Electronic Supplementary Information

$Yb(OTf)_3$ catalyzed new cascade reaction: a facile assembly of fused quinazolinones

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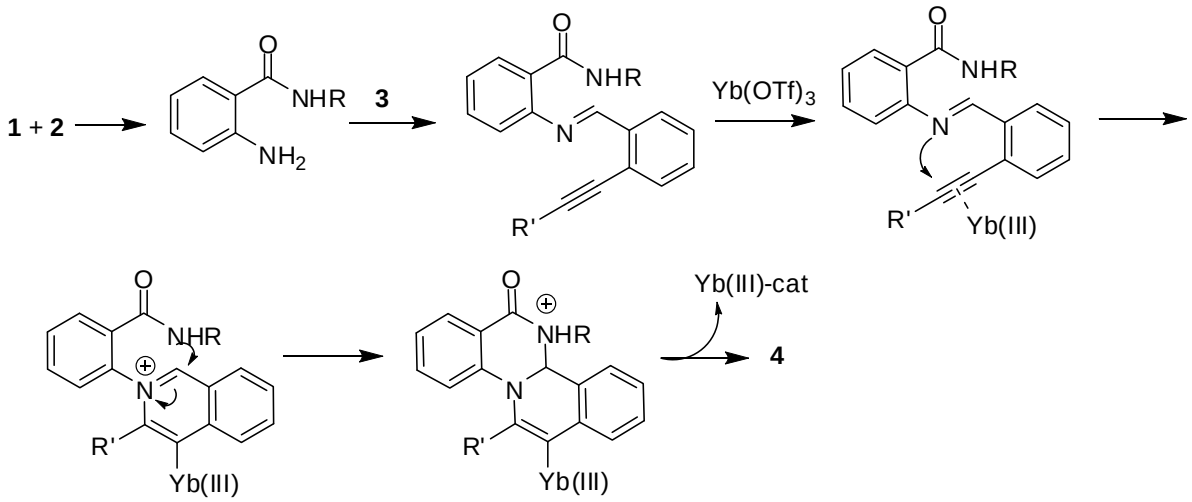
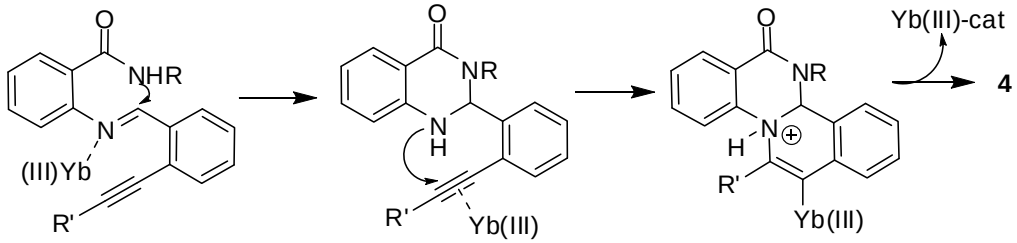
Proposed reaction mechanism for $Yb(OTf)_3$ catalyzed three component reaction

Mechanistically, the reaction seems to proceeds *via* generation of *N*-substituted *o*-aminobenzamide from **1** and **2** *in situ* which on condensation with **3** provides the corresponding imine. A subsequent intramolecular and regiospecific nucleophilic attack of the imine nitrogen at the Yb-coordinated alkyne generates the corresponding (2-(2-carbamoylphenyl)isoquinolinium-4-yl)ytterbium ion which undergoes further nucleophilic attack by the amide nitrogen at C-1 in an intramolecular fashion. The product **4** thus is formed via a tandem process.



Alternative mechanism

Alternatively, 2-(o-alkynylphenyl)-2,3-dihydroquinazolin-4-one intermediate formed through an intramolecular cyclization of iminoalkyne-Yb complex undergoes Yb(III)-assisted regiospecific intramolecular hydroamination reaction leading to the formation of **4**.



Experimental

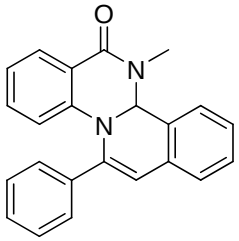
Chemistry

General methods: Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane, ethyl acetate, dichloromethane. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solution by using 400 or 500 and 50 or 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT- IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. High-resolution mass spectra (HRMS) were recorded using electron ionization (EI) mass spectrometry.

Preparation of 4 via $(CF_3SO_3)_3Yb$ catalyzed MCR:

A mixture of isatoic anhydride **1 (**1.0 g, 6.13 mmol), amine **2** (6.75 mmol), 2-(alkynyl) benzaldehyde **3** (6.75 mmol) and $(CF_3SO_3)_3Yb$ (0.38 g, 0.61 mmol) was stirred in DCE at ambient temperature for about 48 h to complete the reaction. After completion of the reaction, the mass was concentrated and the crude product was purified by column chromatography.

5-methyl-12-phenyl-4bH-isoquinolino [2,1-a]quinazolin-6(5H)-one (4a)



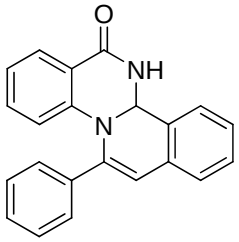
^{1}H NMR ($CDCl_3$, 400 MHz): 7.91(d, *J* = 7.8 Hz, 1H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.45-7.29 (m, 6H), 7.18-7.12 (m, 2H), 6.96 (t, *J* = 7.8 Hz, 1H), 6.77 (t, *J* = 7.8 Hz, 1H), 6.14 (d, *J* = 7.8 Hz, 1H), 5.64 (s, 1H), 3.45 (s, 3H)

^{13}C NMR ($CDCl_3$,100 MHz): 163.2, 141.9, 140.4, 134.5, 133.3, 132.6, 129.3 (2C), 129.1 (2C), 128.4, 128.2, 128.1, 126.1 (2C), 125.6, 123.0 (2C), 120.1, 116.6, 116.4, 74.6, 29.9

IR (KBr): 3158, 2985, 1706, 1625, 1428, 1080 cm^{-1}

HRMS (ESI): calcd for $C_{23}H_{19}N_2O$ $(M+H)^+$ 339.1497, found 339.1493

12-phenyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4b):



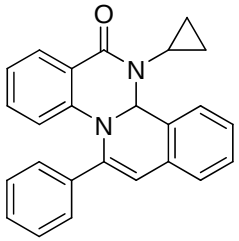
^{1}H NMR (DMSO-d_6, 400 MHz): 9.11 (s, 1H), 7.82 (d, *J* = 7.3 Hz, 2H), 7.69 (d, *J* = 7.3 Hz, 1H), 7.49-7.32 (m, 8H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.77 (t, *J* = 7.3 Hz, 1H), 6.11 (d, *J* = 7.3 Hz, 1H), 5.71 (s, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz):163.8, 143.9, 142.8, 135.2, 133.1 (2C), 132.2, 129.0, 128.9 (2C), 128.7, 128.1 (2C), 127.9, 126.6 (2C), 125.4 (2C), 123.8, 120.8, 116.7, 68.1

IR (KBr): 3492, 3168, 2975, 1722, 1632, 1438, 1078 cm^{-1}

HRMS (ESI): calcd for $C_{22}H_{17}N_2O$ $(M+H)^+$ 325.1341, found 325.1338

5-cyclopropyl-12-phenyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4c):



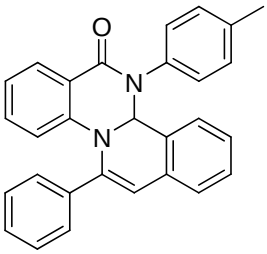
^{1}H NMR ($CDCl_3$, 400 MHz): 7.97(d, *J* = 7.8 Hz, 1H), 7.90 (d, *J* = 7.8 Hz, 2H), 7.62-7.43 (m, 4H), 7.31-7.16 (m, 4H), 6.99 (t, *J* = 7.8 Hz, 1H), 6.79 (t, *J* = 7.8 Hz, 1H), 6.18 (d, *J* = 7.8 Hz, 1H), 5.64 (s, 1H), 2.71-2.62 (m, 1H), 1.12-1.06 (m, 1H), 0.97-0.92 (m, 1H), 0.77-0.71 (m, 2H)

^{13}C NMR ($CDCl_3$, 100 MHz):164.6, 146.6, 142.6, 139.0, 138.5, 136.8, 133.6, 133.2, 131.6 (2C), 129.1, 128.8, 128.7, 128.5 (2C), 128.4 (2C), 126.7, 125.5, 119.1, 114.6, 70.7, 28.7, 11.1, 9.1

IR (KBr): 3167, 2975, 1722, 1632, 1438, 1078 cm^{-1}

HRMS (ESI): calcd for $C_{25}H_{21}N_2O$ $(M+H)^+$ 365.1654, found 365.1636

12-phenyl-5-p-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4d):



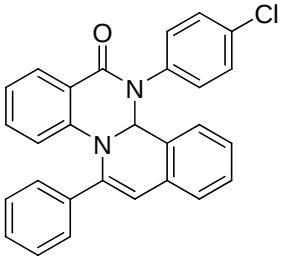
^{1}H NMR ($CDCl_3$, 400 MHz): 8.00 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.48-7.40 (m, 8H), 7.24-7.11 (m, 4H), 7.02 (t, 7.8 Hz, 1H), 6.83 (t, 7.8 Hz, 1H), 6.24 (d, *J* = 7.8 Hz, 1H), 6.06 (s, 1H), 2.36 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz):163.3, 148.2, 145.1, 141.6, 138.3, 136.4, 133.8, 133.0, 131.5 (2C), 129.5 (2C), 129.0, 128.9, 128.7 (2C), 128.6, 128.5, 126.5, 125.7 (2C), 122.2, 120.5, 119.4, 116.7, 114.8, 113.2, 72.3, 21.0

IR (KBr): 3172, 2980, 1718, 1628, 1443, 1084 cm^{-1}

HRMS (ESI): calcd for $C_{29}H_{23}N_2O$ $(M+H)^+$ 415.1810, found 415.1809

5-(4-chlorophenyl)-12-phenyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4e):



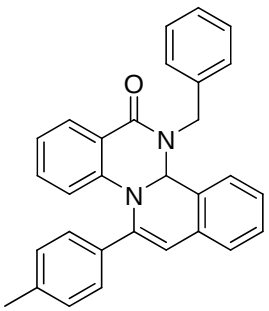
^{1}H NMR ($CDCl_3$, 400 MHz): 8.01(d, J=7.8 Hz, 1H), 7.89 (d, *J* = 7.8 Hz, 2H), 7.54 (d, *J* = 7.8 Hz, 2H), 7.48-7.36 (m, 6H), 7.31-7.18 (m, 4H), 7.02 (t, 7.8 Hz, 1H), 6.82 (t, 7.8 Hz, 1H), 6.25 (d, J=7.8 Hz, 1H), 6.04 (s, 1H)

^{13}C NMR ($CDCl_3$, 100 MHz):163.3, 148.2, 145.2, 141.0, 139.2, 134.1, 133.2, 132.0, 131.6 (2C), 129.2, 129.0 (3C), 128.8, 128.7, 128.6 (2C), 127.1 (2C), 126.5, 122.1, 120.7, 119.6, 116.4, 114.9, 112.8, 72.2

IR (KBr): 3168, 2985, 1716, 1626, 1436, 1082 cm^{-1}

HRMS (ESI): calcd for $C_{28}H_{20}ClN_2O$ $(M+H)^+$ 435.1264, found 435.1245

5-benzyl-12-p-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4f):



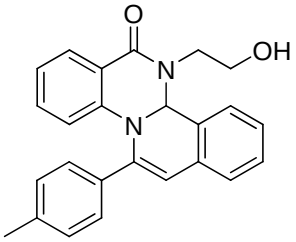
^{1}H NMR ($CDCl_3$, 400 MHz): 8.01 (d, J-7.6 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.36-7.14 (m, 13H), 7.02 (t, *J* = 7.6 Hz, 1H), 6.82 (t, *J* = 7.6 Hz, 1H), 6.22 (d, *J* = 7.6 Hz, 1H), 5.61 (s, 1H), 5.38-5.22 (m, 2H), 2.38 (s, 3H)

^{13}C NMR ($CDCl_3$, 100 MHz):163.5, 146.1, 144.9, 140.8, 139.3, 136.7, 133.6 (2C), 132.8 (2C), 131.4 (3C), 129.2 (3C), 128.7, 128.6, 128.1 (2C), 127.5, 125.6, 121.3, 119.1, 115.6, 114.5, 113.4, 68.2, 47.5, 21.5

IR (KBr): 3164, 2987, 1712, 1624, 1432, 1086 cm^{-1}

HRMS (ESI): calcd for $C_{30}H_{25}N_2O$ $(M+H)^+$ 429.1967, found 429.1962

5-(2-hydroxyethyl)-12-p-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4g):



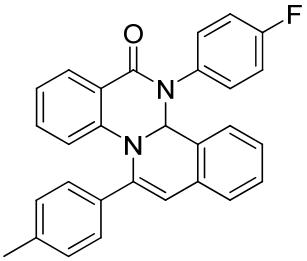
^{1}H NMR ($CDCl_3$,400 MHz): 7.93 (d, *J* = 7.9 Hz, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 7.9 Hz, 2H), 7.34-7.19 (m, 6H), 7.02 (t, *J* = 7.9 Hz, 1H), 6.82 (t, *J* = 7.9 Hz, 1H), 6.24 (d, *J* = 7.9 Hz, 1H), 5.68 (s, 1H), 5.21 (br s, 1H), 4.08-3.83 (m, 3H), 3.28-3.22 (m, 1H), 2.40 (s, 3H)

^{13}C NMR ($CDCl_3$,100 MHz): 165.2, 147.3, 145.0, 140.8, 139.4, 133.8, 132.9, 131.5 (2C), 129.4 (2C), 128.8, 128.7, 128.3, 125.7, 121.1, 119.2, 119.0, 115.6, 114.5, 114.0, 71.0, 61.9, 49.4, 21.5

IR (KBr): 3380, 2970, 1740, 1663, 1497, 1475 cm^{-1}

HRMS (ESI): calcd for $C_{25}H_{23}N_2O_2$ $(M+H)^+$ 383.1760, found 383.1748

5-(4-fluorophenyl)-12-p-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4h):



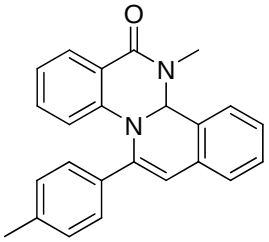
^{1}H NMR ($CDCl_3$,400 MHz): 8.02 (d, *J* = 7.8 Hz, 1H), 7.57-7.20 (m, 12H), 6.99 (t, *J* = 7.8 Hz, 2H), 6.86 (t, *J* = 7.8 Hz, 1H), 6.28 (d, *J* = 7.8 Hz, 1H), 5.70 (s, 1H), 2.40 (s, 3H)

^{13}C NMR ($CDCl_3$,100 MHz): 163.4, 147.9, 145.3, 141.0, 139.5, 136.6, 134.0, 133.0, 131.4 (2C), 129.4 (2C), 129.2, 129.0 (2C), 128.7, 128.6, 127.8, 127.7, 126.5,121.0, 119.5, 119.0, 116.4, 115.8, 115.6, 114.8, 72.4, 21.6

IR (KBr): 2975, 1735, 1665, 1482, 1473 cm^{-1}

HRMS (ESI): calcd for $C_{29}H_{22}FN_2O$ $(M+H)^+$ 433.1716, found 433.1715

5-methyl-12-p-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4i):



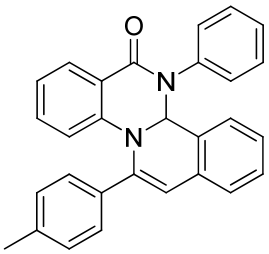
^{1}H NMR ($CDCl_3$,400 MHz): 7.96 (d, *J* = 7.8 Hz, 1H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 7.8 Hz, 2H), 7.33-7.18 (m, 6H), 6.99 (t, *J* = 7.8 Hz, 1H), 6.80 (t, *J* = 7.8 Hz, 1H), 6.22 (d, *J* = 7.8 Hz, 1H), 5.72 (s, 1H), 3.08 (s, 3H), 2.41 (s, 3H)

^{13}C NMR ($CDCl_3$,100 MHz): 163.7, 145.8, 144.9, 140.6, 139.4, 133.3, 133.0, 131.5 (2C), 129.4 (2C), 128.8, 128.6, 128.3, 125.4, 121.3, 119.1, 119.0, 115.7, 114.4, 112.5, 71.3, 32.8, 21.6

IR (KBr): 2973, 1733, 1660, 1478, 1468 cm^{-1}

HRMS (ESI): calcd for $C_{24}H_{21}N_2O$ $(M+H)^+$ 353.1654, found 353.1651

5-phenyl-12-*p*-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4j):



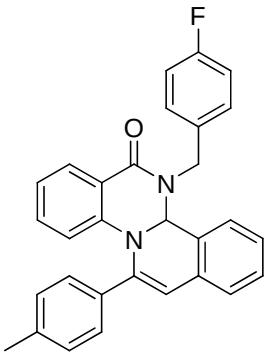
^{1}H NMR ($CDCl_3$,400 MHz): 8.03 (d, J-7.3 Hz, 1H), 7.57-7.53 (m, 2H), 7.45 (d, J-7.3 Hz, 2H), 7.39-7.20 (m, 10H), 7.05 (t, *J* = 7.3 Hz, 1H), 6.85 (t, *J* = 7.3 Hz, 1H), 6.28 (d, *J* = 7.3 Hz, 1H), 5.75 (s, 1H), 2.40 (s, 3H)

^{13}C NMR ($CDCl_3$,100 MHz): 163.3, 146.5, 145.2, 141.4, 140.9, 139.4, 133.9, 133.0, 131.4 (2C), 129.4 (2C), 128.9 (2C), 128.8, 128.7, 128.5, 126.6, 126.5, 125.7 (2C), 120.7, 119.4, 119.0, 116.7, 114.8, 111.8, 72.2, 21.5

IR (KBr): 2980, 1739, 1670, 1485, 1475 cm^{-1}

HRMS (ESI): calcd for $C_{29}H_{23}N_2O$ $(M+H)^+$ 415.1810 , found 415.1805

5-(4-fluorobenzyl)-12-p-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4k)



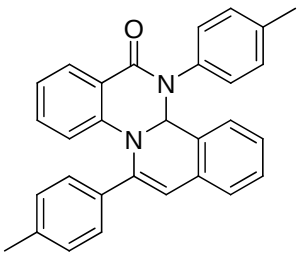
^{1}H NMR ($CDCl_3$,400 MHz): 8.00 (d, J-7.8 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.33-7.16 (m, 11H), 6.96 (t, *J* = 7.8 Hz, 2H), 6.83 (t, *J* = 7.8 Hz, 1H), 6.30 (d, *J* = 7.8 Hz, 1H), 5.80 (s, 1H), 5.32-5.22 (m, 2H), 2.39 (s, 3H)

^{13}C NMR ($CDCl_3$,100 MHz): 163.4, 146.9, 144.9, 140.6, 139.4, 133.7, 132.8, 132.5, 131.4 (2C), 129.9, 129.8, 129.3 (2C), 129.2, 128.7 (2C), 128.6, 125.6, 121.3, 119.1, 118.9, 115.6, 115.5, 115.3, 114.5, 112.5, 68.3, 46.8, 21.5

IR (KBr): 2974, 1742, 1673, 1488, 1478 cm^{-1}

HRMS (ESI): calcd for $C_{30}H_{24}FN_2O$ $(M+H)^+$ 447.1873, found 447.1871

5,12-dip-tolyl-4bH-isoquinolino[2,1-a]quinazolin-6(5H)-one (4l):



^{1}H NMR ($CDCl_3$,400 MHz): 8.00 (d, *J* = 7.3 Hz, 1H), 7.76 (d, *J* = 7.3 Hz, 2H), 7.50-7.46 (m, 3H), 7.29-7.00 (m, 9H), 6.80 (t, *J* = 7.3 Hz, 1H), 6.24 (d, *J* = 7.3 Hz, 1H), 6.05 (s, 1H), 2.41 (s, 3H), 2.35 (s, 3H)

^{13}C NMR ($CDCl_3$,100 MHz): 163.3, 146.4, 145.1, 141.5, 139.4, 138.3, 136.3, 133.7, 132.9, 132.4, 131.4 (2C), 129.5 (2C), 129.4, 129.2, 128.9, 128.5, 126.5, 125.6 (2C), 120.7, 119.4, 119.1, 116.8, 114.8, 112.6, 72.3, 21.6, 21.0

IR (KBr): 2980, 1746, 1677, 1495, 1482 cm^{-1}

HRMS (ESI): calcd for $C_{30}H_{25}N_2O$ $(M+H)^+$ 429.1967, found 429.1965

Single crystal X-ray data

Single crystals suitable for X-ray diffraction of compound **4b** were grown from methanol. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-Kα radiation (0.71073 Å). The crystals were glued to a thin glass fibre using FOMBLIN immersion oil and mounted on the diffractometer. The intensity data were processed using Broker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS.[1] The crystal structure was solved by direct methods using SHELXS-97 and the data was refined by full matrix least-squares refinement on F^2 with anisotropic displacement parameters for non-H atoms, using SHELXL-97.[2]

Crystal data of **4b:** Molecular formula = $C_{22}H_{16}N_2O$, Formula weight =324.37, Crystal system = Monoclinic, space group = $P2_1/n$, a = 9.388(6) Å, b =11.595(8) Å, c = 15.005(10) Å, V = 1627.3(19) Å^3, T = 100(2) K, Z = 4 D_c = 1.324 Mg m^{-3}, μ(Mo-Kα) = 0.08 mm^{-1}, 6728 reflections measured, 3152 independent reflections, 1525 observed reflections [I > 2.0 σ (I)], R_1_obs = 0.079, Goodness of fit = 0.765. Crystallographic data (excluding structure factors) for **4b** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 828186.

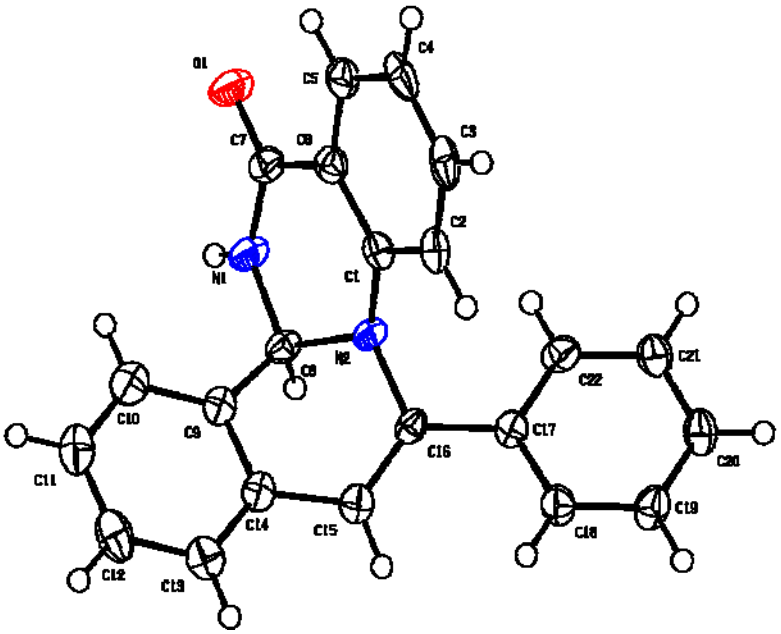


Figure 1. X-ray crystal structure of **4b** (ORTEP diagram). Thermal ellipsoids are drawn at 50% probability level.

Reference

1. Bruker SADABS V2008-1, Bruker AXS.: Madison, WI, USA, **2008**.
2. G. M. Sheldrick,; SHELX-97, Program for Crystal Structure Determination, University of Göttingen, **1997**.

Pharmacology

Materials and Methods

Cells and reagents

RAW 264.7 cells (murine macrophage cell line) were obtained from ATCC (Washington D.C., USA) and routinely maintained in RPMI 1640 medium with 10% fetal bovine serum (Invitrogen Inc., San Diego, CA, USA). Lipopolysaccharide (LPS) was from *Escherichia coli* strain 0127:B8 obtained from Sigma (St. Louis, MO, USA). Mouse TNF-α ELISA kit was procured from R&D Systems (Minneapolis, MN, USA).

TNF-α production assay

The production of TNF-α is measured following a procedure described previously after few modifications.[1] Briefly, RAW 264.7 cells were pre-incubated either with DMSO (vehicle control) or compound for 30 minutes and then stimulated with 1 μg/ml of LPS overnight. Preliminary screening of the compounds was performed at 30 μM and dose response studies were carried out at eight different concentrations (30, 10, 3, 1, 0.3, 0.1, 0.03, 0.01 μM). Post-stimulation, cell supernatants were harvested, centrifuged to clear cell debris and the amount of TNF-α in the supernatants was measured using mouse TNF-α DuoSet ELISA kit from R&D Systems according to manufacturer's recommendations. The percentage of inhibition was calculated using the following formula:

$$\% \text{ inhibition} = 100 - \left| \frac{(\text{LPS stimulated}_{\text{compound}} - \text{unstimulated})}{(\text{LPS stimulated}_{\text{DMSO}} - \text{unstimulated})} \text{ X } 100 \right|$$

Reference:

1. K. V. Parsa, L. P. Ganesan, M. V. Rajaram, M. A. Gavrilin, A. Balagopal, N. P. Mohapatra, M. D. Wewers, L. S. Schlesinger, J. S. Gunn and S. Tridandapani, *PLoS Pathog.* 2006, 2:e71.

Docking study

The docking study was carried out using Glide application of schrodinger software with MASTERO interface 9.2

The compound **4i** was docked in the TNF-α protein and there glide scores and interactions were observed.

Procedure: In the present study the energy minimization and conformational search was performed using the MACROMODEL application in the Schrodinger package. The molecule to be docked was energy minimized for flexibility and the conformational search was followed. We have used OPLS_2005 force field and water as implicit solvent. We have followed the PRCG (Polak-Ribier conjugate gradient) method of minimization with 500 iterations with a threshold gradient on 0.05kJ/mol. The conformational search performed was based on Montecarlo multiple minimum torsional sampling. The ligand was then finally prepared by using LIGPREP application.

The TNF-α protein (3L9J) crystal structure was retrieved from the protein data bank and it was refined with the PROTEIN PREPERATION WIZARD application in which the hydrogen's were added and missing side chains and loops were filled with PRIME application. Water molecules were observed within the 5A distance and other water molecules beyond 5A from het (heteroatom) groups were deleted. Finally the protein was optimized and minimized with impref using OPLS_2005 force filed. GRID based docking was performed in the present study.

Docking of compound **4i** with TNF-α protein: