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Supplementary Material

Dual Targeting of Janus Kinase and Bruton's Tyrosine Kinase: A New Approach to Control the Pathogenesis of Rheumatoid Arthritis

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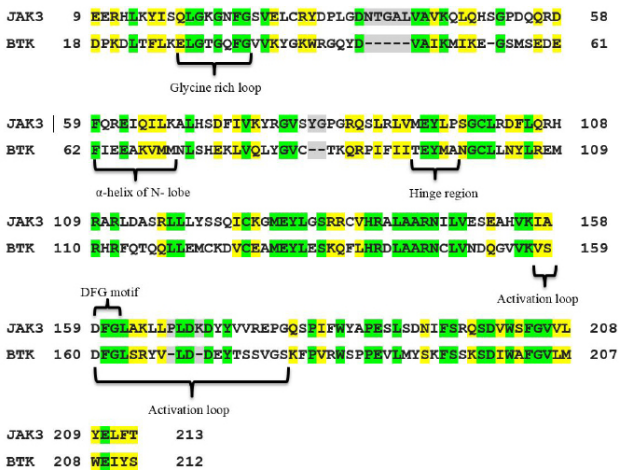
SEQUENCE ALIGNMENT OF JAK3 AND BTK

The pair wise alignment tool EMBOSS Matcher was used to align the sequences. The results obtained show 32.7% identity (67/205), 58.5% similarity (120/205) and 4.9% (10/205) gaps with a score of 266. The basic purpose of this sequence alignment was to identify the regions that have same or equivalent residues and offer similar interactions.



(Pairwise sequence alignment of JAK3 and BTK. Green represents same residues, yellow are equivalent while grey represent the gaps.)

SYNTHESIS PROCEDURE

An equimolar mixture of 4-nitro-o-phenylenediamine (0.025 mol, 3.83 g) and substituted vicinal diketone (0.025 mol) was refluxed in 40 mL of ethanol: acetic acid mixture (1:1) for 2-3 hours. Precipitates were formed on cooling the resulting mixture. The precipitates were filtered, washed with ethanol and dried to obtain pure product of substituted 6-nitroquinoxaline.

A mixture containing substituted 6-nitroquinoxaline (0.02 moles) and 10 % Pd/C in absolute ethanol (30-35 mL) was heated with continuous stirring.in a round bottom flask equipped with a dropping funnel and a reflux condenser flask. 6 mL of hydrazine monohydrate was added at the start of refluxing. The reaction mixture was refluxed for 2 hrs. The contents of the flask were filtered in hot state. Crystals of the corresponding aminoquinoxaline were obtained on cooling which were then filtered, washed with ethanol and dried.

Analytical data of synthesized compounds

Compound 1: 6-Nitroquinoxaline

Physical appearance: Shiny straw-colored crystals
Yield: 92%
Melting Point: 174-175 °C
FT-IR: υ (cm^{-1}): 3060 (Ar-H); 1615 (C=N); 1586, 1550, 1525 (Aromatic ring); 1490, 1346 (NO_2); 872 (C-NO_2)
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: 9.04-9.02 (3H, m, H-2, H-3, H-5), 8.57-8.55 (1H, dd, J = 9.15 Hz, 2.3 Hz, H-7), 8.29 (1H, d, J = 9.15 Hz, H-8)

Compound 2: Quinoxalin-6-amine

Physical appearance: Dirty green crystals
Yield:90 %
Melting Point: 160-162 °C
FT-IR: υ (cm^{-1}): 3388, 3310 (NH_2); 3160 (Ar-H); 1630 (C=N), 1606, 1502, 1460 (Aromatic ring); 1300 (C-N 'amine')
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: 8.66 (1H, d, J = 1.5 Hz, H-3), 8.55 (1H, d, J = 2.3 Hz, H-2), 7.88 (1H, d, J = 9.15 Hz, H-8), 7.18-7.20 (1H, dd, J = 9.2 Hz, J = 3.05 Hz, H-7), 7.14 (1H, d, J = 2.3 Hz, H-5), 4.23 (2H, s, NH_2)

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Compound 3: 2,3-dimethyl-6-nitroquinoxaline
Physical Appearance: Shiny golden pink precipitates
Yield: (94 %)
Melting Point: 129-130 °C
FT-IR: υ (cm^{-1}): 3044 (Ar-H); 1618 (C=N); 1579, 1493, 1451 (Aromatic ring); 1523, 1343 (NO_2); 848 (C-NO_2)
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: 8.95 (1H, s, H-5), 8.48 (1H, d, *J* = 8.4 Hz, H-7), 8.22 (1H, d, *J* = 9.15 Hz, H-8), 2.86 (3H, s, 3H-1'), 2.84 (3H, s, 3H-1'')

Compound 4: 2,3-dimethylquinoxalin-6-amine
Physical appearance: Yellow crystalline solid
Yield: 87 %
Melting Point: 187-188 °C
FT-IR: υ (cm^{-1}): 3326, 3333 (NH_2); 3020 (Ar-H); 1639 (C=N), 1621, 1563, 1503, 1468 (Aromatic ring); 1250 (C-N 'amine')
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: 8.09 (1H, d, *J* = 8.4 Hz, H-8), 7.76 (1H, d, *J* = 8.4 Hz, H-5), 7.08-7.10 (1H, m, H-7), 4.07 (2H, s, NH_2), 2.65-2.67 (6H, m, 3H-1', 3H-1'')

Compound 5: 2,3-di(furan-2-yl)-6-nitroquinoxaline
Physical appearance: Dark brown crystals
Yield: 96 %
Melting Point: 160-161 °C
FT-IR: υ (cm^{-1}): 3097 (Ar-H); 1618 (C=N); 1567, 1523, 1467 (Aromatic ring); 1482, 1339 (NO_2); 828 (C-NO_2)
^{1}H NMR: (DMSO, 500MHz): δ_H: 8.9 (1H, d, *J* = 2 Hz, H-5), 8.59-8.62 (1H, dd, *J* = 9.5 Hz, 2.5 Hz, H-7), 8.31 (1H, d, *J* = 9 Hz, H-8), 7.90-7.91 (2H, two closely merged doublets, H-5', H-5''), 7.03 (1H, d, *J* = 3.5 Hz, H-3'), 7.00 (1H, d, *J* = 3.5 Hz, H-3''), 6.80-6.81 (2H, m, H-4', H-4'')

Compound 6: 2,3-di(furan-2-yl)quinoxalin-6-amine
Physical appearance: Straw-colored crystals
Yield: 88 %
Melting Point: 192-194 °C
FT-IR: υ (cm^{-1}): 3459, 3305 (NH_2); 3199, 3118 (Ar-H); 1630 (C=N), 1596, 1574, 1529, 1486 (Aromatic ring); 1340 (C-N 'amine')
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: 7.92 (1H, d, *J* = 9.0 Hz, H-8), 7.6 (1H, s, H-5''), 7.58 (1H, d, *J* = 0.5 Hz, H-5'), 7.20 (1H, d, *J* = 2.5 Hz, H-3''), 7.15-7.17 (1H, dd, *J* = 9.0 Hz, *J* = 2.5 Hz, H-3'), 6.52-6.56 (4H, m, H-5, H-7, H-4', H-4''), 4.27 (2H, s, NH_2)

Compound 7: 2,3-diphenyl-6-nitroquinoxaline
Physical appearance: Shiny off-white precipitates
Yield: 95 %
Melting Point: 192-194 °C
FT-IR: υ (cm^{-1}): 3051 (Ar-H); 1616 (C=N); 1515, 1337 (NO_2); 837 (C-NO_2)
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: 9.08 (1H, d, *J* = 2.3 Hz, H-5), 8.55-8.53 (1H, dd, *J* = 9.15 Hz, 2.3 Hz, H-7), 8.3 (1H, d, *J* = 9.15, H-8), 7.59-7.55 (4H, m, H-3', H-5', H-3'', H-5''),7.43-7.36 (6H, m, H-2', H-4', H-6', H-2'', H-4'', H-6'')

Compound 8: 2,3-diphenylquinoxalin-6-amine
Physical appearance: Yellow crystals
Yield: 86 %
Melting Point: 180-181 °C
FT-IR: υ (cm^{-1}): 3445, 3317 (NH_2); 3212 (Ar-H); 1638 (C=N), 1613, 1490 (Aromatic ring); 1349 (C-N 'amine')
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: 8.00 (1H, d, *J* = 9.15 Hz, H-8), 7.52-7.59 (4H, m, H-3', H-5', H-3'', H-5''), 7.44-7.45 (2H, m, H-4', H-4''), 7.24-7.39 (8H, m, H-5, H-7, H-2', H-6', H-2'', H-6'', NH_2)

Compound 9: 7-Nitro-11H-indeno[1,2-b]quinoxalin-11-one (9a) / 8-Nitro-11H-indeno[1,2-b]quinoxalin-11-one (9b)
Physical appearance: Yellow orange precipitates
Yield: 91 %
Melting Point: 292-294 °C
FT-IR: υ (cm^{-1}): 3070 (Ar-H); 1721 (C=O); 1607, 1573, 1501 (Aromatic ring); 1536, 1350 (NO_2)
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: "9.12 (1H, d, *J* = 2.5Hz, H-8_a), 9.02 (1H, d, *J* = 2.5Hz, H-11_b), 8.59-8.61 (1H, dd, *J* = 9Hz, 2.5Hz, H-10_a), 8.51-8.53 (1H, dd, *J* = 9Hz, 2.5Hz, H-9_b), 8.41 (1H, d, *J* = 9Hz, H-8_b), 8.28 (1H, d, *J* = 9Hz, H-11_a), 8.19-8.21 (2H, dd, *J* = 7.5Hz, 0.5Hz, H-5_a, H-5_b), 8.01 (2H, d, *J* = 7.5Hz, H-2_a, H-2_b), 7.85-7.88 (2H, dt, *J* = 7.5Hz, 1Hz, H-3_a, H-3_b), 7.71-7.74 (2H, dt, *J* = 7.5Hz, 1Hz, H-4_a, H-4_b)"

Compound 10: 8-Amino-11H-indeno[1,2-b]quinoxalin-11-one (10a) / 7-Amino-11H-indeno[1,2-b]quinoxalin-11-one (10b)
Physical appearance: Orange precipitates
Yield: 85 %
Melting Point: 238-240 °C
FT-IR: υ (cm^{-1}): 3322, 3300 (NH_2); 3160 (Ar-H); 1630 (C=N), 1587, 1578, 1479 (Aromatic ring); 1342 (C-N 'amine')
^{1}H NMR: ($CDCl_3$, 500MHz): δ_H: "8.18 (1H, d, *J* = 6Hz, H-5_b), 8.07 (1H, d, *J* = 7.5Hz, H-5_a), 7.94-7.97 (2H, m, H-2_a, H-2_b), 7.81 (1H, d, *J* = 7.5Hz, H-3_a), 7.63 (1H, d, *J* = 6Hz, H-3_b), 7.42-7.50 (4H, m, H-4_a, H-4_b, H-8_b,H-11_a), 7.16-7.19 (2H, m, H-8_a, H-10_a), 7.13 (1H, d, *J* = 8.5Hz, H-9_b), 6.88 (1H, s, H-11_b), 4.17 (2H, s, $NH_{2\ a}$), 4.09 (2H, s, $NH_{2\ b}$)"