Supporting Information

Figures

NMR characterization of the influence of zinc(II) ions on the structural and dynamic behavior of the New-Delhi Metallo-β-lactamase-1 and on the binding with flavonols as inhibitors

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Figure S1. Superposition of the 800 MHz $^1$H-$^{15}$N HSQC spectra recorded on NDM-1 (332 μM) in absence of Zn(II) (red) and in presence of 3 molar equivalent of Zn(II) (dark blue) at 20°C in solution in 0.1 mM bis tris pH 7.0, 150 mM NaCl.
Figure S2. 800 MHz $^{1}\text{H}$-$^{15}\text{N}$ HSQC spectra recorded on NDM-1 (550 μM) in absence of Zn(II) at 20°C in solution in 50mM phosphate buffer pH 7.0.
Figure S3. Zoom of the 800 MHz $^1$H-$^{15}$N HSQC spectra recorded on NDM-1 (550 μM) in absence of Zn(II) at 20°C in solution in 50mM phosphate buffer pH 7.0.
Figure S4. 600 MHz $^1$H-$^{15}$N HSQC spectra recorded on NDM-1 (996 μM) in presence of 2 molar equivalents of Zn(II) at 27°C in solution in 0.1 M bis tris pH 7.0, 150 mM NaCl.
Figure S5. Zoom of the 600 MHz $^1$H-$^{15}$N HSQC spectra recorded on NDM-1 (996 μM) in presence of 2 molar equivalents of Zn(II) at 27°C in solution in 0.1 M bis tris pH 7.0, 150 mM NaCl.
a) NDM-1 in absence of zinc

|  | A1 | A2 | A3 |
|---|---|---|---|
| X-ray | MGE | MEIPTIQQMTE | MGE |
| DATA SEQUENCE | TGMHRQGLY | TGQRFQDGLV | TGMHRQGLY |
| DATA PREDICTED_SS | XX | LLLLLEEGEE | LLLLLEEGEE |
| DATA CONFIDENCE | 007 | 7998788789 | 9915396816 |
| | 911 | 101 | 131 |

b) NDM-1 in presence of 2 molar equivalents of Zn²⁺

|  | B1 | B2 | B3 |
|---|---|---|---|
| X-ray | MGE | MEIPTIQQMTE | MGE |
| DATA SEQUENCE | TGMHRQGLY | TGQRFQDGLV | TGMHRQGLY |
| DATA PREDICTED_SS | XX | LLLLLEEGEE | LLLLLEEGEE |
| DATA CONFIDENCE | 007 | 7998788789 | 9915396816 |
| | 911 | 101 | 131 |

S7
**Figure S6.** Comparison of the NDM-1 secondary structures predictions derived from its backbone NMR chemical shifts by TALOS+, with those observed in the X-ray structures and the Yao et al results (1) (a) in absence (PDB ID: 3RKK, 3SBL, 3RKJ and 3PG4) and (b) in presence of zinc (PDB ID: 4TZE, 4TZF, 3SPU and 4TYF). The red and yellow boxes highlight the helix and strand predictions, the purple boxes the residues that belong to α-helices in several X-ray structures (PDB ID: 4TZE and 4TZF) and not in others (PDB ID: 3SPU and 4TYF) and the green boxes the residues that belong to β-strands in several X-ray structures (PDB ID: 3SPU) and not in others (PDB ID: 4TZE, 4TYF and 4TZF). DATA PREDICTED_SS and DATA CONFIDENCE are our results from TALOS+. H, E and L are for Helix, Strand and Coil predictions. X indicate the amino acids for which no resonance has been assigned in our NMR spectra and grey boxes those for which the amide proton cannot be assigned, and the blue boxes the resonances that could not be assigned by Yao et al. (1). The loops are highlighted in (a) (L1 to L16 in red) with the residues underlined. L3 corresponds to ASL1, L7 to ASL2, L12 to ASL3, L14 to ASL4 and L16 to ASL5.
Figure S7. (a) NMR relaxation parameters of NDM-1. $^{15}$N R1, R2 relaxation and heteronuclear $^{1}H-^{15}$N NOE parameters obtained for NDM-1 in the absence of Zn(II) (phosphate buffer 50 mM, pH 7.0 and 150 mM NaCl), (b) in the presence of 2 molar equivalents of Zn(II) (0.1 mM bis tris, pH 7.0, 150 mM NaCl), at 600 MHz $^{1}H$ and 25°C. The light grey bars represent either the unassigned residues or the residues for which the R1, R2 and $^{1}H-^{15}$N NOE values could not be obtained due to superimposition of several cross-peaks. * indicate the residues implicated in the binding of Zn(II).
Figure S8. Synthetic route to compounds MG188c (4a), MG187c (4b) and MG195c (6a + 6b)
Figure S9. Synthetic route to compound MG194c2 (4c)

Figure S10. Synthetic route to compound MG219F3 (9)
Figure S11. Experimental NMR titration curves, of amide proton and nitrogen for three residues of di-Zn(II) NDM-1 (L65, D66, Q123), by (a) morin and (b) myricetin. The protein, concentrated to 320 µM, was solubilized in 0.1 mM Bis-Tris buffer pH 7, supplemented by 150 mM NaCl. The flavonols were dissolved in 100% DMSO at a concentration of 20 mM. We titrated 320 µM of NDM-1 with the flavonols (0 to 4 molar equivalents for morin and 0 to 3 molar equivalents for myricetin. Each HSQC spectrum was collected with 8 scans per increment with spectral widths of 9.6 kHz for 1H and 3.2 kHz for 15N as well as 256 data points in the indirect dimension. The experiments used the 3-9-19 watergate sequence (2) for water suppression.
Figure S12. Selected regions of the $^1$H-$^{15}$N HSQC spectra probing the NDM-1:flavonol interactions in absence of Zn(II). Panels a) and b) show that addition of DMSO (black in absence of DMSO and red in presence of the quantity of DMSO corresponding to that was added to have 2 and 4 molar equivalents of flavonol relative to the NDM-1 concentration: 5.72 and 11.44 μl DMSO respectively) do not induce significant chemical shift variations of the NDM-1 resonances. Titrations of NDM-1 (in black in absence of flavonol) by 2 molar equivalent (c) and 4 molar equivalents (d) (in red) of morin showing the influence of the flavonols on several NDM-1 resonances. The spectra were recorded at 20 °C and 800 MHz $^1$H frequency on a sample buffer containing 0.1 mM bis tris (pH 7.0) and 150 mM NaCl with increasing concentrations of DMSO as the ligand is incremented. The concentration of NDM-1 is 357 μM.
Figure S13. NDM-1 ASL1 and ASL4 loop flexibilities. (a) Superimposition of ten art-apo NDM-1 (PDB: 4EXS, 4EY2, 4EYL, 4RL0, 4RL2, 4EYB, 4EYF, 4RBS, 4RAM, 4HL2) in pink. (b) Superimposition of five apo-NDM-1 (PDB: 3S0Z, 3SPU, 4TYF, 4TZE, 4TZF) in blue. (c) Superimposition of the ten art-apo NDM-1 (PDB: 4EY2, 4EYL, 4RL0, 4RL2, 4EYB, 4EYF, 4RBS, 4RAM, 4HL2) in pink and the five apo-NDM-1 (PDB: 3S0Z, 3SPU, 4TYF, 4TZE, 4TZF) in blue used for molecular-docking simulations in the data-driven program HADDOCK, showing the flexibility of the ASL1 and ASL4 loops relative to the global structure of NDM-1. (d) Superimposition of the art-apo NDM-1 structures with the Zn1-Zn2 distances ranging from 4.5 to 4.6 Å (PDB: 4EY2, 4EYB, 4HL2, 4RL2, 4EYF) in the starting structures. (e) Superimposition of the art-apo NDM-1 structures with the Zn1-Zn2 distances ranging from 3.1 to 4.1 Å (PDB: 4EXS, 4EYL, 4RAM, 4RBS, 4RL0) in the starting structures. The superimposition were done on the 45-271 backbone atoms of the PDB:4EY2 structure. The residues implicated in the flavonol recognitions are highlighted by sticks.
Figure S14 Models of the NDM-1/morin complexes generated using the HADDOCK webserver with the four metal-free apo crystal structures of NDM-1 (PDB: 3PG4, 3SBL, 3RKJ, 3RKK). Only the two top clusters are shown: clusters 4 and 2 for 4PG4, clusters 2 and 5 for 3SBL, clusters 3 and 2 for 3RKJ and clusters 2 and 1 for 3RKK. The top clusters are the most reliable according to HADDOCK. The statistics of the all clusters are shown in Table S4. The Fraction of native contacts (FCC) clustering were used to clustered the structures. The number of structures in each cluster over the HADDOCK clustered structures (which represent the number of the water-refined HADDOCK generated structures) is written in brackets. The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive” residues and the flavonol as “active” residue (as define in the HADDOCK software).
4TYF: cluster 3 (54/390 structures)

4TYF: cluster 1 (212/390 structures)

3S0Z: cluster 2 (50/393 structures)

3S0Z: cluster 1 (336/393 structures)

4TZF: cluster 2 (65/398 structures)

4TZF: cluster 7 (9/398 structures)
Figure S15. Models of the NDM-1/morin complexes generated using the HADDOCK webserver with five apo crystal structure of NDM-1 (PDB:4TYF, 3S0Z, 4TZF, 4TZE, 3SPU). The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive” residues and the flavonol as “active” residue (as defined in the HADDOCK software). Only the two top clusters are shown: clusters 3 and 1 for 4TYF, clusters 2 and 1 for 3S0Z, clusters 2 and 7 for 4TZF, clusters 3 and 2 for 4TZE and clusters 2 and 4 for 3SPU. The top clusters are the most reliable according to HADDOCK. The statistics of the all clusters are shown in Table S5. The Fraction of native contacts (FCC) clustering were used to clustered the structures. The number of structures in each cluster over the HADDOCK clustered structures (which represent the number of the water-refined HADDOCK generated structures) is written in brackets.
4EY2: cluster 2 (130/392 structures)
4EYB: cluster 2 (83/389 structures)
4EYF: cluster 2 (96/393 structures)
4EYL: cluster 2 (83/390 structures)
4HL2: cluster 2 (94/387 structures)
4RAM: cluster 2 (142/390 structures)
Figure S16. Models of the NDM-1/morin complexes generated using the HADDOCK webserver with ten crystal structure of NDM-1 in complex with a ligand. (PDB: 4EXS, 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RBS, 4RL2, 4RL0). These structures were used for docking after removing of the ligand localized in the active site of the NDM-1 crystallographic structures. They were named “artificial apo” (art-apo) NDM-1 structures. The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive" residues and the flavonol as “active" residue (as defined in the HADDOCK software). Only the top clusters are shown: clusters 2 for 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RL2, 4RL0 and cluster1 for 4RBS. The top clusters are the most reliable according to HADDOCK. The statistics of the all clusters are shown in Table S6. The Fraction of native contacts (FCC) clustering were used to clustered the structures. The number of structures in each cluster over the HADDOCK clustered structures (which represent the number of the water-refined HADDOCK generated structures) is written in brackets.
Figure S17. Models of the NDM-1/morin complexes generated using the HADDOCK webserver using five apo crystal structure of NDM-1 (PDB: 4TYF, 3S0Z, 4TZF, 4TZE, 3SPU). (a) Only the top clusters are shown: clusters 2 for 4TYF, 4TZF, clusters 1 for 3S0Z, 4TZE and cluster 3 for 3SPU. The top clusters are the most reliable according to HADDOCK. The ranking of the clusters is based on the average score of the top 4 members of each cluster. The «ligand interface RMSD» was used to clustered the structures. The statistics of the all clusters are shown in Table S7. The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive” residues, the two Zn(II) ions and and the flavonol as “active” residues (as define in the HADDOCK software). (b) The morin target the Zn(II) ions through polar contacts and is localized in the active site except for PDB: 3S0Z for which the morin is partially outside the active NDM-1 site. (c) Zoom on several other observed orientations of the morin in the active site of NDM-1.
Figure S18. Models of the NDM-1/morin complexes generated using the HADDOCK webserver with ten crystal structures of NDM-1 in complex with a ligand. (PDB: 4EXS, 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RBS, 4RL2, 4RL0). (a) Superimposition of the ten crystal structures of NDM-1 used for the docking, showing the flexibility of the ASL1 loop (on the left). On the right only the seven most similar structures are represented (PDB: 4EY2, 4EYB, 4EYF, 4HL2, 4RAM, 4RL2, 4RL0). All these structures were used for docking after removing of the ligand localized in the active site of the NDM-1 crystallographic structures. The residues defined as «passive» during the docking simulations are highlighted (L65 in light blue, M67, Q123, N220 in blue, W93 in pink, F70 in green) as well as the Zn(II) ligands (H120, H122, D124, H189, C208 and H250 in yellow). (b) The best generated models during the docking simulations, were selected on the basis of the HADDOCK score and shown: clusters 1 for 4EXS, 4EYB, 4EYL, 4HL2, 4RBS, 4RL2 and clusters 2 for 4EY2, 4EYF, 4RAM, 4RL0 (Table S6). The top clusters are the most reliable according to HADDOCK. The ranking of the clusters is based on the average score of the top 4 members of each cluster. The «ligand interface RMSD» was used to clustered the structures. The statistics on the top cluster is shown in table S7. The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive” residues, the two Zn(II) ions as “active” residues and the flavonol as “active” residue (as define in the HADDOCK software). (c) Superimposition of 21 structures over the of the 40 structures clustered in the top clusters generated using the ten art-apo NDM-1 crystallographic structures showing the majority orientation of the morin in the active site of NDM-1 (orientation-1). These 21 structures adopt two slight different orientations in the active site, in 11 dark pink and 10 in light pink. (d) A minority orientation (orientation-2) is also observed in the docking generated strutures (on the left: PDB: 4RAM (cluster 2: structure 2 and 3) and 4RBS (cluster 1: structure 4); and on the right: PDB: 4RBS (cluster 1: structures 1 and 3).
Figure S19. Models of the NDM-1/morin complexes generated using the HADDOCK webserver with ten crystal structures of NDM-1 in complex with a ligand: (PDB: 4EXS, 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RBS, 4RL2, 4RL0). All these structures were used for docking after removing of the ligand localized in the active site of the NDM-1 crystallographic structures. The best generated models during the docking simulations, were selected on the basis of the HADDOCK score and shown: clusters 1 for 4EXS, 4EYB, 4EYL, 4HL2, 4RBS, 4RL2 and clusters 2 for 4EY2, 4EYF, 4RAM, 4RL0 (Table S7). The top clusters are the most reliable according to HADDOCK. The ranking of the clusters is based on the average score of the top 4 members of each cluster.

This figure highlights the deviations of Zn1 and Zn2, in the active site, induced by docking with morin, compared to their positions in the starting crystal structures, after superimposition on the (45-271) backbone atoms of the ten art-apo NDM-1 crystal structures and those generated during the docking simulations. Zn1 and Zn2 are in dark green and orange respectively in the crystal structures and in light green and yellow in NDM-1/morin complexes generated using the HADDOCK. The Zn(II) ligands are highlighted by green sticks in the crystal structures and in yellow in the docking generated structures.
a) PDB: 4EYB + Morin

b) PDB: 4EYB + Quercetin

c) PDB: 4EYB + Myricetin
Figure S20. Comparison of models of the NDM-1/morin, NDM-1/quercetin and NDM-1/myricetin complexes generated using the HADDOCK. The best generated models during the docking simulations, using the PDB: 4EYB crystallographic structure, were selected on the basis of the HADDOCK scores and shown, in presence of morin (a), quercetin (b) and myricetin (c). While only one orientation (orientation-1) is observed for morin in complex with PDB: 4EYB (a) orientation-1 (b1 and c1) and orientation-2 (b2 and c2), which results from a 180° rotation of the ligand in the active site of NDM-1 from orientation-1, is also observed in presence of quercetin and myricetin. Superimposition on the NDM-1 backbone atoms showing the relative orientation of the three flavonols (morin in pink, quercetin in blue and myricetin in cyan) in orientation-1 (d1) and orientation-2 (d2).
Figure S21. Comparison of models of the NDM-1/quercetin and NDM-1/quercetin conjugates generated using HADDOCK. (a) Structures of the quercetin conjugates. The best generated models during the docking simulations, using the PDB: 4EY2 crystallographique structure, were selected on the basis of the HADDOCK scores and shown, in presence of quercetin (b), quercetin with OMe in position 5 (quercetin-CH3A) (c), quercetin with OMe in position 3’ (quercetin-CH3B) (d) and quercetin with OMe in position 3 (quercetin-CH3C) (e).
Figure S22. NDM-1 ligand interactions. (a) Interaction of the hydrolyzed benzylpenicillin in the NDM-1 active site (PDB: 4EYF). The polar interactions of the ligand with NDM-1 are highlighted by black dash lines. (b) Superimposition of one NDM-1/morin structure obtained after docking with the art-apo NDM-1 PDB:4EYB (morin in dark blue) and the crystallographic structures of NDM-1 in complex with ligand in pink (PDB: 4EY2 (hydrolysed methicillin), 4EYL (hydrolysed meropenem), 4RL0 (hydrolysed cefuroxime), 4RL2 (hydrolysed cefalexin), 4EYB (hydrolysed oxacillin), 4EYF (hydrolysed benzylpenicillin), 4RBS (hydrolysed meropenem), 4HL2 (hydrolysed ampicillin). The residues implicated in the flavon recognition are highlighted by sticks. Zn1 is in orange and Zn2 in green.
Supplemental materials and Methods

Chemistry synthesis of the compounds: MG188c, MG187c, MG195c, MG194c2 and MG219F3

Chemistry synthesis.

All chemical reagents were of analytical grade, obtained from Acros, Alfa Aesar, or Aldrich, and used without further purification. Solvents were obtained from SDS or VWR-Prolabo. Chromatography was performed using silica gel (35-70 μm, Merck). Analytical TLC was performed using Silica Gel 60 F254 pre-coated aluminum plates (Merck). NMR spectra were collected on Bruker DRX 250 (1H at 250 MHz and 13C at 62.5 MHz), DRX 300 (1H at 300 MHz and 13C at 75 MHz) or DRX 360 (1H at 360 MHz and 13C at 90 MHz) spectrometers and analyzed using MestReNova software. Chemical shifts are reported in ppm (δ) and coupling constants in Hz (J). Multiplicity is given as follow: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singulet. 1H NMR spectra were performed in CDCl3, (CD3)2CO, CD3OD or D2O. High-resolution mass spectrometry (HRMS) analyses were performed by electrospray with positive or negative ionization mode (ESI+ or ESI-).

The synthetic routes are presented on Figures S8, S9 and S10

Procedure for benzylation

A-Benzylation of quercetin (Fig. S8)

To a solution of quercetin 1 (CAS 6151-25-3) in anhydrous DMF (c=1.9mol/L) under argon and at room temperature were added 4 eq of potassium carbonate (K2CO3). The solution was then cooled at 0°C for the dropwise addition of 4 eq. of benzylbromide (BnBr). The resulting solution was then stirred at 0°C and then allowed to return to room temperature for 15h. AcOEt was added, the resulting solution was washed twice with HCl (1M), with distilled water (untill neutral pH) and twice with brine. The organic layer was dried over MgSO4 and filtered. The solvent was removed under reduced pressure to give a brown oil. Flash chromatography (silica, elution cyclohexane/acetonitrile, 3/1) yielded to three pure fractions: Tetrabenzylated quercetin (F1, 2a 14%), Tribenzylated quercetin (F2, 2b 46%) and Dibenzyalted quercetin (F3, 12%).

2a: 1H NMR ((CD3)2CO): 12.78 (s, 1H, OH 5), 7.88 (d, J=2.1, 1H, H2'), 7.72 (d*d, J=2.1, J=8.6, 1H, H6'), 7.57-7.21 (m, 21H, Bn and H5'), 6.74-6.45 (m, 1H, H8), 6.45 (m, 1H, H6), 5.29-5.27 (m, 4H, CH2Bn), 5.18 (s, 2H, CH2Bn), 5.04 (s, 2H, CH2Bn). HRMS m/z Found: 663.2354 [M+H]+. Calcd for C43H33O7: 663.2377.

2b: 1H NMR ((CD3)2CO): 12.68 (s, 1H, OH 5), 7.68-6.72 (m, 1H, H6'), 7.45-7.30 (m, 16H, Bn and H2'), 6.98 (d, J=9.2, 1H, H5'), 6.54-6.52 (m, 1H, H8), 6.47-6.45 (m, 1H, H6), 5.71 (s, 1H, OH), 5.18 (s, 2H, CH2Bn), 5.11 (s, 2H, CH2Bn), 5.04 (s, 2H, CH2Bn). HRMS m/z Found: 573.1894 [M+H]+. Calcd for C36H29O7: 573.1908.

B-Benzylation of Rutin [1] (Fig. S9)

To a solution of dried rutin 7 (CAS 153-18-4) in anhydrous DMF (c=1.9mol/L) under argon and at room temperature were added 4.5 eq of cesium carbonate (Cs2CO3). 6 eq. of benzylbromide (BnBr) were added dropwise to the solution at room temperature. The resulting solution was kept under stirring 16h at room temperature then heated to 60°C for 4 h. The solution was then poured in an ice-cold solution of acetic acid (0.7mol/L). The precipitate was diluted in AcOEt. The resulting solution was washed twice with distilled water and twice with brine. The organic layer was dried over MgSO4. The solvent was removed under reduced pressure to give orange solid. This solid was dissolved in ethanolic solution of hydrochloric acid (1.8mol/L) and heated to 75°C for 6h. The mixture was poured into ice-cold distilled water. The precipitate was filtered off and washed with distilled water to yield a bright yellow solid. This solid was then diluted in DCM, the resulting organic layer was dried over MgSO4 and filtered. The solvent was evaporated under reduced pressure. The title compound 2c was isolated as a yellow solid after flash chromatography (silica, elution cyclohexane/AcOEt, 85/15 to 80/20) with 32% yield.

2c: 1H NMR (CDCl3): 7.88 (d, J=1.9, 1H, H2'), 7.78 (d*d, J=1.9, J=8.5, 1H, H6'), 7.52-7.31 (m, 20H, Bn), 7.03 (d, J=8.6, 1H, H5'), 6.59 (d, J=2.0, 1H, H8) 6.48 (d, J=1.9, 1H, H6), 5.26-5.24 (m, 6H, CH2Bn), 5.12-5.15 (m, 3H, CH2Bn et OH3). HRMS m/z Found: 663.2369 [M+H]+. Calcd for C43H33O7: 663.2377.

Procedure for methylation [2] (Fig. S8 and S9)

Benzylated quercetin 2a, 2b or 2c (c=0.08mol/L) and 2eq cesium carbonate (Cs2CO3) were dissolved in anhydrous DMF under argon. Methyl iodide (2eq) was added, and the resulting mixture was stirred at
room temperature and under argon for 16h (2a, 2b) or 24h (2c). The mixture was then diluted in DCM and washed with distilled water, diluted HCl (1M), distilled water again and brine. The organic layer was dried over MgSO4 and filtered. The solvent was evaporated under reduced pressure.

3a was isolated after Flash chromatography (silica, elution cyclohexane/acetone 7/3) with 99% yield.

1H NMR (CDCl3): 7.75 (d, J=1.9, 1H, H2'), 7.56 (d', d, J=1.9, J=8.6, 1H, H6'), 7.47-7.19 (m, 20H, Bn), 6.95 (d, J=8.6, 1H, H5'), 6.53 (d, J=2.1, 1H, H8), 6.44 (d, J=2.0, 1H, H6), 5.24 (s, 2H, CH2Bn), 5.14 (s, 2H, CH2Bn), 5.07 (s, 2H, CH2Bn), 4.97 (s, 2H, CH2Bn), 3.97 (s, 3H, OCH3). HRMS m/z Found: 677.2525 [M+H]+. Calcd for C31H36NaO10: 676.2461.

3b was isolated after Flash chromatography (silica, elution cyclohexane/acetone 9/1 to 8/2) with 50% yield.

1H NMR (CDCl3): 7.63 (d, J=2.1, 1H, H2'), 7.54 (d', d, J=2.1, J=8.6, 1H, H6'), 7.48-7.23 (m, 15H, Bn), 6.93 (d, J=8.6, 1H, H5'), 6.51 (d, J=2.2, 1H, H8), 6.44 (d, J=2.2, 1H, H6), 5.25 (s, 2H, CH2Bn), 5.13 (s, 2H, CH2Bn), 5.06 (s, 2H, CH2Bn), 3.72 (s, 1H, OCH3). HRMS m/z Found: 609.1874 [M+Na]+. Calcd for C29H30NaO9: 609.1864.

3c was isolated after Flash chromatography (silica, elution cyclohexane/acetone 7/3) with 98% yield.

1H NMR (CDCl3): 7.77 (d, J=2.0, 1H, H2'), 7.66-7.62 (m, 3H, Bn and H6'), 7.50-7.20 (m, 18H, Bn), 7.02 (d, J=8.6, 1H, H5'), 6.53 (d, J=2.1, 1H, H8), 6.46 (d, J=2.1, 1H, H6), 5.27-5.25 (m, 4H, CH2Bn), 5.23 (s, 2H, CH2Bn), 5.10 (s, 2H, CH2Bn), 3.71 (s, 3H, OCH3). HRMS m/z Found: 677.2524 [M+H]+. Calcd for C30H33NaO10: 677.2524.

**Procedure for (3'-O)-alkylation (Fig. S8)**

Benzylated quercetin 2b (c=0.06mol/L in acetone), 12 eq of potassium carbonate, and 0.4 eq of terabutylammonium iodide were dissolved in acetone. 4.5 eq of chloromethyl-isopropyl carbonate were added to the mixture then DMF (1/1 to acetone) to solubilize the starting materials. The solution was kept under stirring and at 50°C for 24h. The reaction was quenched by the addition of distilled water and equal volume of DCM. The organic layer was washed with distilled water and brine, the solvent evaporated under reduced pressure and the residue was purified by flash chromatography (silica, elution cyclohexane/acetone 9/1). Compounds 5a and 5b (conversion 65%) were co-éluted in these conditions and were not further separated. The proportions of each compound were evaluated by NMR to 60% of 5a and 40% of 5b.

5a+5b: 1H NMR (CDCl3): 12.70 (5b, OH5), 12.69 (5a OH5), 7.92 (d', d, J=2.1, J=8.7, 5a, H6'), 7.88 (d, J=2.2, 5a, H2'), 7.82 (d, J=2.1, 5b, H2'), 7.73 (d', d, J=2.2, J=8.7, 5b, H6'), 7.49-7.21 (m, 5a+5b, Bn), 7.05 (d, J=8.7, 5a, H5'), 6.98 (d, J=8.7, 5b, H5'), 6.53-6.51 (m, 5a+5b, H8), 6.48-6.46 (m, 5a+5b, H6), 5.72 (s, 5b, OCH3), 5.23-5.11 (m, 5a+5b, CH2Bn), 5.04-4.94 (m, 5b, CH-i-Pr), 4.95-4.86 (m, 5b, CH-i-Pr), 1.34 (d, J=6.2, 5a, CH3-i-Pr), 1.28 (d, J=6.2, 5b, CH3-i-Pr). HRMS m/z Found: 681.2078 [M+Na]+. Calcd for C31H36NaO10: 681.2095 5a. HRMS m/z Found: 711.2188 [M+H]+. 5b. Calcd for C31H36NaO10: 711.2201 5b.

**Procedure for hydrogenolysis (debenzylation) (Fig. S8 and S9)**

Compounds 3a, 3b, 3c, (5a + 5b) were all debenzylated by catalytic hydrogenation. Benzylated compound was added to a degassed solution of EtOH/THF (1v/1v) (3a or 3b) or DCM/MeOH (1v/1v) (3c and (5a + 5b)) containing 5% of Pd/C. The reaction mixture was stirred for 16h under hydrogen atmosphere. The solution was filtered over celite, washed with THF or MeOH and evaporated.

The crude compound 4a was then washed with Et2O and filtered. Pure 4a was isolated as a yellow solid with 50% yield.

1H NMR (CD3OD): 7.68 (d, J=1.8, 1H, H2'), 7.58 (d', d, J=1.8, J=8.4, 1H, H6'), 6.84 (d, J=8.5, 1H, H5'), 6.46 (d, J=1.6, 1H, H8), 6.34 (d, J=1.5, 1H, H6), 3.87 (s, 3H, OCH3). HRMS m/z Found: 339.0472 [M+Na]+. Calcd for C16H22NaO4: 339.0475.

The crude compound 4b was then washed with Et2O and filtered. Pure 4b was isolated as a dark yellow solid with quantitative yield.

1H NMR (CD3OD): 7.87 (s, 1H, H2'), 7.74 (d, J=7.4, 1H, H6'), 6.93 (d, J=7.3, 1H, H5'), 6.41 (s, 1H, H8), 6.19 (s, 1H, H6), 3.77 (s, 3H, OCH3). HRMS m/z Found: 315.0513 [M-H]-. Calcd for C16H11O7: 315.0510.

The crude compound 4c was dissolved in chloroform then precipitated with pentane. The precipitate was filtered off and pure 4c was isolated as a brown/green solid with quantitative yield.

1H NMR (CD3OD): 7.61 (s, 1H, H2'), 7.52 (d, J=8.4, 1H, H6'), 6.88 (d, J=8.5, 1H, H5'), 6.32 (s, 1H, H8), 6.14 (s, 1H, H6), 3.94 (s, 3H, OCH3). HRMS m/z Found: 315.0514 [M-H]-. Calcd for C16H11O7: 315.0510.

The mixture of compounds 6a + 6b were isolated without further treatment as a dark green solid.
\(^1\)H NMR (CD\(_3\)OD /D\(_2\)O): 7.99 (s, 5b, H2'), 7.92-7.88 (m, 5b, H6'), 7.75 (s, 5a, H2'), 7.65 (d, J=8.0, 5a, H6'), 7.06-7.00 (m, 5b, H5'), 6.93 (d, J=8.3, 5a, H5'), 6.43-6.40 (m, 5a + 5b, H8), 6.19-6.21 (m, 5a + 5b, H6), 5.81 (s, 5b, OCH\(_2\)O), 1.38 (d, J=6.1, 5b, CH\(_3\)i-Pr); 1.28 (d, J=6.1, 5a, CH\(_3\)i-Pr). The CH i-Pr are masked by water residual pic.

6a HRMS \(m/z\) Found: 411.0880 [M+Na]+ Calcd for C\(_{19}\)H\(_{16}\)NaO\(_9\): 411.0614. and Found : 441.0772 [M+Na]+ Calcd for C\(_{20}\)H\(_{18}\)NaO\(_{10}\): 411.0792.

**Synthesis of the oxime of Naringenin** (Fig. S10)
Commercially available naringenin \(^8\) (CAS 67604-48-2) was dissolved in EtOH (c=0.04mol/L), 30 eq of aqueous hydroxylamine and 20 eq of pyridine were added. The resulting mixture was stirred at 65°C for 6h. The reaction mixture was evaporated to dryness and the residue was purified by flash chromatography (silica, elution DCM to DCM/MeOH (2%)) to afford 9 in 52 % yield.

9. \(^1\)H NMR ((CD\(_3\))\(_2\)CO): 11.04 (s, 1H, OH 5), 10.35 (bs, 1H, OH), 8.69 (bs, 1H, OH), 7.41, 7.38, 6.93, 6.89 (Ar Syst AA'BB', 4H, H2' and H3'), 6.01 (d, J=2.3, 1H, H8), 5.98 (d, J=2.3, 1H, H6), 5.80 (d* d, J\(_1\)=3.1, J\(_2\)=12.0, 1H, H2), 3.47 (d* d, J\(_1\)=3.1, J\(_2\)=17.1, 1H, H3a), 3.13 (bs, 1H, OH), 2.79 (d* d, J\(_2\)=12.0, J\(_3\)=17.1, 1H, H3b). HRMS m/z Found: 286.0720 [M-H]+. Calcd for C\(_{15}\)H\(_{12}\)O\(_5\): 286.0721.

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[2] Mattarei A, Biasutto I, Marotta E, De Marchi U, Sassi N, Garbisa S, et al. A mitochondriotropic derivative of quercetin: a strategy to increase the effectiveness of polyphenols. Chembiochem. 2008;9:2633-42.
Supporting Information

Tables

NMR characterization of the influence of zinc(II) ions on the structural and dynamic behavior of the New-Delhi Metallo-β-lactamase-1 and on the binding with flavonols as inhibitors

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Table S1 Effect of Zn(II) on NDM-1 thermal stability. The thermal denaturation temperature of NDM-1 was determined by thermal Shift assay in presence and in absence of EDTA and ZnCl₂

| ZnCl₂ (µM) | EDTA (mM) | Td1/2 NDM-1 (°C) |
|------------|-----------|------------------|
| 0          | 0         | 40.5±0.3         |
| 3.12       | 2.5       | 41.5±0.4         |
| 6.25       | 7.5       | 46.6±0.3         |
| 12.5       | 12.5      | 49.8±0.2         |
| 25         | 17.5      | 52.8±0.2         |
| 50         | 20        | 56.2±0.4         |
| 25         | 2.5       | 41.1±0.3         |

Table S2 Average of the relaxation parameters of the flexible and rigid domains of NDM-1

| Domain       | -Zn(II) | +Zn(II) |
|--------------|---------|---------|
| RIGID*       | 0.73 ± 0.08 | 0.64 ± 0.05 |
| ASL1 (64-74) | 0.73 ± 0.06 | 0.67 ± 0.04 |
| ASL2 (118-123) | Not shown | 0.61 ± 0.04 |
| L9 (154-158) | Not shown | 0.62 ± 0.04 |
| ASL3 (184-195) | 0.74 ± 0.08 | 0.65 ± 0.05 |
| ASL4 (209-225) | 0.85 ± 0.10 | 0.68 ± 0.06 |
| α3 helix (226-239) | 0.75 ± 0.09 | 0.62 ± 0.05 |
| RIGID*       | 24.22 ± 1.69 | 31.19 ± 1.27 |
| ASL1 (64-74) | 19.73 ± 0.91 | 25.56 ± 0.67 |
| ASL2 (118-123) | Not shown | 30.54 ± 1.18 |
| L9 (154-158) | Not shown | 32.40 ± 1.13 |
| ASL3 (184-195) | 24.14 ± 2.26 | 30.45 ± 1.70 |
| ASL4 (209-225) | 22.64 ± 1.71 | 31.01 ± 2.15 |
| α3 helix (226-239) | 24.95 ± 1.65 | 32.28 ± 1.59 |
| hetNOE       | 0.87 ± 0.15 | 0.85 ± 0.12 |
| ASL1 (64-74) | 0.65 ± 0.10 | 0.70 ± 0.09 |
| ASL2 (118-123) | Not shown | 0.86 ± 0.13 |
| L9 (154-158) | Not shown | 0.84 ± 0.10 |
| ASL3 (184-195) | 0.83 ± 0.30 | 0.87 ± 0.17 |
| ASL4 (209-225) | 0.74 ± 0.19 | 0.82 ± 0.16 |
| α3 helix (226-239) | 0.85 ± 0.15 | 0.87 ± 0.16 |

RIGID*: average calculation on the folded domain. The values from the 28-44 (N-terminal domain), 64-74 (ASL1), 118-123 (ASL2), 154-158 (L9), 184-195 (ASL3), 209-225 (ASL4) and 226-239 (α3 helix) were removed from the calculation.
**Table S3** LogP estimation of morin, quercetin and myricetin*

|       | Morin | Quercetin | Myricetin |
|-------|-------|-----------|-----------|
| Log P | 0.35  | 0.35      | -0.04     |

*ChemBioDraw was used to predict values for LogP*

**Table S4** Estimated Kd values of morin and myricetin in complex with NDM-1

| residues | Kd morin (µM) (5eq final) | Kd Myricetin (µM) (3eq final) |
|----------|---------------------------|--------------------------------|
| L65      | 359.2±35.3                | 375.4±66.6                     |
| D66      | 229.5±23.82               | 139.6±48.5                    |
| W93      | 1495±472                  | Not determined                 |
| Q123     | 1586.0±280.8              | 524.1±54.5                    |
| D124     | 988.7±513.1               | Not determined                 |
| L218     | 330.8±68.1                | 1745.0±261.4                  |
| G222     | 433.3±83.7                | 268.2±17.1                    |
| H250     | 2136±436.5                | Not determined                 |
Table S5 Zn1-Zn2 distances in structures of NDM-1 in complex with different products.

| PDB number | Product in complex                     | Zn1-Zn2* distance Å | Zn1-Zn2+ distance Å after docking with morin |
|------------|---------------------------------------|---------------------|-----------------------------------------------|
| 4EY2       | hydrolyzed methicillin                | 4.6                 | Cluster1 (4.0, 4.4, 4.3, 4.5) Cluster2 (4.6, 4.0, 4.6, 4.6) |
| 4EYB       | hydrolyzed oxacillin                  | 4.5                 | Cluster1 (4.3, 4.0, 4.3, 4.4) Cluster2 (4.9, 5.4, 6.7, 4.3) |
| 4EXS       | L-captopril                           | 3.6                 | Cluster1 (4.9, 5.3, 3.6, 5.0) Cluster2 (5.0, 2.6, 2.6, 2.6) |
| 4EYL       | hydrolyzed meropenem                  | 4.0                 | Cluster1 (2.6, 2.6, 3.9, 2.6) Cluster2 (4.3, 2.6, 2.6, 5.1) |
| 4RBS       | hydrolyzed meropenem                  | 4.0                 | Cluster1 (4.2, 4.1, 4.2, 4.9) Cluster2 (4.2, 4.0, 4.4, 4.5) |
| 4EYF       | hydrolyzed benzylpenicillin           | 4.6                 | Cluster1 (4.2, 4.4, 5.0, 4.4) Cluster2 (4.5, 4.9, 4.4, 4.4) |
| 4RL0       | hydrolyzed cephalosporins             | 3.8                 | Cluster1 (4.2, 4.3, 4.3, 4.4) Cluster2 (4.2, 4.4, 4.6, 3.9) |
| 4RL2       | hydrolyzed cephalosporins             | 4.5                 | Cluster1 (4.4, 4.4, 2.6, 5.0) Cluster2 (4.4, 2.6, 3.7, 4.9) |
| 4RAM       | hydrolyzed penicillin G               | 4.1                 | Cluster1 (4.2, 4.0, 4.4, 4.5) Cluster2 (5.0, 3.9, 4.6, 5.1) |
| 4HL2       | hydrolyzed ampicillin                 | 4.6                 | Cluster1 (4.5, 4.5, 4.5, 4.4) Cluster2 (4.5, 7.1 4.1, 6.8) |

* Zn1-Zn2 distances measured in ten NDM-1 crystal structures in complex with products.
† Zn1-Zn2 distances measured in the NDM-1/morin complexes generated using HADDOCK
Table S6 NDM-1/MORIN docking using the molecular-docking simulations in the data-driven program HADDOCK.

The art-apo NDM-1 structures (from PDB: 4EXS, 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RBS, 4RL2, 4RL0) used for docking.
The two Zn(II) were not defined as either passive or active residues. Only the results for the top clusters are shown.

|       | 4EXS | 4EY2 | 4EYB | 4EYF | 4EYL | 4HL2 | 4RAM | 4RBS | 4RL2 | 4RL0 |
|-------|------|------|------|------|------|------|------|------|------|------|
| nb structures | 398  | 392  | 389  | 393  | 390  | 387  | 390  | 395  | 398  | 394  |
| nb clusters   | 3    | 7    | 6    | 6    | 5    | 6    | 6    | 7    | 7    | 6    |
| HADDOCK score|      |      |      |      |      |      |      |      |      |      |
| Cluster 1  | -58 +/- 9 | -75 +/- 4 | -74 +/- 3 | -74 +/- 1 | -75 +/- 3 | -71 +/- 2 | -75 +/- 3 | -68 +/- 2 | -64 +/- 4 |
| Cluster 2  | -75 +/- 4 | -74 +/- 3 | -74 +/- 1 | -75 +/- 3 | -71 +/- 2 | -75 +/- 3 | -68 +/- 2 | -64 +/- 4 | -64 +/- 4 |
| Cluster 2  | -71 +/- 2 | -75 +/- 3 | -71 +/- 2 | -75 +/- 3 | -68 +/- 2 | -64 +/- 4 | -64 +/- 4 | -64 +/- 4 |
| Cluster 2  | -75 +/- 3 | -71 +/- 2 | -75 +/- 3 | -68 +/- 2 | -64 +/- 4 | -64 +/- 4 | -64 +/- 4 |
| Cluster 2  | -68 +/- 2 | -64 +/- 4 |
| Cluster 2  | -64 +/- 4 |
| RMSD from the overall lowest-energy structure | 0.3 +/- 0.2 | 0.2 +/- 0.1 | 0.3 +/- 0.2 | 0.4 +/- 0.2 | 0.3 +/- 0.2 | 0.2 +/- 0.1 | 0.2 +/- 0.1 | 0.3 +/- 0.2 | 0.3 +/- 0.2 | 0.3 +/- 0.0 |
| Van der Waals energy | -14 +/- 4 | -23 +/- 3 | -17 +/- 5 | -20 +/- 4.0 | -18 +/- 2 | -19 +/- 5 | -19 +/- 4 | -17 +/- 5 | -20 +/- 5 | -21 +/- 4 |
| Electrostatic energy | -9 +/- 4 | -47 +/- 18 | -63 +/- 18 | -25 +/- 28 | -30 +/- 34 | -80 +/- 39 | -61 +/- 45 | -64 +/- 19 | -36 +/- 37 | -44 +/- 49 |
| Desolvation energy | -10 +/- 4 | -6 +/- 3 | -9 +/- 4 | -11 +/- 2 | -11 +/- 5.0 | -8 +/- 2 | -5 +/- 4 | -11 +/- 2 | -6 +/- 4 | -3 +/- 3 |
| Restraints violation energy | 0.0 +/- 0.0 | 0.0 +/- 0.0 | 0.1 +/- 0.1 | 0.0 +/- 0.1 | 0.2 +/- 0.2 | 0.1 +/- 0.1 | 0.1 +/- 0.2 | 0.0 +/- 0.1 | 0.0 +/- 0.1 | 0.5 +/- 0.1 |
| Buried Surface Area | 348 +/- 74 | 518 +/- 23 | 487 +/- 9 | 513 +/- 17 | 461 +/- 48 | 477 +/- 12 | 479 +/- 17 | 536 +/- 16 | 481 +/- 22 | 526 +/- 35 |
| Z-Score | -1.4 | -1.6 | -1.9 | -1.3 | -1.7 | -1.6 | -1.7 | -1.3 | -1.5 | -1.7 |
Apo form of NDM-1 (PDB: 4TYF, 3S0Z, 4TZF, 4TZE, 3SPU) used for docking.

|                   | 4TYF | 3S0Z | 4TZF | 4TZE | 3SPU |
|-------------------|------|------|------|------|------|
| nb structures     | 390  | 393  | 398  | 392  | 390  |
| nb clusters       | 9    | 3    | 6    | 5    | 8    |

| Cluster          | 3     | 2     | 2     | 3     | 2     |
|------------------|-------|-------|-------|-------|-------|
| HADDOCK score    | -77 +/- 6 | -74 +/- 7 | -67 +/- 7 | -64 +/- 3 | -76 +/- 5 |
| Cluster size     | 54    | 50    | 65    | 49    | 87    |

| Van der Waals energy | -22 +/- 3 | -17 +/- 3 | -18 +/- 1 | -22 +/- 5 | -14 +/- 4 |
|----------------------|-----------|-----------|-----------|-----------|-----------|
| Electrostatic energy | -76 +/- 4 | -27 +/- 9 | -26 +/- 9 | -74 +/- 32 | -74 +/- 40 |
| Desolvation energy   | -4 +/- 2  | -21 +/- 3 | -5 +/- 3  | -2 +/- 4  | -7 +/- 5  |
| Restraints violation energy | 0.0 +/- 0.0 | 0.0 +/- 0.0 | 0.0 +/- 0.0 | 0.0 +/- 0.0 | 0.0 +/- 0.0 |
| Buried Surface Area  | 527 +/- 3 | 469 +/- 67 | 440 +/- 16 | 522 +/- 27 | 465 +/- 18 |
| Z-Score             | -1.6     | -1.1     | -1.2     | -1.4     | -2.1     |

The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive” residues and morin as “active” residue.

The two Zn(II) were not defined as either passive or active residues.

The fraction of native conctats (FCC) clustering were used to clustered the structures.

**nb structures**: is the water-refined models HADDOCK generated

**nb clusters**: is the number of clusters in which all the water-refined models HADDOCK generated are clustered.

**HADDOCK score**: is calculated as function of the intermolecular van der Waals energy, the intermolecular electrostatic energy, the empirical desolvation energy term and the Ambiguous Interaction Restraints (AIRs) energy.

**Z-Score**: indicates how many standard deviations from the average this cluster is located in terms of score.
Metal-free apo form of NDM-1 (PDB: 3PG4, 3RKJ, 3RKK, 3SBL) used for docking.

The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as "passive" residues and morin as "active" residue.

There is no zinc ions in these RX structures

The fraction of native contacts (FCC) clustering were used to clustered the structures.

|       | 3PG4 | 3RKJ | 3RKK | 3SBL |
|-------|------|------|------|------|
| nb structures | 385  | 392  | 389  | 389  |
| nb clusters | 8    | 6    | 6    | 7    |
| HADDOCK score | Cluster 4 | Cluster 3 | Cluster 2 | Cluster 2 |
|              | -78 +/- 4    | -58 +/- 4   | -59 +/- 2  | -70 +/- 2 |
| Cluster size | 27     | 36     | 91     | 118    |
| RMSD from the overall lowest-energy structure | 0.4 +/- 0.2   | 0.4 +/- 0.0  | 0.4 +/- 0.0 | 0.3 +/- 0.0 |
| Van der Waals energy | -18 +/- 3    | -13 +/- 4   | -21 +/- 2  | -21 +/- 4 |
| Electrostatic energy | -35 +/- 20    | -9 +/- 8    | -14 +/- 4  | -15 +/- 5 |
| Desolvation energy | -7 +/- 4     | -3 +/- 2    | -2 +/- 3   | -7 +/- 3  |
| Restraints violation energy | 0.0 +/- 0.0  | 0.0 +/- 0.0 | 0.0 +/- 0.0 | 0.0 +/- 0.0 |
| Buried Surface Area | 468 +/- 22    | 404 +/- 28  | 491 +/- 41 | 545 +/- 34 |
| Z-Score | -1.7   | -1.0   | -1.2   | -1.4   |

**nb structures:** is the water-refined models HADDOCK generated

**nb clusters:** is the number of clusters in which all the water-refined models HADDOCK generated are clustered.

**HADDOCK score:** is calculated as function of the intermolecular van der Waals energy, the intermolecular electrostatic energy, the empirical desolvation energy term and the Ambiguous Interaction Restraints (AIRs) energy.

**Z-Score:** indicates how many standard deviations from the average this cluster is located in terms of score.
Table S7 NDM-1/MORIN docking using the molecular-docking simulations in the data-driven program HADDOCK. The art-apo NDM-1 structures (PDB: 4EXS, 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RBS, 4RL2, 4RL0) used for docking. The two Zn(II) were defined as active residues. Only the results for the top clusters are shown.

|        | 4EXS | 4EY2 | 4EYB | 4EYF | 4EYL | 4HL2 | 4RAM | 4RBS | 4RL2 | 4RL0 |
|--------|------|------|------|------|------|------|------|------|------|------|
| nb struct  | 194  | 195  | 198  | 200  | 186  | 198  | 191  | 197  | 193  | 193  |
| nb clusters| 4    | 5    | 6    | 4    | 4    | 6    | 5    | 4    | 5    | 4    |

|        | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 |
|--------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| HADDOCK | -83 +/- 3 | -81 +/- 4 | -79 +/- 1 | -79 +/- 2 | -82 +/- 7 | -81 +/- 3 | -78 +/- 6 | -78 +/- 2 | -78 +/- 5 | -70 +/- 2 |        |
| K score |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Cluster | 85        | 74        | 107       | 71        | 70        | 71        | 65        | 155       | 65        | 74        |           |           |           |           |           |
| size    |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| RMSD    | 0.4 +/- 0.0 | 0.3 +/- 0.2 | 0.5 +/- 0.0 | 0.4 +/- 0.0 | 0.2 +/- 0.1 | 0.3 +/- 0.2 | 0.2 +/- 0.2 | 0.4 +/- 0.0 | 0.3 +/- 0.2 | 0.3 +/- 0.1 |           |           |           |           |           |
| from the overall lowest-energy structure |           |           |           |           |           |           |           |           |           |           |           |           |           |           |           |
| Van der Waals energy | -19 +/- 5 | -18 +/- 6.0 | -15 +/- 3 | -17 +/- 3 | -17.4 +/- 0.2 | -16.4 +/- 0.4 | -18 +/- 6 | -20 +/- 7 | -15 +/- 3 | -15 +/- 4 |           |           |           |           |           |
| Electrostatic energy | -64 +/- 26 | -61 +/- 20 | -70 +/- 4 | -55 +/- 24 | -107 +/- 21 | -77 +/- 5 | -48 +/- 28 | -68.8 +/- 9.0 | -84.8 +/- 21.4 | -73.8 +/- 0.8 |           |           |           |           |           |
| Desolvation energy | -8.0 +/- 3.1 | -11.1 +/- 1.4 | -11.5 +/- 1.6 | -14.1 +/- 0.9 | -11.0 +/- 1.9 | -10.7 +/- 2.1 | -8.0 +/- 2.3 | -9 +/- 2 | -4 +/- 1 | -3 +/- 3 |           |           |           |           |           |
| Restraints violation energy | 0.2 +/- 0.2 | 0.1 +/- 0.1 | 0.1 +/- 0.1 | 0.0 +/- 0.1 | 0.1 +/- 0.2 | 0.0 +/- 0.1 | 0.4 +/- 0.5 | 0.0 +/- 0.1 | 0.1 +/- 0.1 | 0.5 +/- 0.4 |           |           |           |           |           |
| Buried Surface Area | 486 +/- 12 | 501 +/- 12 | 494 +/- 14 | 493 +/- 16 | 495 +/- 33 | 509 +/- 18 | 491 +/- 25 | 527 +/- 30 | 481 +/- 5 | 491 +/- 24 |           |           |           |           |           |
| Z-Score | -0.9     | -1.3     | -1.7     | -0.7     | -1.0     | -1.3     | -1.1     | -1.5     | -1.6     | -1.2     |           |           |           |           |           |
Apo form of NDM-1 (PDB: 4TYF, 3S0Z, 4TZF, 4TZE, 3SPU) used for docking.

|                | 4TYF | 3S0Z | 4TZF | 4TZE | 3SPU |
|----------------|------|------|------|------|------|
| nb structures  | 197  | 195  | 188  | 193  | 200  |
| nb clusters    | 4    | 5    | 4    | 5    | 5    |

HADDOCK score:
- Cluster 2: -80 +/− 9
- Cluster 1: -89 +/− 4
- Cluster 2: -75 +/− 4
- Cluster 1: -76 +/− 3
- Cluster 3: -76 +/− 3

Cluster size:
- Cluster 2: 34
- Cluster 1: 105
- Cluster 2: 53
- Cluster 1: 110
- Cluster 3: 26

RMSD from the overall lowest-energy structure:
- 0.2 +/− 0.1
- 0.5 +/− 0.1
- 0.3 +/− 0.2
- 0.4 +/− 0.1
- 0.3 +/− 0.2

Van der Waals energy:
- -23 +/− 5
- -22 +/− 1
- -22 +/− 6
- -22 +/− 2
- -15 +/− 4

Electrostatic energy:
- -78 +/− 31
- -0.0 +/− 2.3
- -95 +/− 32
- -73 +/− 36
- -28 +/− 36

Desolvation energy:
- -7 +/− 2
- -24 +/− 3
- -5 +/− 4
- -1 +/− 2
- -14 +/− 5

Restraints violation energy:
- 0.2 +/− 0.3
- 1.5 +/− 1.2
- 0.7 +/− 0.7
- 0.0 +/− 0.1
- 0.8 +/− 0.4

Buried Surface Area:
- 542 +/− 9
- 537 +/− 17
- 530 +/− 20
- 528 +/− 7
- 463 +/− 36

Z-Score:
- -1.1
- -0.9
- -1.2
- -1.2
- -1.1

L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive” residues and morin as “active” residue.

The two Zn(II) were defined as “active” residues.

The «ligand interface RMSD» was used to clustered the structures.

nb structures: is the water-refined models HADDOCK generated

nb clusters: is the number of clusters in which all the water-refined models HADDOCK generated are clustered.

HADDOCK score: is calculated as function of the intermolecular van der Waals energy, the intermolecular electrostatic energy, the empirical desolvation energy term and the Ambiguous Interaction Restraints (AIRs) energy.

Z-Score: indicates how many standard deviations from the average this cluster is located in terms of score.
Table S8 Rmsd calculated on the morin atoms after superimposition on the (45-271) backbone atoms of the ten art-apo NDM-1 structures after docking simulation.

| PDB number | rmsd cluster 1 (Å) | rmsd cluster 2 (Å) |
|------------|---------------------|---------------------|
| 4EY2       | 1.2 ± 0.3           | 1.1 ± 0.7           |
| 4EYB       | 0.8 ± 0.2           | 4.2 ± 3.5           |
| 4EXS       | 5.4 ± 2.4           | 3.5 ± 2.9           |
| 4EYL       | 3.2 ± 3.5           | 6.6 ± 0.2           |
| 4RBS       | 3.6 ± 3.1           | 6.1 ± 2.0           |
| 4EYF       | 1.0 ± 0.4           | 1.4 ± 0.2           |
| 4RL0       | 0.7 ± 0.3           | 1.1 ± 0.4           |
| 4RL2       | 2.6 ± 2.4           | 3.5 ± 2.9           |
| 4RAM       | 1.1 ± 0.7           | 5.0 ± 3.0           |
| 4HL2       | 0.6 ± 0.4           | 3.6 ± 2.5           |

The rmsd were calculated within each cluster for cluster 1 and cluster 2 for each PDB starting structures. The top cluster of each series is in bold (either the cluster 1 or the cluster 2 depending on the PDB starting structures); the ranking of the clusters is based on the average score of the top 4 members of each cluster. The HADDOCK score is calculated as function of the intermolecular van der Waals energy, the intermolecular electrostatic energy, the empirical desolvation energy term and the Ambiguous Interaction Restraints (AIRs) energy.
Table S9 NDM-1/QUERCETIN docking using the molecular-docking simulations in the data-driven program HADDOCK.

The "artificial apo" NDM-1 structures (PDB: 4EXS, 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RBS, 4RL2, 4RL0) used for docking
The two Zn(II) were defined as active residues. Only the results for the top clusters are shown.

| quercetin | 4EXS | 4EY2 | 4EYB | 4EYF | 4EYL | 4HL2 | 4RAM | 4RBS | 4RL2 | 4RL0 |
|-----------|------|------|------|------|------|------|------|------|------|------|
| nb structures | 194 | 200 | 194 | 193 | 198 | 193 | 198 | 200 | 199 | 194 |
| nb clusters | 3 | 4 | 3 | 3 | 5 | 4 | 4 | 5 | 5 | 4 |

| Cluster 2 | Cluster 1 | Cluster 1 | Cluster 2 | Cluster 2 | Cluster 2 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 1 |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| HADDOCK score | -85 +/- 6 | -80 +/- 3 | -77 +/- 4 | -83 +/- 1 | -78 +/- 1.0 | -82 +/- 5 | -75 +/- 6 | -77 +/- 2 | -74 +/- 4 | -69 +/- 4 |
| Cluster size | 78 | 131 | 85 | 71 | 83 | 75 | 45 | 118 | 59 | 87 |
| RMSD from the overall lowest-energy structure | 0.4 +/- 0.2 | 0.4 +/- 0.0 | 0.3 +/- 0.2 | 0.3 +/- 0.0 | 0.4 +/- 0.1 | 0.4 +/- 0.2 | 0.4 +/- 0.1 | 0.5 +/- 0.1 | 0.3 +/- 0.2 | 0.3 +/- 0.2 |
| Van der Waals energy | -15 +/- 2 | -12 +/- 1 | -15 +/- 4 | -17 +/- 3 | -15 +/- 2 | -18 +/- 5 | -15 +/- 2 | -14 +/- 1 | -15 +/- 3 |
| Electrostatic energy | -117 +/- 5 | -72 +/- 5 | -72 +/- 3 | -51 +/- 34 | -111 +/- 7 | -79 +/- 5 | -63 +/- 20 | -74 +/- 2 | -80 +/- 12 | -90 +/- 22 |
| Desolvation energy | -9 +/- 3 | -11 +/- 1 | -11 +/- 4 | -12 +/- 2 | -8 +/- 3.0 | -11 +/- 3 | -8 +/- 2 | -9 +/- 3 | -4 +/- 3 |
| Restraints violation energy | 0.1 +/- 0.1 | 0.0 +/- 0.0 | 0.1 +/- 0.1 | 0.1 +/- 0.2 | 0.1 +/- 0.0 | 0.2 +/- 0.0 | 9.4 +/- 14.9 | 0.1 +/- 0.1 | 0.3 +/- 0.3 | 0.7 +/- 0.2 |
| Buried Surface Area | 499 +/- 12 | 468 +/- 17 | 484 +/- 11 | 485 +/- 14 | 489 +/- 33 | 486 +/- 36 | 470 +/- 23 | 507 +/- 13 | 453 +/- 18 | 505 +/- 21 |
| Z-Score | -1.3 | -1.6 | -1.1 | -1.2 | -0.9 | -1.6 | -1.2 | -0.8 | -1.1 | -0.9 |

- The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as “passive” residues and quercetin as “active” residue.
- The «ligand interface RMSD» was used to clustered the structures.
- nb structures: is the water-refined models HADDOCK generated
- nb clusters: is the number of clusters in which all the water-refined models HADDOCK generated are clustered.
Table S10 NDM-1/MYRICETIN docking using the molecular-docking simulations in the data-driven program HADDOCK.

| MYRICETIN | 4EXS | 4EY2 | 4EYB | 4EYF | 4EYL | 4HL2 | 4RAM | 4RBS | 4RL2 | 4RL0 |
|-----------|------|------|------|------|------|------|------|------|------|------|
| nb structures | 199 | 198 | 197 | 198 | 194 | 198 | 198 | 196 | 198 | 198 |
| nb clusters | 5 | 5 | 4 | 5 | 5 | 4 | 3 | 5 | 5 | 5 |

| Cluster 1 | Cluster 2 | Cluster 1 | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 1 | Cluster 2 | Cluster 2 | Cluster 2 |
|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| HADDOCK score | -85 +/- 4 | -77 +/- 8 | -77 +/- 2 | -82 +/- 2 | -82 +/- 4 | -82 +/- 3 | -78 +/- 4 | -78 +/- 2 | -73 +/- 5 | -73 +/- 4 |
| Cluster size | 70 | 92 | 104 | 94 | 55 | 85 | 76 | 103 | 26 | 77 |
| RMSD from the overall lowest-energy structure | 0.2 +/- 0.1 | 0.2 +/- 0.2 | 0.5 +/- 0.1 | 0.5 +/- 0.1 | 0.3 +/- 0.2 | 0.3 +/- 0.2 | 0.3 +/- 0.1 | 0.3 +/- 0.2 | 0.2 +/- 0.1 |
| Van der Waals energy | -13 +/- 3 | -15 +/- 2 | -15 +/- 5 | -16 +/- 2 | -15 +/- 2 | -15 +/- 3 | -15 +/- 3 | -20 +/- 3 | -15 +/- 5 | -17 +/- 4 |
| Electrostatic energy | -120 +/- 5 | -71 +/- 3 | -84 +/- 25 | -83 +/- 21 | -107 +/- 21 | -83 +/- 22 | -80 +/- 19 | -60 +/- 17 | -71 +/- 40 | -89 +/- 27 |
| Desolvation energy | -11 +/- 4 | -10 +/- 3 | -8 +/- 2 | -11 +/- 1 | -6 +/- 3 | -9 +/- 3 | -7 +/- 3 | -9 +/- 2 | -5 +/- 2 | -3 +/- 4 |
| Restraints violation energy | 0.0 +/- 0.1 | 0.0 +/- 0.0 | 0.1 +/- 0.1 | 0.0 +/- 0.0 | 0.1 +/- 0.1 | 0.1 +/- 0.1 | 0.3 +/- 0.2 | 0.0 +/- 0.0 | 0.3 +/- 0.1 | 0.5 +/- 0.2 |
| Buried Surface Area | 486 +/- 27 | 514 +/- 23 | 483 +/- 30 | 511 +/- 19 | 499 +/- 28 | 462 +/- 24 | 486 +/- 18 | 534 +/- 14 | 460 +/- 16 | 509 +/- 13 |
| Z-Score | -1.0 | -1.0 | -1.1 | -1.4 | -1.2 | -1.2 | -0.8 | -1.2 | -0.9 | -1.2 |

The "artificial apo" NDM-1 structures (PDB: 4EXS, 4EY2, 4EYB, 4EYF, 4EYL, 4HL2, 4RAM, 4RBS, 4RL2, 4RL0) used for docking.
The two Zn(II) were defined as active residues. Only the results for the top clusters are shown.

- The L65, M67, F70, W93, H122, Q123, D124, N220, H250 NDM-1 residues were defined as "passive" residues and myricetin as "active" residue.

- The «ligand interface RMSD» was used to cluster the structures.

- nb structures: is the water-refined models HADDOCK generated

- nb clusters: is the number of clusters in which all the water-refined models HADDOCK generated are clustered.
Table S11 NDM-1/QUERCETIN and QUERCETIN derivatives docking using the molecular-docking simulations in the data-driven program HADDOCK. In CH3A, CH3B and CH3C an OCH3 group replace 3’OH, 3OH and 5OH respectively. The "artificial apo" NDM-1 structures (PDB: 4EYB and 4EY2) used for docking. The two Zn(II) were defined as active residues. Only the results for the top clusters are shown.

| 4EYB | quercetin | CH3A | CH3B | CH3C | 4EY2 | quercetin | CH3A | CH3B | CH3C |
|------|-----------|------|------|------|------|-----------|------|------|------|
| nb struct | 194 | 196 | 197 | 196 | nb struct | 200 | 200 | 196 | 196 |
| nb clusters | 3 | 4 | 4 | 4 | nb clusters | 4 | 5 | 4 | 3 |
| HADDOC K score | Cluster 1 | Cluster 1 | Cluster 2 | Cluster 1 | HADDOC K score | Cluster 1 | Cluster 1 | Cluster 2 | Cluster 1 |
| RMSD from the overall lowest-energy structure | 0.3 +/- 0.2 | 0.3 +/- 0.0 | 0.3 +/- 0.2 | 0.6 +/- 0.1 | RMSD from the overall lowest-energy structure | 0.4 +/- 0.0 | 0.4 +/- 0.0 | 0.3 +/- 0.2 | 0.4 +/- 0.2 |
| Van der Waals energy | -15 +/- 4 | -13 +/- 2 | -12 +/- 5 | -23 +/- 5 | Van der Waals energy | -12 +/- 1 | -12 +/- 4 | -11 +/- 2 | -18 +/- 5 |
| Electrostatic energy | -71 +/- 3 | -75 +/- 7 | -73 +/- 4 | -42 +/- 29 | Electrostatic energy | -72 +/- 6 | -76 +/- 7 | -70 +/- 5 | -47 +/- 33 |
| Desolvation energy | -11 +/- 4 | -11 +/- 2 | -10 +/- 2 | -14 +/- 5 | Desolvation energy | -11 +/- 1 | -11 +/- 2 | -11 +/- 3 | -13 +/- 5 |
| Restraints violation energy | 0.1 +/- 0.1 | 0.1 +/- 0.2 | 0.1 +/- 0.1 | 0.5 +/- 0.4 | Restraints violation energy | 0.0 +/- 0.0 | 0.4 +/- 0.4 | 0.1 +/- 0.1 | 0.5 +/- 0.4 |
| Buried Surface Area | 484 +/- 11 | 511 +/- 15 | 497 +/- 13 | 533 +/- 6 | Buried Surface Area | 468 +/- 17 | 527 +/- 31 | 506 +/- 20 | 517 +/- 12 |
| Z-Score | -1.1 | -1.1 | -1.1 | -0.9 | Z-Score | -1.6 | -1.5 | -1.5 | -1.0 |

- The L65, M67, F70, W93, H122, Q123, D124, N220, defined as “passive” residues and quercetin as “active” residue.
- The “ligand interface RMSD” was used to cluster the structures.
- nb structures: is the water-refined models HADDOCK generated.
- nb clusters: is the number of clusters in which all the water-refined models HADDOCK generated are clustered.
References

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