01, 126.92, 128.04, 129.62, 129.90, 130.33, 135.62, 139.44, 140.33, 150.70, 162.70.

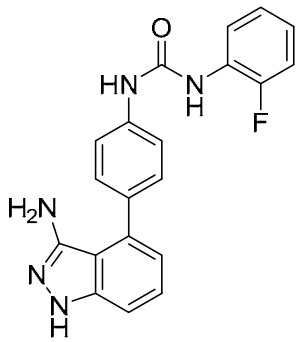
Synthesis of 1-(2-fluorophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



6a

Dai, Yujia, et al. "Discovery of N-(4-(3-Amino-1 H-indazol-4-yl) phenyl)-N'-(2-fluoro-5-methylphenyl) urea (ABT-869), a 3-Aminoindazole-Based Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitor." *Journal of medicinal chemistry* 50.7 (2007): 1584-1597.

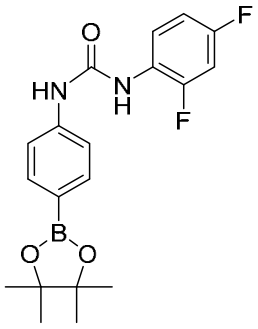
Synthesis of 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2-fluorophenyl)urea



6

Dai, Yujia, et al. "Discovery of N-(4-(3-Amino-1 H-indazol-4-yl) phenyl)-N'-(2-fluoro-5-methylphenyl) urea (ABT-869), a 3-Aminoindazole-Based Orally Active Multitargeted Receptor Tyrosine Kinase Inhibitor." *Journal of medicinal chemistry* 50.7 (2007): 1584-1597.

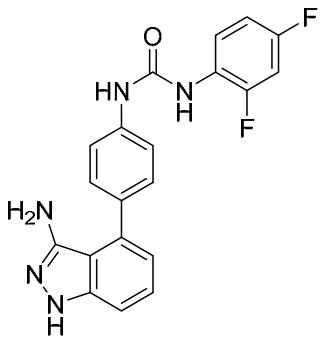
Synthesis of 1-(2,4-difluorophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea



7a

By the method outlined for compound **2a**, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (238 mg, 2 mmol) and 2,4-difluorophenyl isocyanate (310 mg, 2 mmol) were reacted together in DCM to give 1-(2,4-difluorophenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea (710 mg, 95 %). MS: m/z (HRMS) calculated for $C_{19}H_{21}BF_2N_2O_3$ 375.1613 [M+H], MS found: 375.1327 [M+H]. ^{1}H NMR (400 MHz, $CHCl_3$-d_1): δ 1.35 (s, 12H), 7.06 (m, 3H), 7.19 (s, 2H), 7.37 (d, J = 8.50 Hz, 2H), 7.77 (s, 1H), 8.07 (s, 1H).

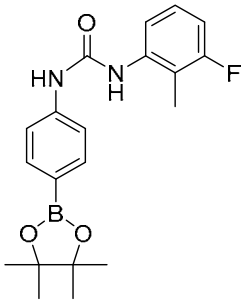
Synthesis of 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2,4-difluorophenyl)urea



7

By the method outlined for compound **2,** the 4-iodo-1H-indazol-3-amine **1a** (259 mg, 1 mmol), urea boronate **14** (449 mg, 1.2 mmol) were reacted to give 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(2,4-difluorophenyl)urea (197 mg, 52 %). MS: m/z (HRMS) calculated for $C_{20}H_{15}F_2N_5O$ 380.1244 [M+H], MS found: 380.1337 [M+H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.33 (s, 2H), 6.79 (dd, J = 2.07 Hz, J = 5.87 Hz, 1H), 7.08 (t, J = 7.93 Hz, 1H), 7.27 (m, 2H), 7.33 (m, 1H), 7.41 (d, J = 8.52 Hz, 2H), 7.59 (d, J = 8.65 Hz, 2H), 8.12 (m, 1H), 8.57 (s, 1H), 9.18 (s, 1H), 11.72 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 104.01, 104.26, 104.51, 109.12, 111.05, 111.42, 111.63, 118.43, 119.57, 126.74, 129.88, 133.40, 135.90, 139.48, 142.54, 148.62, 152.84.

Synthesis of 1-(3-fluoro-2-methylphenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea

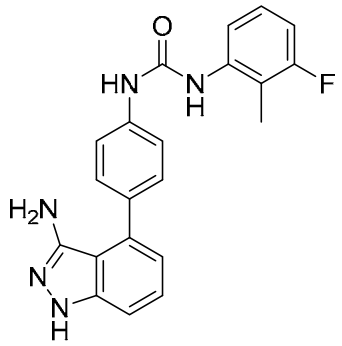


8a

By the method outlined for compound **2a**, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (238 mg, 2 mmol) and 2-methyl-3-fluorophenyl isocyanate (302 mg, 2 mmol) were

reacted together in DCM to give 1-(3-fluoro-2-methylphenyl)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)urea (710 mg, 95 %). MS: m/z (HRMS) calculated for $C_{20}H_{24}BFN_2O_3$ 371.1864 [M+H], MS found: 371.1778 [M+H]. ^{1}H NMR (400 MHz, $CHCl_3$-d_1): δ 1.36 (s, 12H), 2.17 (s, 3H), 6.52 (s, 1H), 6.80 (s, 1H), 6.93 (t, J = 8.38 Hz, 1H), 7.19 (m, 1H), 7.36 (d, J = 8.62 Hz, 3H), 7.75 (s, 1H), 7.77 (s, 1H).

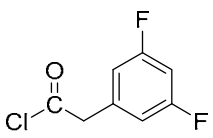
Synthesis of 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(3-fluoro-2-methylphenyl)urea



8

By the method outlined for compound **2,** the 4-iodo-1H-indazol-3-amine **1a** (259 mg, 1 mmol), urea boronate **16** (444 mg, 1.2 mmol) were reacted to give 1-(4-(3-amino-1H-indazol-4-yl)phenyl)-3-(3-fluoro-2-methylphenyl)urea (197 mg, 52 %). MS: m/z (HRMS) calculated for $C_{21}H_{18}FN_5O$ 376.1495 [M+H], MS found: 375.9643 [M+H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.18 (s, 3H), 4.34 (s, 2H), 6.79 (dd, J = 2.03 Hz, J = 5.83 Hz, 1H), 6.87 (t, J = 8.83 Hz, 1H), 7.19 (m, 1H), 7.27 (m, 2H), 7.41 (d, J = 8.56 Hz, 2H), 7.61 (d, J = 8.56 Hz, 2H), 7.72 (d, J = 8.28 Hz, 1H), 8.16 (s, 1H), 9.21 (s, 1H), 11.72 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 19.9, 109.02, 109.81, 111.01, 115.31, 117.52, 118.39, 119.51, 126.70, 127.23, 129.81, 133.19, 135.92, 139.70, 142.55, 148.59, 152.99, 159.84, 162.23.

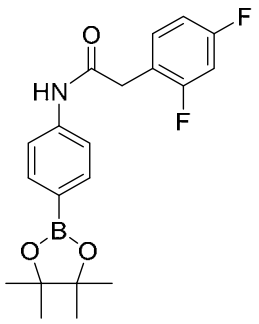
Synthesis of 2-(3,5-difluorophenyl)acetyl chloride



9a

To a solution of 2-(3,5-difluorophenyl)acetic acid (344 mg, 2 mmol) in dichloromethane (2 mL) was added thionyl chloride (218 μL, 3 mmol) and dimethylformamide (16 μL, 0.2 mmol) and the mixture stirred at room temperature for 3 hours. The solvent was removed under vacuum to give 2-(3,5-difluorophenyl)acetyl chloride as a yellow oil, which was used without further purification.

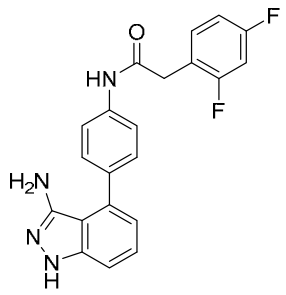
Synthesis of 2-(3,5-difluorophenyl)-N-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)acetamide



9b

To a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (438 mg, 2 mmol) in dichloromethane (20 mL) cooled with an ice-water bath was added triethylamine (418 μL, 3 mmol) and 2-(3,5-difluorophenyl)acetyl chloride **9a** (217 μL, 2 mmol). The mixture was stirred overnight, washed with water, dried (MgSO4), filtered, and concentrated to give the product as a white solid (708 mg, 95%). MS: m/z (HRMS) calculated for $C_{20}H_{22}BF_2NO_3$ 374.1660 [M+H], MS found: 374.1731 [M+H]. ^{1}H NMR (400 MHz, $CHCl_3$-d_1): δ 1.35 (s, 12H), 3.75 (s, 2H), 6.90 (m, 2H), 7.37 (m, 1H), 7.54 (d, J = 8.28 Hz, 2H), 7.62 (m, 1H), 7.75 (s, 1H), 7.77 (s, 1H).

Synthesis of N-(4-(3-amino-1H-indazol-4-yl)phenyl)-2-(2,4-difluorophenyl)acetamide



9

The 4-iodo-1H-indazol-3-amine **1a** (259 mg, 1 mmol), acetamide **9b** (373 mg, 1 mmol) and Na_2CO_3 (78 mg, 0.7 mmol) were suspended in dimethoxyethane/water (36:12) mixture. The Palladium was then added and the reaction was allowed to stir at 85 °C overnight. TLC indicated complete conversion of the starting material. The solution was cooled, evaporated, extracted twice with ethyl acetate/water twice and dried over $MgSO_4$. The EtOAc extract was concentrated down and purified by flash column chromatography (20-80 % MeOH/DCM) to get the product as light brown solid (210 mg, 56 %). MS: m/z (HRMS) calculated for $C_{21}H_{16}F_2N_4O$ 379.1292 [M+H], MS found: 379.1398 [M+H]. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.77 (s, 2H), 4.30 (s, 2H), 6.69 (m, 1H), 6.78 (q, 1H), 7.13 (m, 3H), 7.42 (d, J = 8.60 Hz, 2H), 7.47 (d, J = 6.86 Hz, 1H), 7.73 (d, J = 8.60 Hz, 2H), 10.37 (s, 1H), 11.72 (s, 1H). ^{13}C NMR (125 MHz, DMSO-d_6): δ 36.23, 104.05, 109.16, 111.00, 111.57, 111.77, 114.14, 119.39, 119.58, 126.69, 129.77, 129.90, 133.43, 134.56, 135.82, 139.09, 142.55, 148.57, 168.48.

S2. Preparation of gels and vial inversion

Gels were prepared for both linifanib (**1**) and its analogues (**compounds 2-9**) as shown in Table 1

Table 1: Amounts of compound, DMSO and water required for gel preparation

Final concentration % (w/v)	Compound (mg)	DMSO µL	Water µL	Final volume µL	% (v/v) DMSO
0.5	2.5	25	475	500	5
0.5	2.5	50	450	500	10
0.5	2.5	75	425	500	15
0.5	2.5	100	400	500	20
0.5	2.5	125	375	500	25
0.5	2.5	150	350	500	30
0.5	2.5	180	300	500	40
0.5	2.5	230	250	500	50

Note: The 0.25 % and 0.125 % (w/v) were prepared by using 1.25 mg and 0.625 mg of the weight of compound respectively.

The compounds were measured into individual vials (Fisherbrand screw top wide opening 9mm short thread 2 mL, 12 mm x 32 mm). DMSO was then added and the vials were heated up to 60 °C in a bespoke heating mantle for a period of time to ensure solubilisation. Pre-heated (70 °C) purified water was added before removal of the vials from the heating block and then allowed to cool down to room temperature.

S3. Rheological measurements were performed using an Anton Paar modular compact rheometer MCR302 equipped with a temperature controlled peltier. Cup and vane accessories were used to complete all amplitude sweeps (see K. J. Skilling *et al.*, Gelation properties of

self-assembling N-acyl modified cytidine derivatives. *Journal of Materials Chemistry B* **2**, 8412 (2014) for further details). All gels were prepared in a similar manner as described in S3 above to make a 2 ml volume in sterilin vials. All strain amplitude measurements were performed in the range of 0.01 % – 100 % strain and at a frequency of 10 rad s^{-1}. All measurements were performed on three replicate samples.

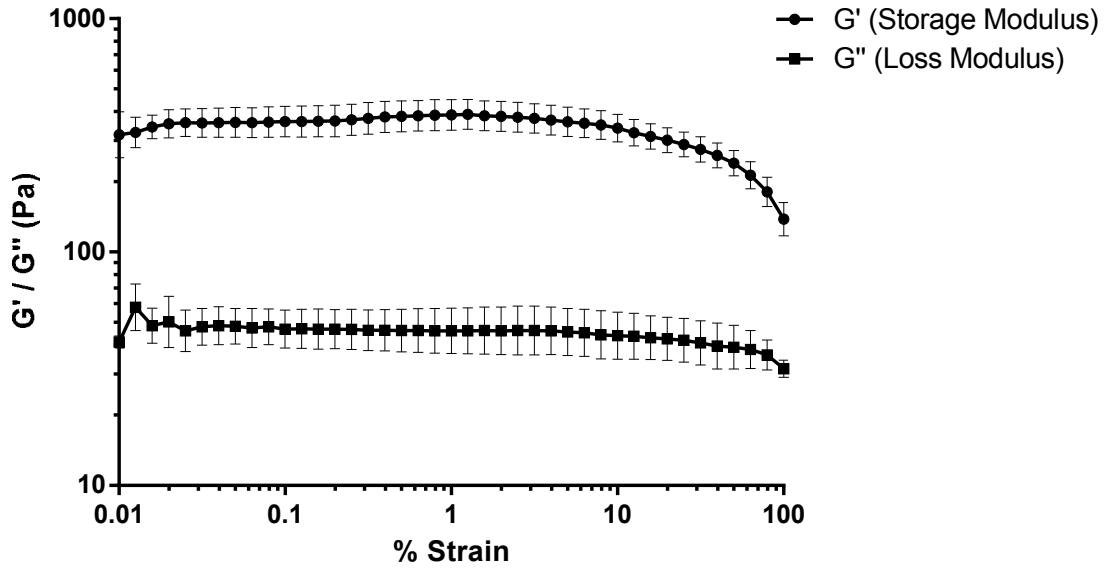


Figure 11: Strain amplitude data of analogue **3** gels at 0.25% (w/v)) in 5% (v/v) DMSO/water. Storage modulus (G′) and loss modulus (G″) shown.

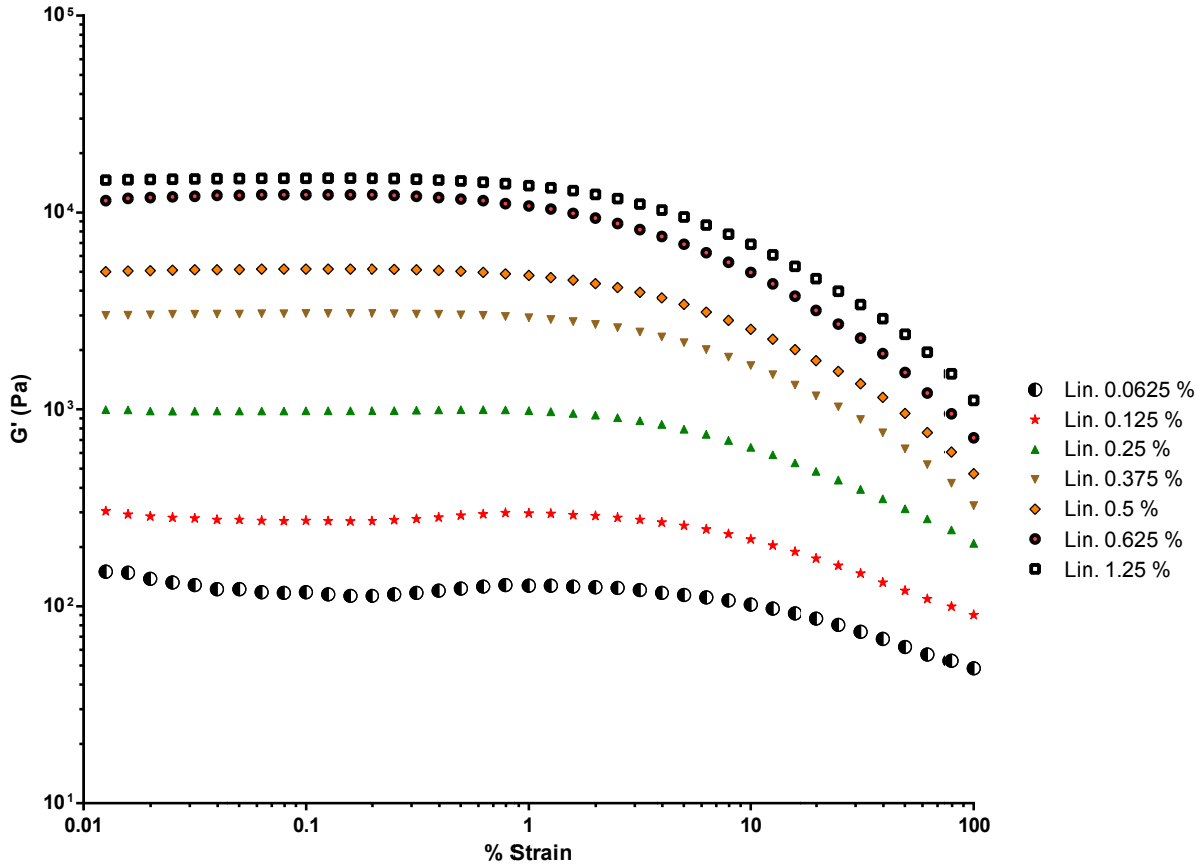


Figure 2: Strain amplitude data of linifanib gels at different concentrations (0.0625 to 1.25% (w/v)) in 5% (v/v) DMSO/water. Only storage modulus (G′) shown.

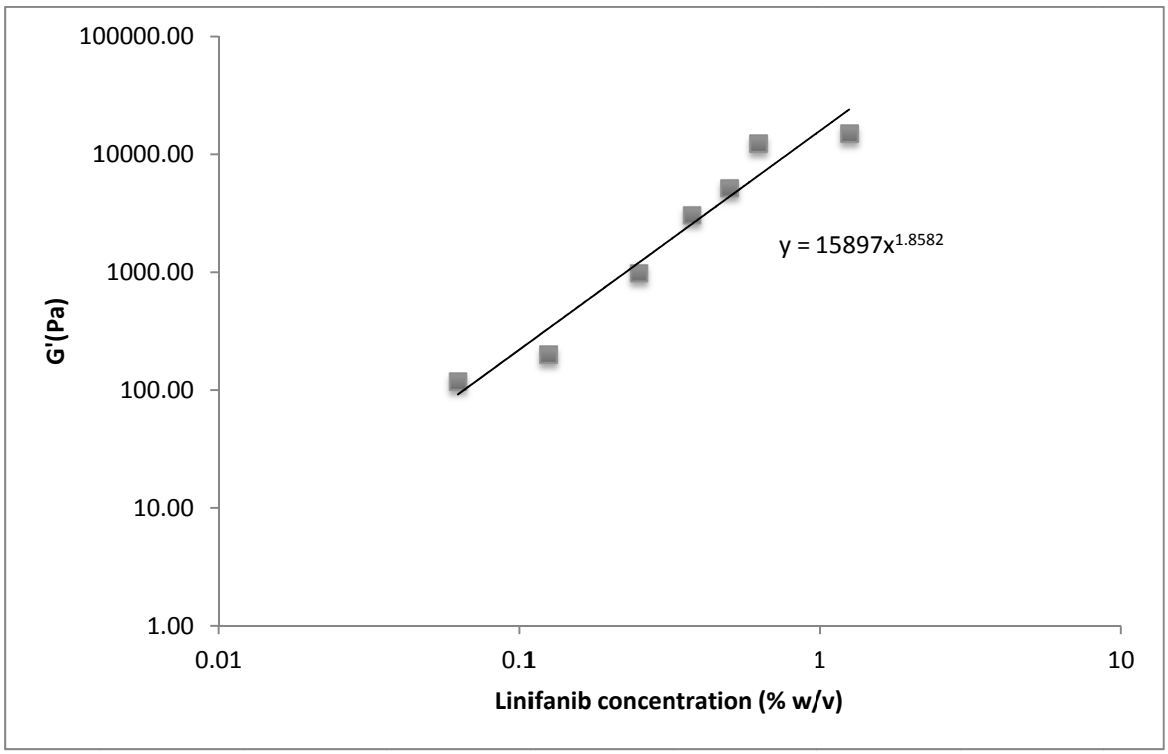


Figure 3: Storage modulus (G') versus linifanib concentration (% (w/v) in 0.5% (v/v) DMSO/water. All measurements were carried out in triplicate and the mean calculated. Slope = 1.86 to 2 decimal places.

S4. Transmission electron microscope (TEM) imaging was undertaken using a JEOL JEM – 2000FXII transmission electron microscope. 4 μL of sample was placed on a carbon coated copper grid (300 mesh, Agar Scientific) for 30 seconds. The sample was blotted with Whatman 50 filter paper, the grid was then inserted in the microscope through a sample holder and a high vacuum was applied. Images were subsequently taken at an accelerating voltage of 100 kV.