Supporting Information

Confirmation of Bosutinib Structure; Demonstration of Controls to Ensure Product Quality-- Supporting Information

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Contents

Preparation of 1: [4-Methoxy-3-[3-(4-methylpiperazin-1-yl)propoxy]phenyl]amine disuccinate:
Note: Compound **1** was previously disclosed[10], but upon conducting a literature search a CAS number was not found, thus preparation information is provided herein for completeness.

Following the method described in OPRD 2013, see reference 11, a solution of 4-methoxy-3-(3-(4-methylpiperazin-1-yl)propoxy)aniline, CAS# [846023-55-0] in isopropanol was obtained following the filtration of the palladium on carbon catalyst. The [846023-55-0] (990 mL, 93 gA, 1.0 eq., 333 mmol) was treated in a 3-L vessel by dropwise addition to succinic acid (83 g, 2.1 eq., 699 mmol) in IPA (650 mL), over 25 minutes. The mixture exothermed to 58 ˚C as it was stirred for 45 minutes. Heating was removed permitting the slurry to then slowly cool to room temperature, then the slurry as stirred for several hours. The product was collected by filtration and the cake washed with isopropanol. The product was dried in a vacuum oven at 55 ˚C. Isolated **1** 164.3 g, 95.7% weight yield.

^{1}H NMR (DMSO-d_6, 600 MHz, 25 ˚C): δ 7.67 (b), 6.63 (d, *J* = 8.5 Hz, 1H), 6.25 (d, *J* = 2.5 Hz, 1H), 6.06 (dd, *J* = 8.5, 2.5 Hz, 1H), 3.87 (t, *J* = 6.4 Hz, 2H), 3.60 (s, 3H), 2.56-2.38 (b, 4H), 2.57-2.37 (b, 4H), 2.46 (t, *J* = 7.1 Hz, 2H), 2.39 (s, 8H), 2.28 (s, 3H), 1.85 (m, 2H).
^{13}C NMR (DMSO-d_6, 150.9 MHz, 25 ˚C): δ 173.9x4, 149.2, 143.4, 140.2, 115.1, 105.3, 101.2, 66.2, 56.9, 54.2, 54.1x2, 51.9x2, 44.9, 29.3x4, 26.2.

Figure S1: IR of **2b** compared to **2a**
The mid-IR spectral differences between **2a** and **2b** can be attributed to the positions of the ether and chlorine's on the phenyl ring. The proximity of the chlorine next to the secondary amide causes bands to shift, particularly the Amide I band from 1697 cm^{-1} to 1669 cm^{-1} and bands in the CH deformation vibration region from 900-700 cm^{-1}. The band position at 869 cm^{-1} in the spectrum of **2a** is indicative of the CH out-of-plane deformation of a 1,2,4,5-tetrasubstituted ring. Whereas the band positions at 846 cm^{-1} and 814 cm^{-1} in the spectrum of **2b** is indicative of the CH out-of-plane deformation of a 1,2,4-trisubstituted ring.

2a (blue) vs 2b (red)

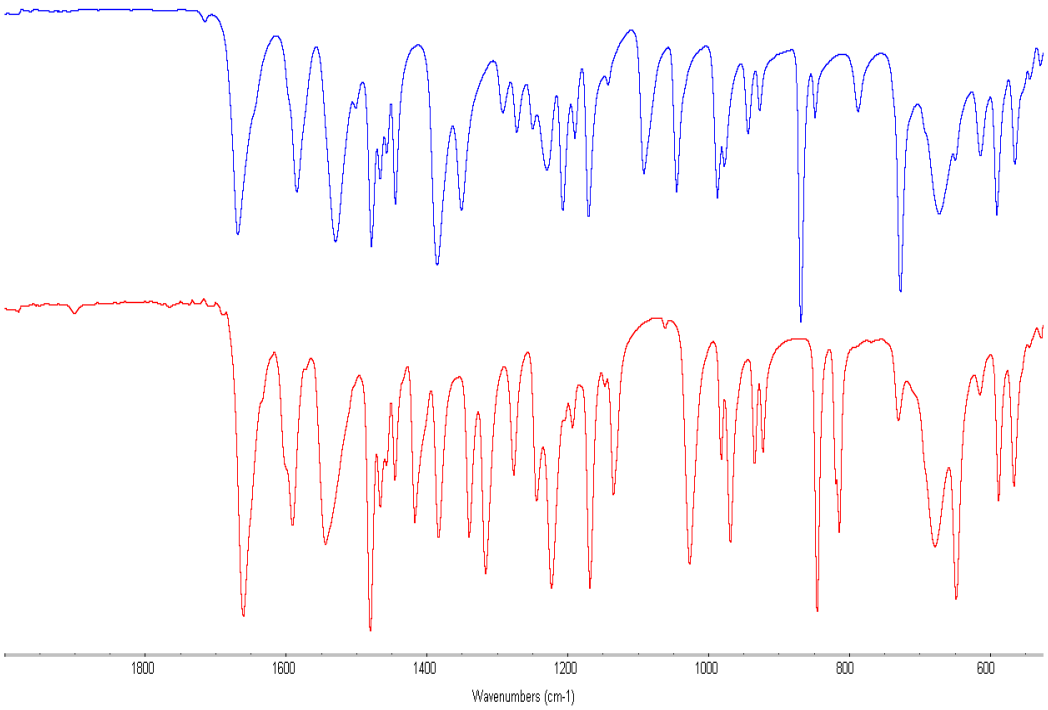


Figure S2: IR of **2c** compared to **2a**
The mid-IR spectral differences between **2a** and **2c** can be attributed to the positions of the ether and chlorine's on the phenyl ring. The proximity of the chlorine next to the secondary amide causes bands to shift, particularly the Amide I band from 1697 cm^{-1} to 1669 cm^{-1} and bands in the CH deformation vibration region from 900-700 cm^{-1}. The band position at 869cm^{-1} in the spectrum of **2a** is indicative of the CH out-of-plane deformation of a 1,2,4,5-tetrasubstituted ring. Whereas the band positions at 854 cm^{-1} and 801 cm^{-1} in the spectrum of **2c** is indicative of the CH out-of-plane deformation of a 1,2,4-trisubstituted ring.

2a (blue) vs 2c (red)

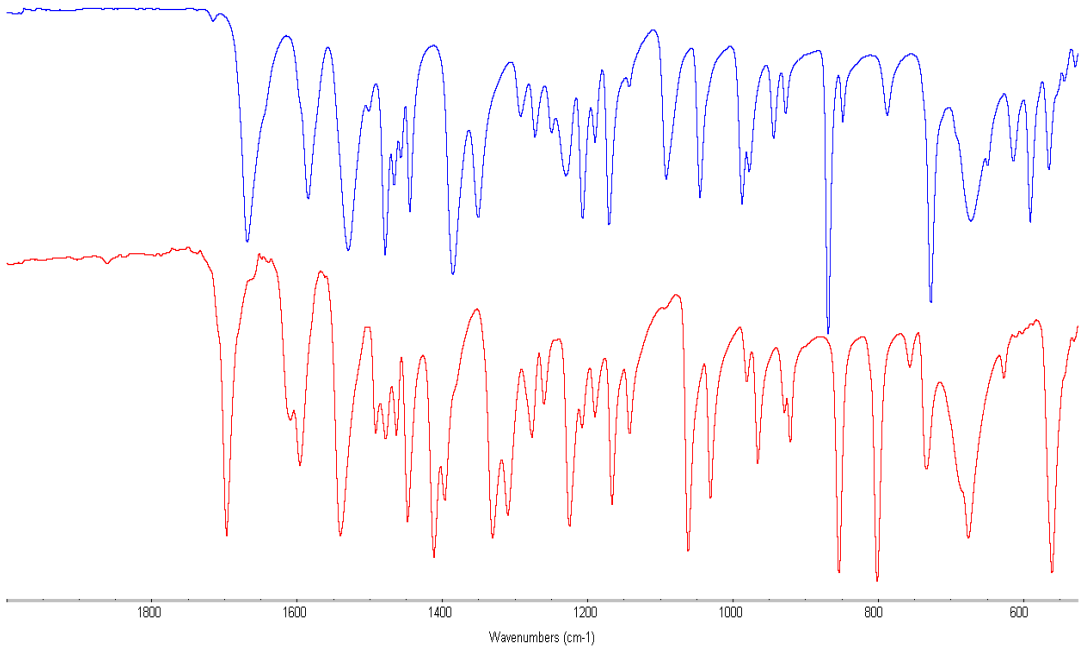


Figure S3: IR of **2e** compared to **2a**
The mid-IR spectral differences between **2a** and **2e** can be attributed to the positions of the bands in the CH deformation vibration region from 900-700 cm^{-1}. The band position at 869 cm^{-1} in the spectrum of **2a** is indicative of the CH out-of-plane deformation of a 1,2,4,5-tetrasubstituted ring. Whereas the band positions at 814 cm^{-1} and 803 cm^{-1} in the spectrum of **2e** is indicative of the CH out-of-plane deformation of a 1,2,3,4-tetrasubstituted ring.

2a (blue) vs 2e (red)

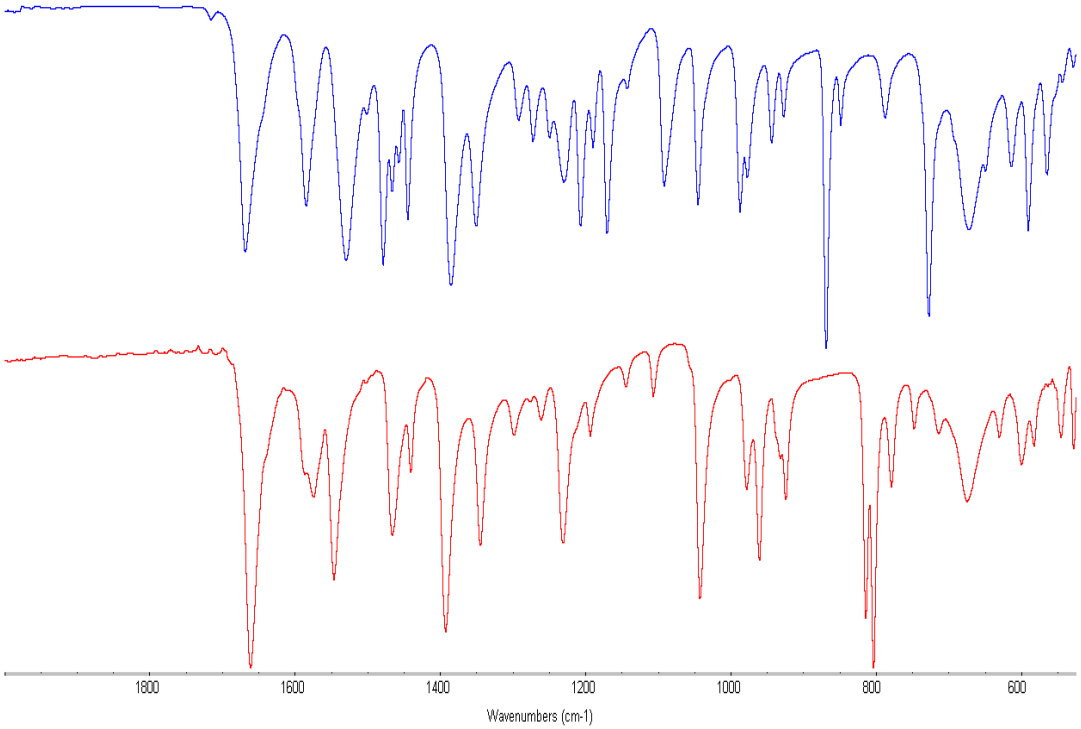


Figure S4: IR of **2f** compared to **2a**
The mid-IR spectral differences between **2a** and **2f** can be attributed to the positions of the bands in the CH deformation vibration region from 900-700 cm^{-1}. The band position at 869 cm^{-1} in the spectrum of **2a** is indicative of the CH out-of-plane deformation of a 1,2,4,5-tetrasubstituted ring. Whereas the band positions at 815 cm^{-1} and 801 cm^{-1} in the spectrum of **2f** is indicative of the CH out-of-plane deformation of a 1,2,3,4-tetrasubstituted ring.

2a (blue) vs 2f (red)

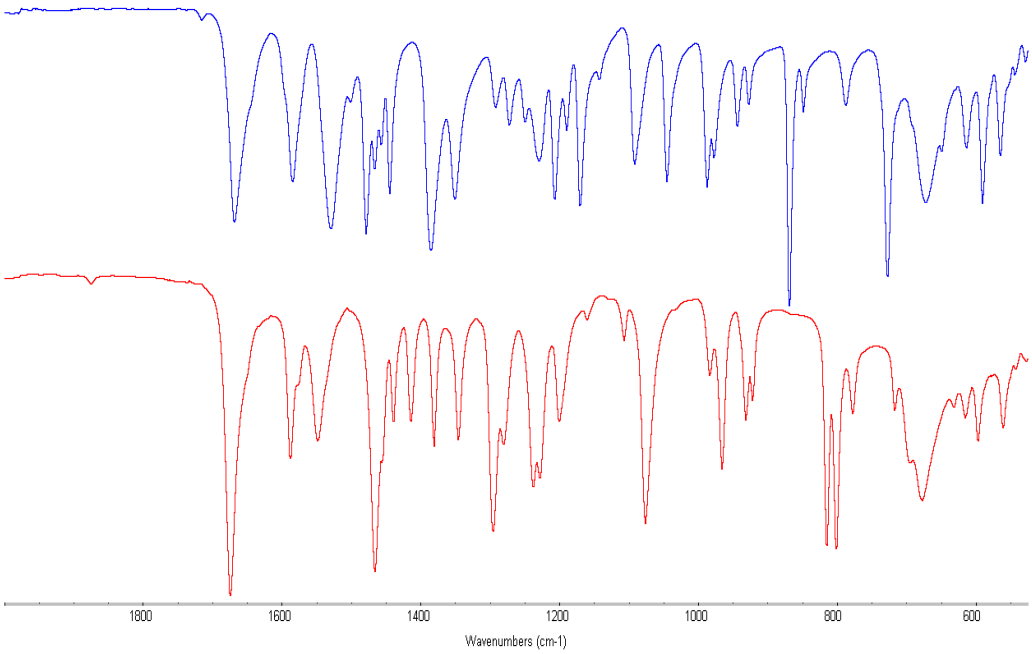


Figure S5: IR of **2g** compared to **2a**
The mid-IR spectral differences between **2a** and **2g** can be attributed to the positions of the ether and chlorine's on the phenyl ring. The proximity of the chlorine next to the secondary amide causes bands to shift, particularly the Amide I band from 1703 cm^{-1} to 1669 cm^{-1} and bands in the CH deformation vibration region from 900-700 cm^{-1}. The band position at 869 cm^{-1} in the spectrum of **2a** is indicative of the CH out-of-plane deformation of a 1,2,4,5-tetrasubstituted ring. Whereas the band position at 851 cm^{-1} and 835 cm^{-1} in the spectrum of **2g** is indicative of the CH out-of-plane deformation of a 1,2,3,5-tetrasubstituted ring.

2a (blue) vs 2g (red)

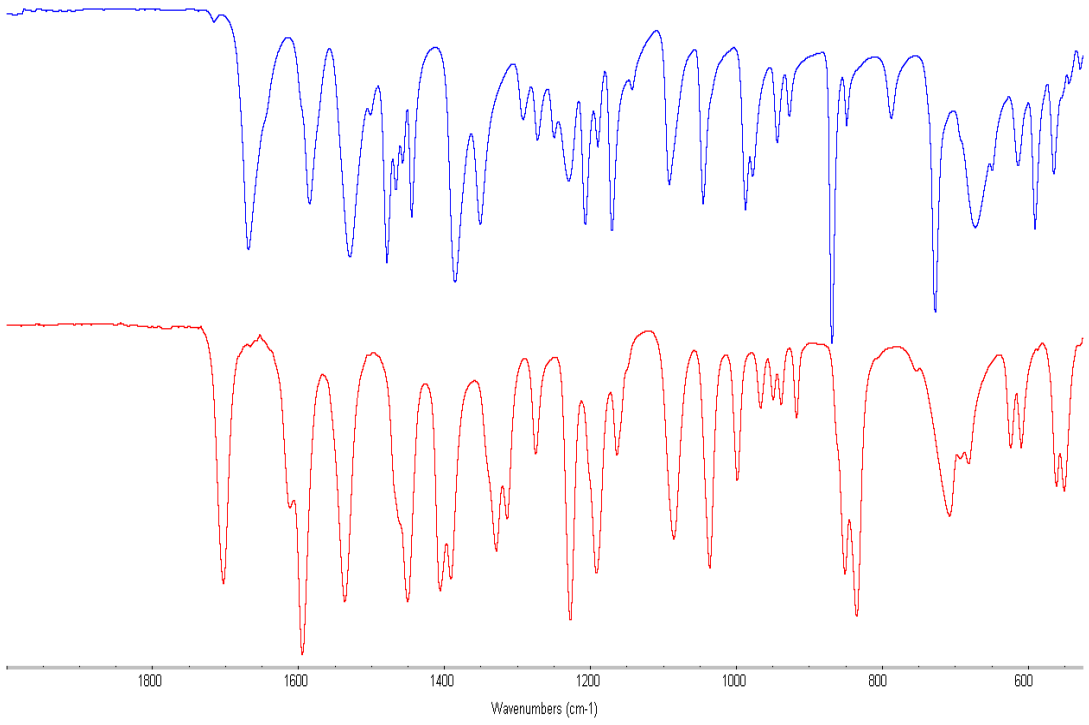
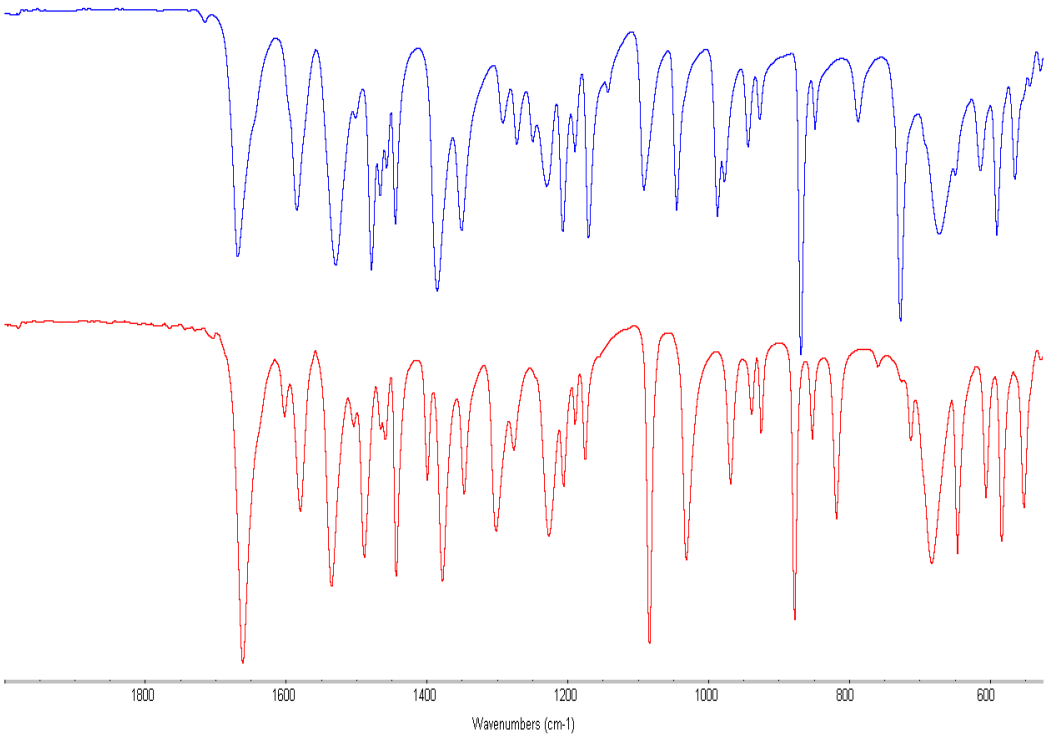


Figure S6: IR of **2h** compared to **2a**
The mid-IR spectra of **2a** and **2h** are very similar due the similarities in structure. Specific bands related to the observed structural differences make it very difficult to differentiate between **2a** and **2h**. However, certain areas of the spectra that are expected to shift due to substitutions on the phenyl ring happen from 1000-700 cm^{-1} in the spectra.

2a (blue) vs 2h (red)



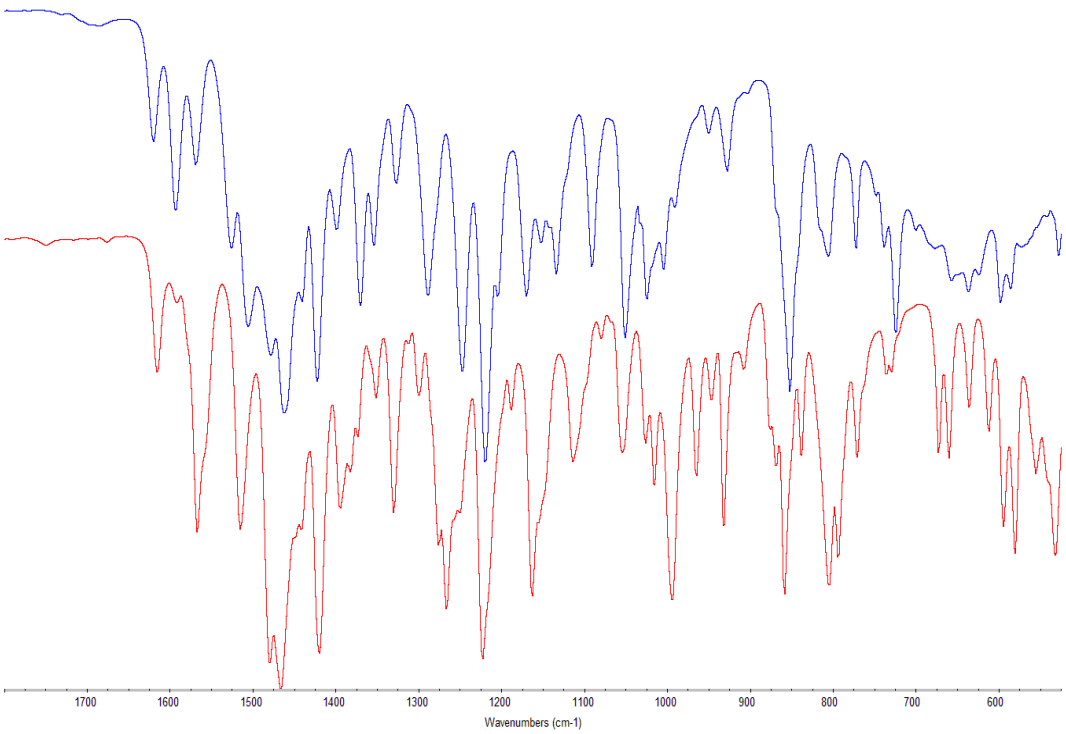


Figure S7: IR of **4a** (bosutinib) compared to **4d** ("bosutinib isomer")

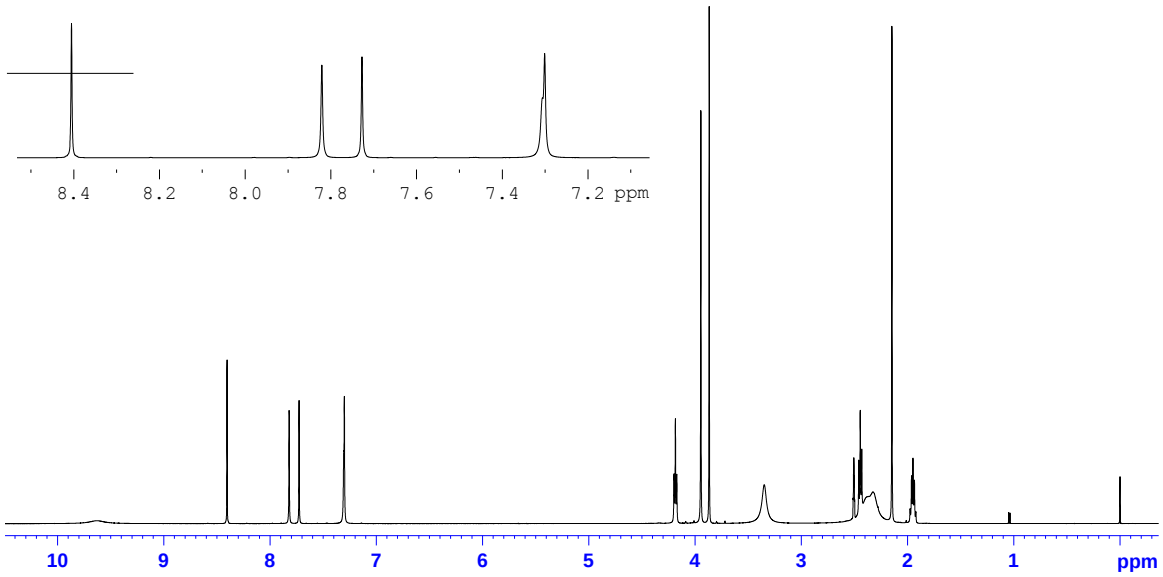
Bosutinib (red line) is clearly distinguished from "bosutinib isomer" (blue line).

Table S1: Crystallographic Parameters Determined for Bosutinib

Crystal Parameters	Results
Crystal system	Orthorhombic
Space group	Pbca
Unit cell dimensions	a = 12.3486(11) Å b = 14.0074(12) Å c = 30.165(3) Å
Final R indices [I>2sigma(I)]	R1 = 0.0830
R indices (all data)	R1 = 0.0875

X-ray structure shown in body of paper.

^{1}H NMR Spectrum of Pfizer bosutinib (aromatic region expanded)



^{13}C NMR Spectrum of Pfizer bosutinib

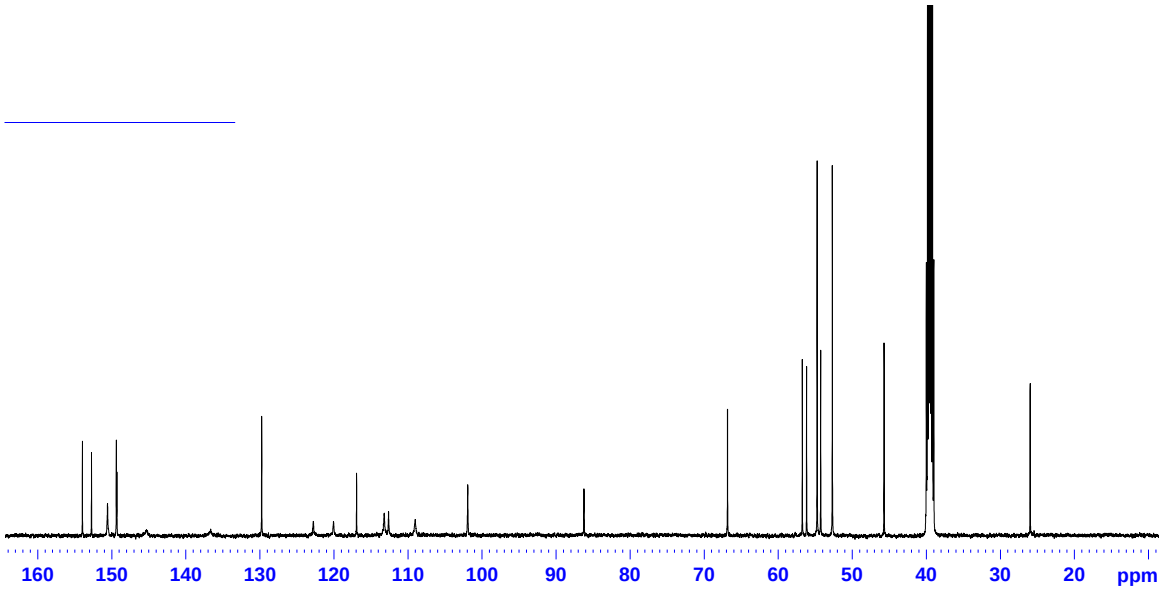


Figure S8: ^{1}H NMR Spectrum and ^{13}C NMR Spectrum

The 1D proton spectrum of bosutinib shows the expected chemical shifts, multiplicities, and integrations that are consistent with the bosutinib structure. The 1D carbon spectrum is consistent with the structure based upon chemical shifts. The 2D spectra show the expected heteronuclear ^{1}H-^{13}C and homonuclear ^{1}H-^{1}H correlations (not shown). Proton and carbon assignments for bosutinib are listed in Table S2.

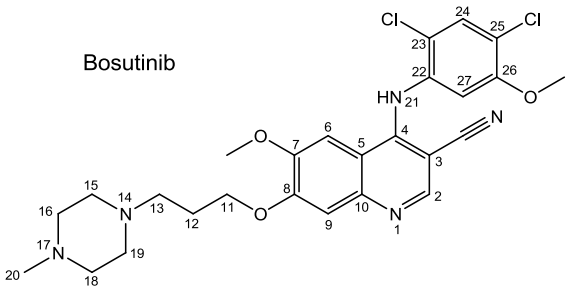
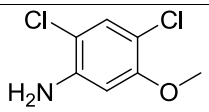
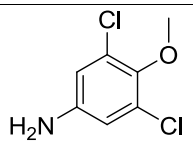


Table S2: Peak Listing

Atom Number	**^{1}H chemical shift (ppm)**	**^{13}C chemical shift (ppm)**
2	**8.40**	**150.6**
3	-	86.2
3-CN	-	116.9
4	-	149.4
5	-	112.6
6	**7.82**	**101.9**
7	-	149.3
7-OCH_3	3.94	56.2
8	-	152.7
9	**7.30**	**109.0**
10	-	145.3
11	4.18	66.8
12	1.95	26.0
13	2.45	54.3
15/19	2.39	52.7
16/18	2.33	54.7
20	2.15	45.7
21	9.63	-
22	-	136.6
23	-	120.1
24	**7.73**	**129.8**
25	-	122.8
26	-	154.0
26- OCH_3	3.87	56.8
27	**7.31**	**113.2**

Table S3: Comparison of Dichloromethoxy Aniline Isomers

Structure		
Chemical Name	2,4-dichloro-5-methoxyaniline	3,5-dichloro-4-methoxyaniline
CAS#	[98446-49-2]	[32407-11-7]
Literature mp	**50.5-51.5° C**	80-80.5° C

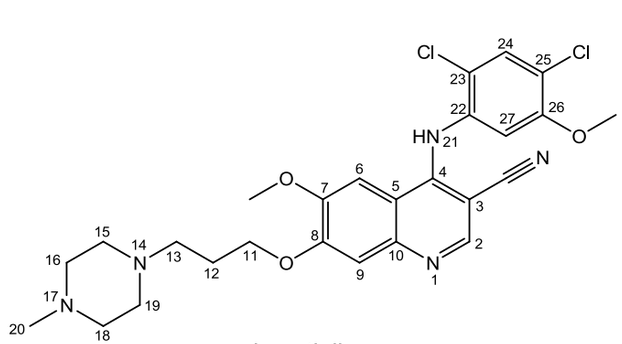
The melting point of the desired aniline isomer for the synthesis of bosutinib is emphasized in bold.

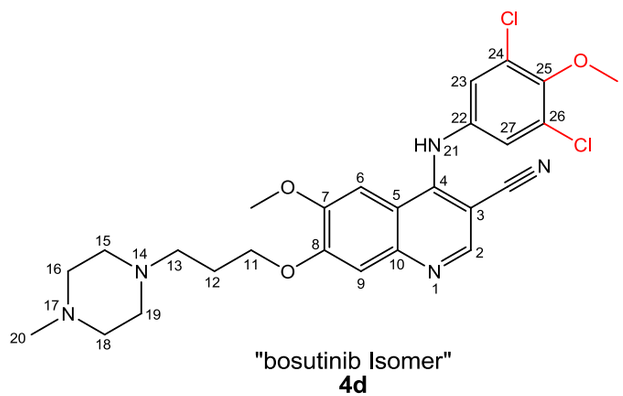
Table S4: Comparison of 4d ^{13}C NMR spectra from Pfizer and from Molecules 2010[1]

Source	**Solvent**	C25	C2	C4	C7	C8	C10	C22	C24 & 26	C23& 27	C3-<u>C</u>N	C5	C9	C6	C3	C11	C25-O<u>C</u>H$_3$	C7-O<u>C</u>H$_3$	C16& C18	C13	C15& C19	C20	C12
Pfizer produced **4d**, "Bosutinib Isomer"	D_6-DMSO	**153.1**	150.6	149.5	148.6	148.2	146.3	137.9	128.3	123.1 123.1	117.3	114.0	109.2	101.7	89.1	66.9	60.8	56.1	54.8	54.3	52.7	45.8	26.0
Molecules 2010 published data[1,2]	$CDCl_3$	153.03	150.43	149.50	148.51	148.13	146.15	137.88	128.27	122.99 122.99	117.17	113.95	109.12	101.66 101.66	89.10	66.87	60.71	56.01	54.72 54.72	54.24	52.67 52.67	45.68	25.96

All shifts in ppm

1. Ref: Li, Fei, *et. al*. *Molecules* **2010**, *15*, 4261-4266 (experimental **10**).
2. Li reported to 2 decimal places, our data rounded to 1 decimal based upon reporting practices.





Blue: More than 2 ppm shift in peak from bosutinib standard. As expected the differences in the shifts are most pronounced on the aniline ring. Carbons 23 and 27 along with carbons 24 and 26 showing the same shift (single peak) indicate symmetry and that both samples are "Bosutinib isomer".