Chemicals Targeting an HIV-1 Nef/Host Cell Kinase Complex as Novel Anti-Retroviral Compounds

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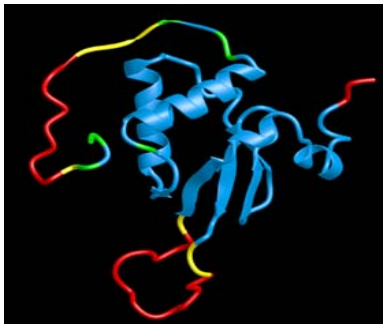
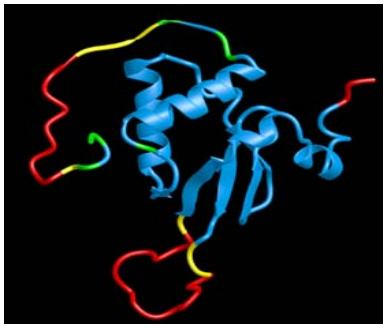
HIV-AIDS



- HIV-**H**uman **I**mmunodeficiency **V**irus
- AIDS- **A**cquired **I**mmune **D**eficiency **S**yndrome (NOW A PANDEMIC)
- Mechanism- T-cell, Macrophages, Dendritic cells, etc.
- 33.2 million cases in 2007
- 2.1 million deaths (330,000) annually
- US Center for Disease Control and Prevention.

Accessory Protein

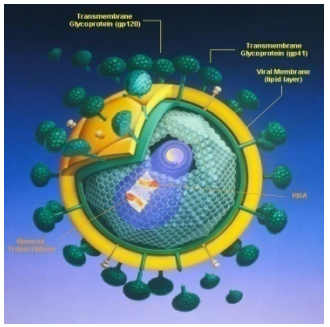


- Nef
- Nef is an HIV capsid and RNA-encoded protein essential in the pathogenesis of AIDS; it is a good, new target for anti-HIV drug discovery.
- Nef interacts with Src family kinases, including Hck, altering their sites and regulationof signal transduction.
- These Nef-mediated interactions optimize viral replication and contribute to the immune cell invasion, as well as survival of infected cells.

High-Throughput Screening Assay

- Inhibitors of Nef in a complex with one of its host cell binding partners.
- 10,000 discrete chemical compounds were screened and two classes of inhibitors for the protein-protein interaction were identified.
- Identified these two substructures as a valuable probes of HIV Nef function and as potential pharmacophores for future AIDS drug discovery and development.

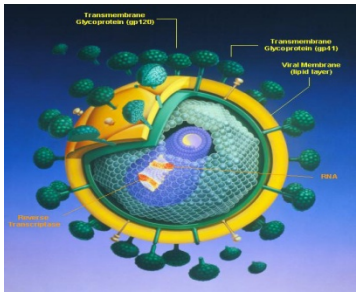
MECHANISM



- Nef binds to the Src 3 homology 3 (SH3) domains of the Src family members (Fyn, Hck, Lyn, Kyn, and c-Src).
- Growing evidence shows that Nef:SFK interaction is an important interaction for HIV replication and AIDS progression.

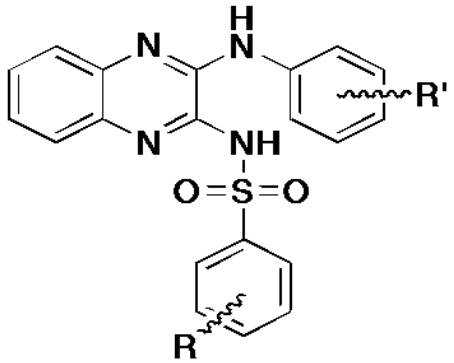
APPROACH

- Nef catalytic function has yet to be fully elucidated.
- A different approach that couples Nef to the activation of Hck.
- These compounds represent valuable chemical probes for Nef-dependent HIV-1 replication in vitro.



PURPOSE

- Inhibiting the function of the Nef protein and other HIV accessory factors and their interaction with host cell target proteins may accelerate the discovery of new anti-HIV agents.



2-sulfonamido-3-arylaminequinoxalines

GOALS

1. Synthesis of one or more derivatives that will inhibit the interaction of Nef and Hck (2-sulfonamido-3-arylaminequinoxalines)

    -Organic synthesis

    -Purification

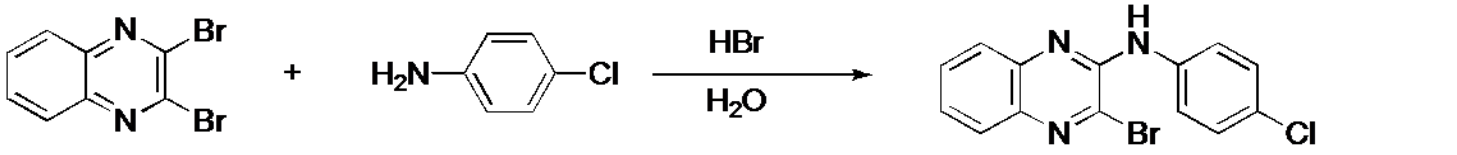
    -Column Chromatography

    -NMR

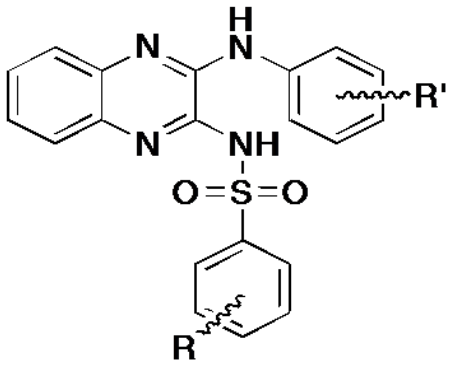
    -LC-ESI-MS

2. Test the chemical(s) for inhibitory activities

3-Bromo-*N*-(4-chlorophenyl)quinoxalin-2-amine



2,3-Dibromoquinoxaline 4-Chloroaniline 3-Bromo-*N*-(4-chlorophenyl)quinoxalin-2-amine

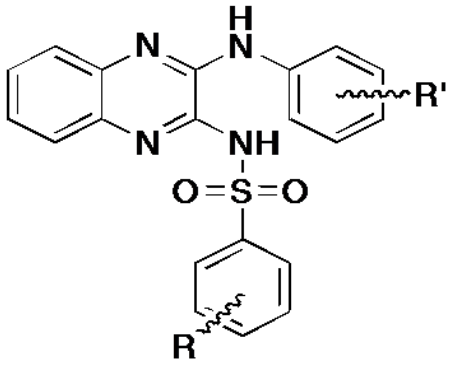


2-sulfonamido-3-arylaminequinoxalines

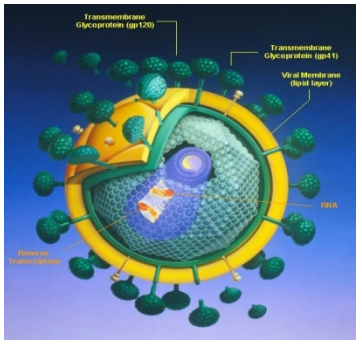
Sulfonamide

First trial

- 0.001 mmole
- 2,3-Dibromoquinoxaline (288 mg)
- *p*-Chloroaniline (127 mg)
- Hydrobromic acid (0.113 mL)
- Water (10 mL)



2-sulfonamido-3-arylaminequinoxalines

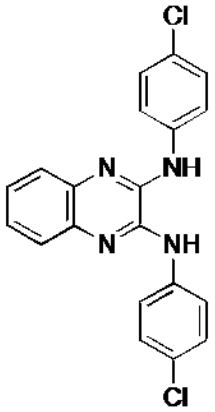


SULFONAMIDE

- Refluxed -For a total of 9 hours.
- Extraction- 20 mL of NaOH (Sodium hydroxide) and 20 mL of CH_2Cl_2 (Dichloromethane).

RESULTS

- Disubstituted product obtained.
- 19% Product and 81% starting material

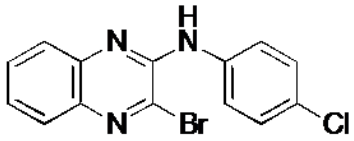


N^2,N^3-bis(4-chlorophenyl)quinoxaline-2,3-diamine

Sulfonamide

Second trial

- 0.0005 mmole
- 2,3-dibromoquinoxaline (144 mg)
- *p*-Chloroaniline (63.5 mg)
- Hydrobromic acid (0.565 mL)
- Water (5 mL)



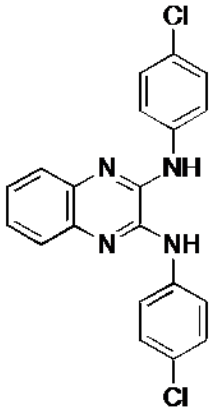
3-Bromo-*N*-(4-chlorophenyl)quinoxalin-2-amine

Sulfonamide

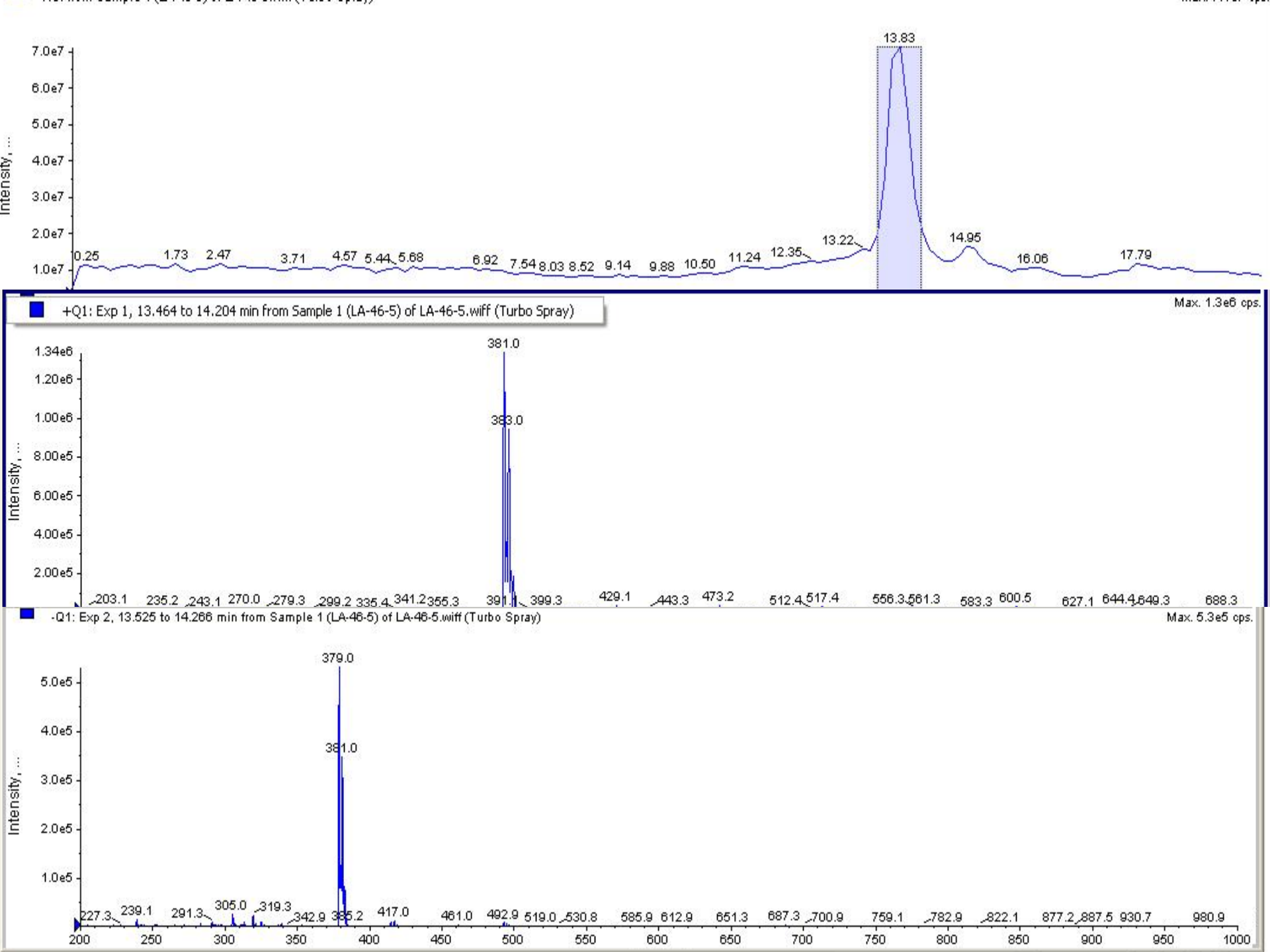
- Refluxed - For a total of 20 hours.
- Extraction - 20 mL of NaOH (Sodium hydroxide) and 20 mL of CH_2Cl_2 (Dichloromethane).
- Temperature was monitored, 88 °C – 90 °C

RESULTS

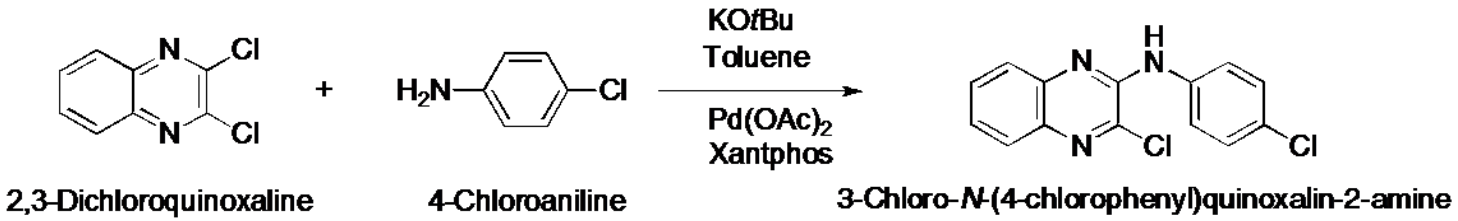
- Disubstituted product obtained.
- 36% Product and 64% starting material

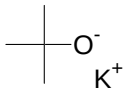
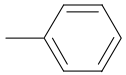
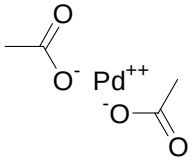
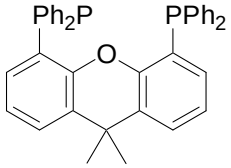


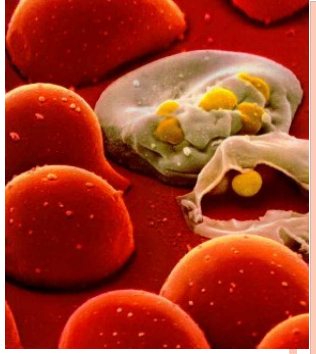
N^2,N^3-bis(4-chlorophenyl)quinoxaline-2,3-diamine


Intensity, ...
7.0e7
6.0e7
5.0e7
4.0e7
3.0e7
2.0e7
1.0e7
13.83
0.25
1.73
2.47
3.71
4.57
5.44
5.68
6.92
7.54
8.03
8.52
9.14
9.88
10.50
11.24
12.35
13.22
14.95
16.06
17.79
+Q1: Exp 1, 13.464 to 14.204 min from Sample 1 (LA-46-5) of LA-46-5.wiff (Turbo Spray)
Max. 1.3e6 cps.
1.34e6
1.20e6
1.00e6
8.00e5
6.00e5
4.00e5
2.00e5
381.0
383.0
203.1
235.2
243.1
270.0
279.3
299.2
335.4
341.2
355.3
391.1
399.3
429.1
443.3
473.2
512.4
517.4
556.3
561.3
583.3
600.5
627.1
644.4
649.3
688.3
-Q1: Exp 2, 13.525 to 14.266 min from Sample 1 (LA-46-5) of LA-46-5.wiff (Turbo Spray)
Max. 5.3e5 cps.
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
379.0
381.0
227.3
239.1
291.3
305.0
319.3
342.9
385.2
417.0
461.0
492.9
519.0
530.8
585.9
612.9
651.3
687.3
700.9
759.1
782.9
822.1
877.2
887.5
930.7
980.9
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000

0.001 mmole

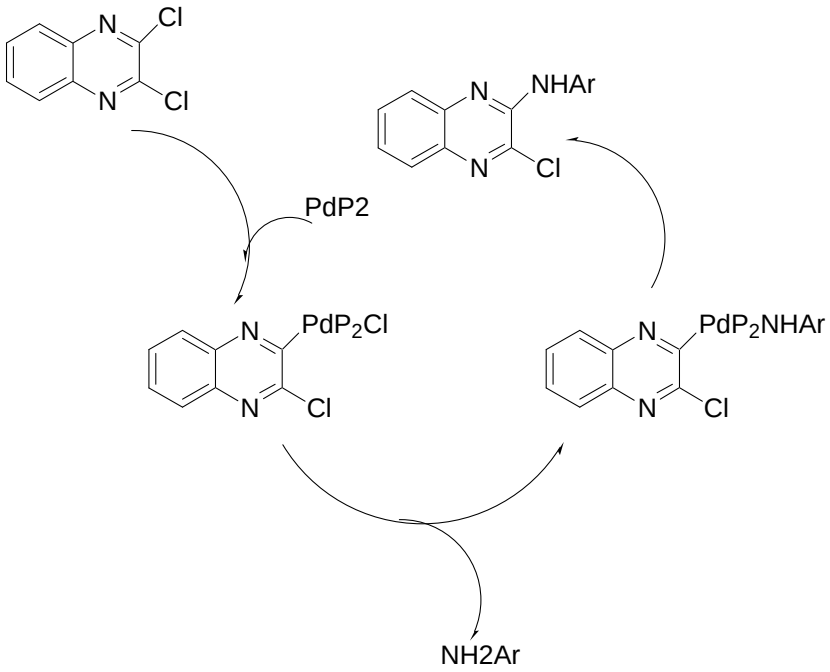

KO*t*Bu
Toluene
Pd(OAc)2
Xantphos
2,3-Dichloroquinoxaline
4-Chloroaniline
3-Chloro-*N*-(4-chlorophenyl)quinoxalin-2-amine

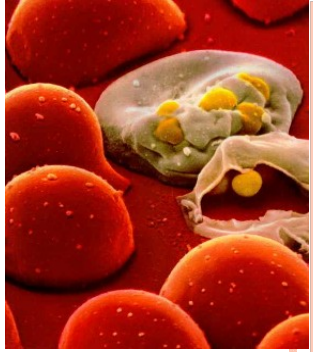
Reagents	Structure	Purpose
KO*t*Bu (Potassium tert-butoxide)		Used to deprotonate amine
Toluene	 toluene	Solvent
$Pd(OAc)_2$ (Palladium(II) acetate)		Catalyst
Xantphos (Phosphorous Ligand)		Trapped palladium catalyst inserted into C-halogen bond.



Third trial

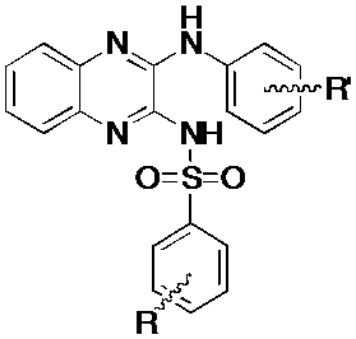
- 0.001 mmole
- 2,3-dichloroquinoxaline (144 mg)
- *p*-Chloroaniline (63.5 mg)
- KO*t*Bu - Potassium *tert*-butoxide (1.2 equivalents)
- Toluene - Solvent (15 mL)
- $Pd(OAc)_2$ -Palladium(II) acetate (5 molar %)
- Xantphos – Phosphorous ligand (7.5 molar %)


N
Cl
N
Cl
PdP2
N
NHAr
N
Cl
N
PdP2Cl
N
Cl
N
PdP2NHAr
N
Cl
NH2Ar



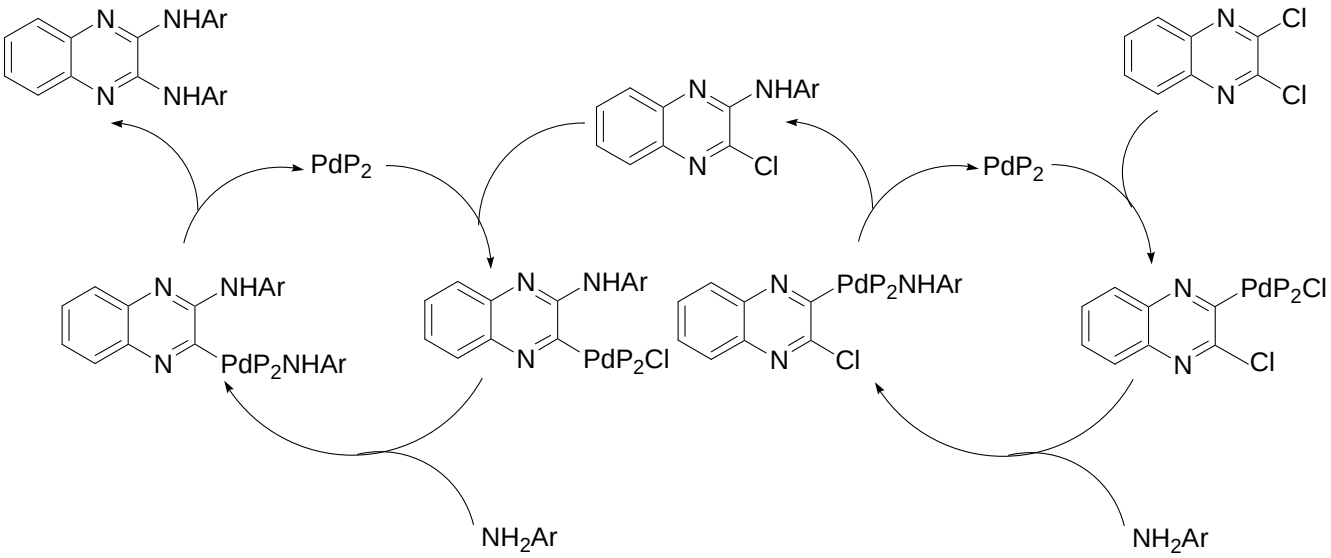
Sulfonamide group

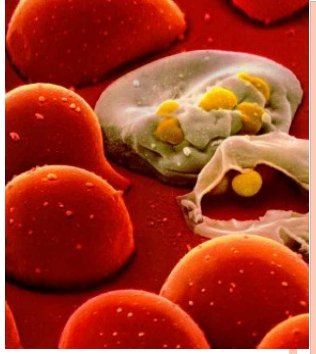
- Cesium Carbonate - Cs_2CO_3 (1.2 equivalents)
- DMF anhydrous - Dimethylformamide
- Reflux under N_2 atmosphere



2-sulfonamido-3-arylaminequinoxalines

Palladium Catalysis





- Refluxed - For a total of 22 hours.
- Extraction

  15 mL of NH_4Cl (Ammonium chloride)

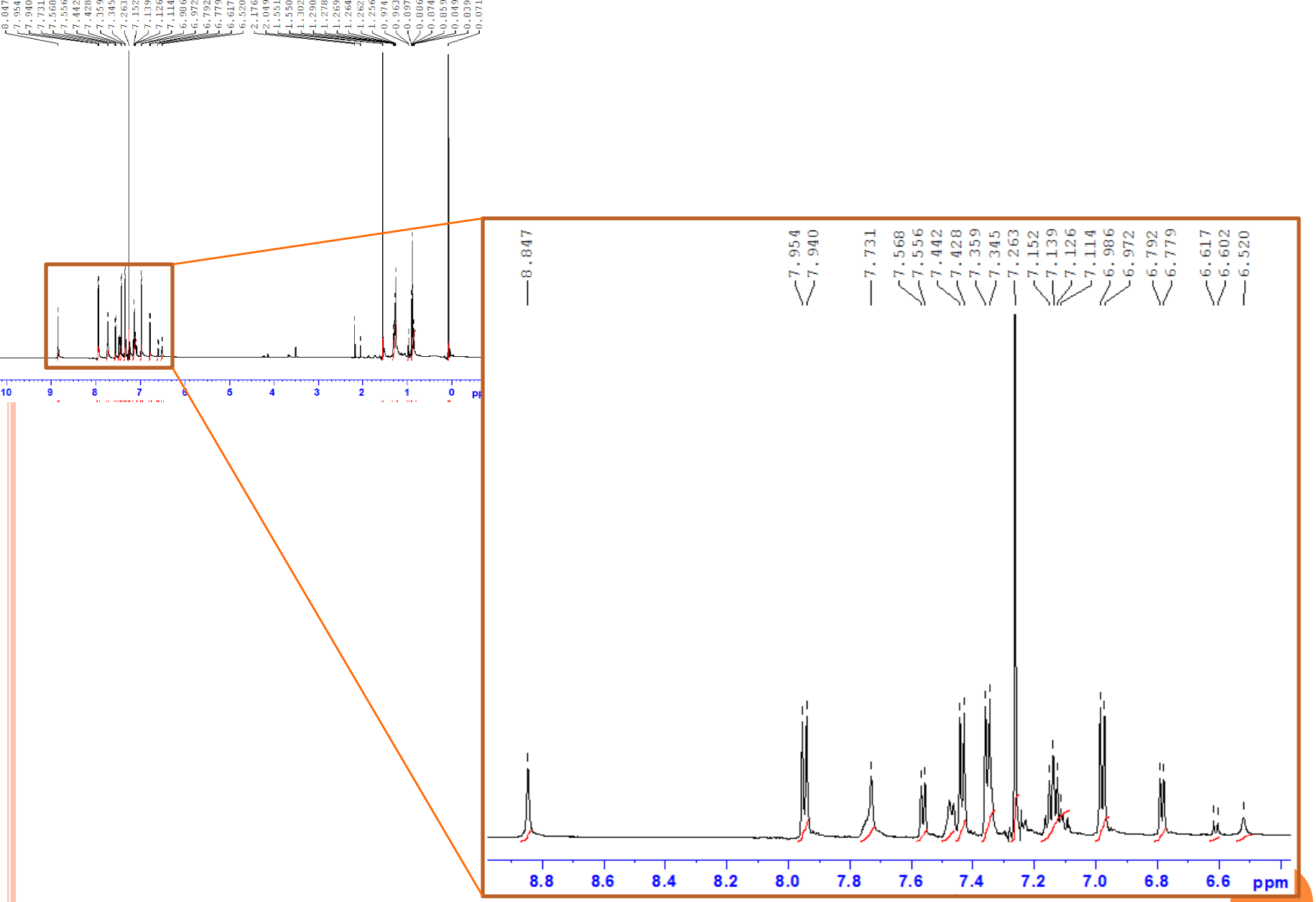
  20 mL of CH_2Cl_2 (Dichloromethane)

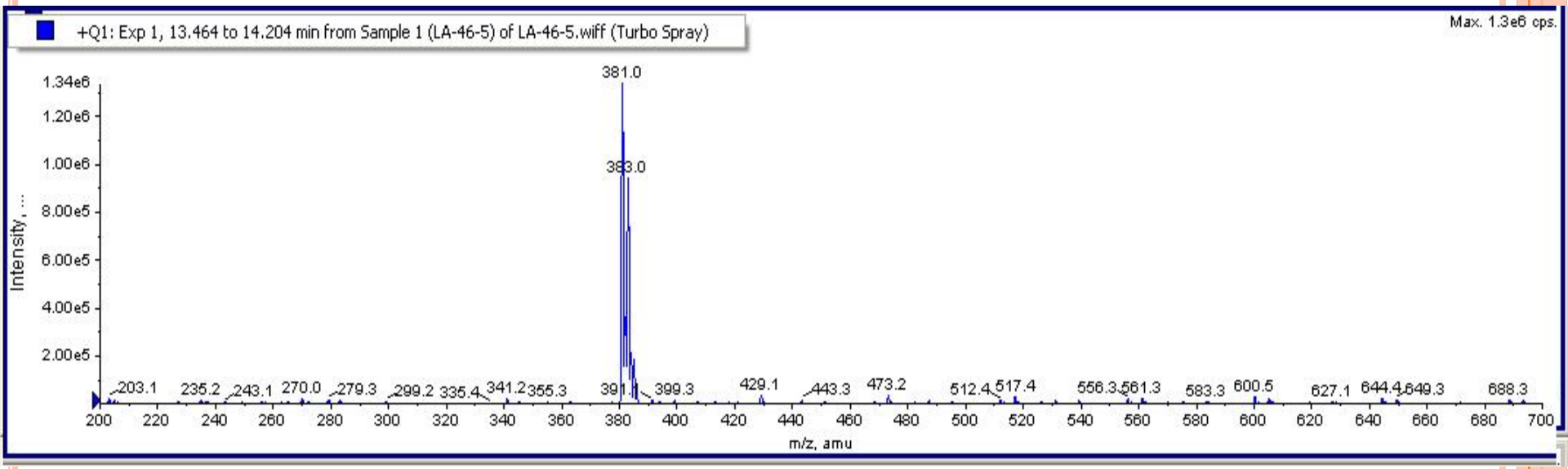
  10 mL of water

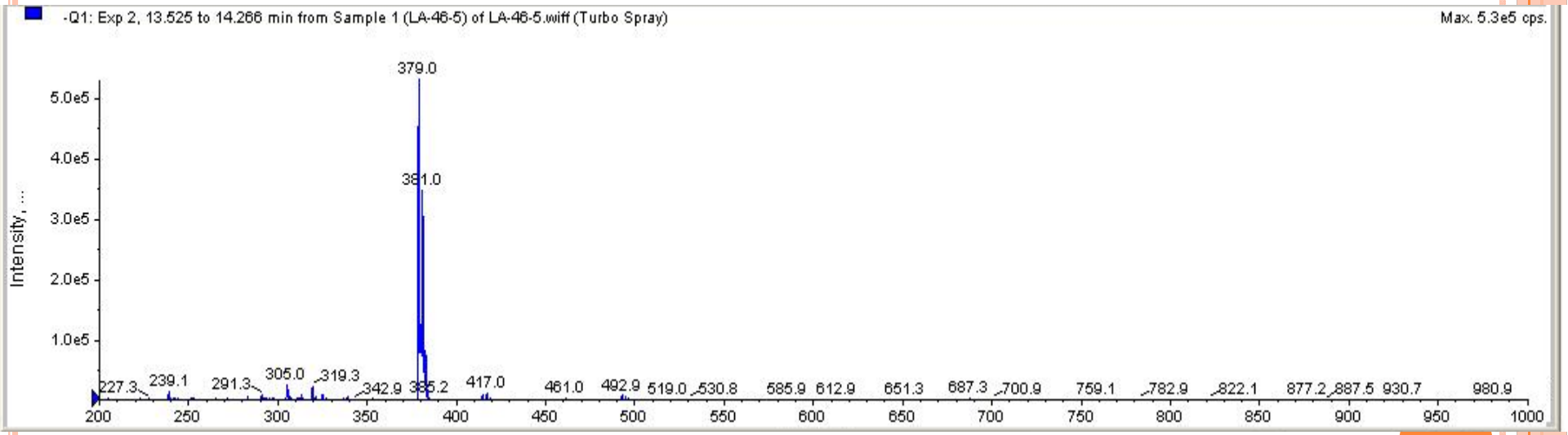
  25 mL of saturated aqueous NaCl (Sodium chloride)
- Dried with magnesium sulfate for 1 hour.

RESULTS

- Disubstituted product obtained.

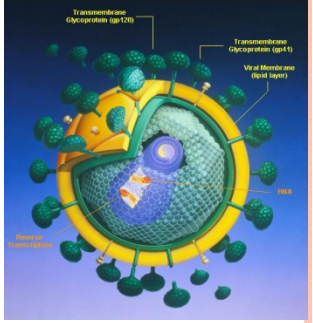

8.847
7.954
7.940
7.731
7.568
7.556
7.442
7.428
7.359
7.345
7.263
7.152
7.139
7.126
7.114
6.986
6.972
6.792
6.779
6.617
6.602
6.520
8.8
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
ppm


+Q1: Exp 1, 13.464 to 14.204 min from Sample 1 (LA-46-5) of LA-46-5.wiff (Turbo Spray)
Max. 1.3e6 cps.
Intensity, ...
m/z, amu
381.0
383.0


-Q1: Exp 2, 13.525 to 14.266 min from Sample 1 (LA-46-5) of LA-46-5.wiff (Turbo Spray)
Max. 5.3e5 cps.
Intensity, ...
379.0
381.0

Isolation

- Flash Column Chromatography
- NMR (Nuclear Magnetic Resonance)
- HPLC-MS (High Performance Liquid Chromatography-Mass Spectrometry)
- Characterization
  - 35 mg of isolated product
  - melting point: 225 °C – 235 °C



Testing

- Time didn't allow

Future Plans

Test the compound on Nef protein, Hck protein and Nef:Hck interaction through high-throughput biochemical and high information content cell-based screening assays.

Acknowledgements

- MARC
- BBSI
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- Duquesne University
- Billy Day, Ph.D.
- Vasiliy Korotchenko, Ph.D.
- NIH

ANY QUESTIONS ????