

Table of Contents

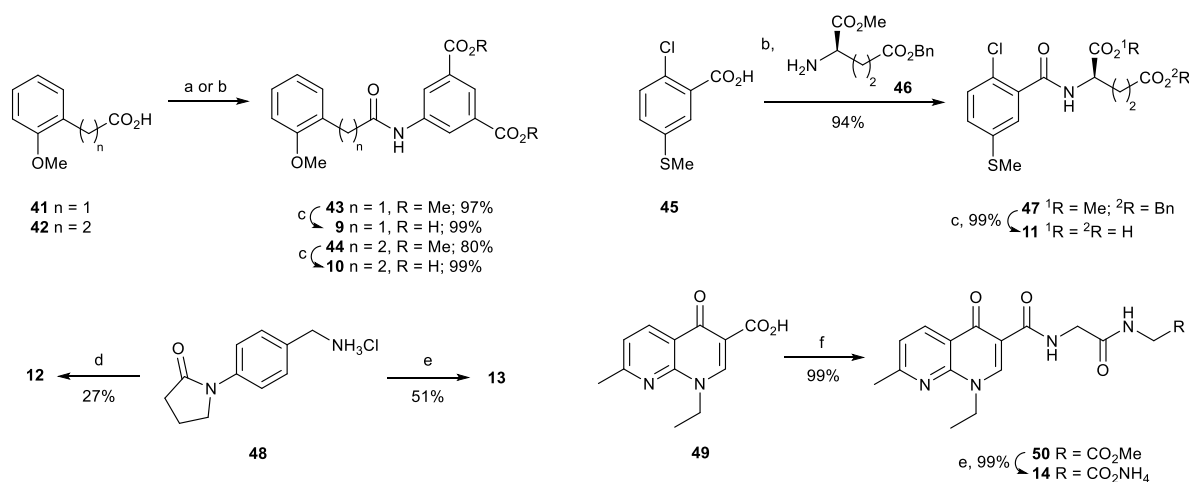
1. Additional Experimental Section	S2
1.1 Synthesis of Compounds 9–14	S2
1.2 Fragment-based screening.....	S7
1.3 Dehydroquinase assays.....	S7
1.4 Molecular Dynamics Simulations.....	S7
2. References	S8
3. Figure S1	S10
4. Figure S2	S11
5. Figure S3	S12
6. Figure S4	S13
7. Table S1	S14
8. NMR spectra	S19
9. Dixon plots	S29

1. Additional Experimental Section

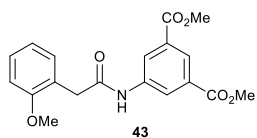
General – All starting materials and reagents were commercially available and were used without further purification. ^1H NMR spectra (250, 300, 400 and 500 MHz) and ^{13}C NMR spectra (63, 75, 100 and 125 MHz) were measured in deuterated solvents. J values are given in Hertz. NMR assignments were carried out by a combination of 1D, COSY, and DEPT-135 experiments. FT-IR spectra were recorded as NaCl plates or KBr discs in a PerkinElmer Two FTIR spectrometer with attenuated total reference. $[\alpha]_D^{20}$ values are given in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$. MilliQ deionized water was used in all the buffers. All procedures involving the use of ion-exchange resins were carried out at room temperature using Milli-Q deionized water. Amberlite IR-120 (H^+) (cation exchanger) was washed alternately with water, NaOH (10%), water, HCl (10%), and finally water before use. The spectroscopic measurements were made on a Varian Cary 100 UV-Vis spectrophotometer with a 1 cm pathlength cell fitted with a Peltier temperature controller. The purity of the reported compounds was analyzed by HPLC and by NMR. HPLC was performed on either a Bio-Rad Aminex ion exclusion HPX-87H organic acids column (300 mm \times 16 mm), eluting with 100 mM aqueous formic acid at a flow rate of 0.6 mL min^{-1} or a Phenomenex Luna 5 μM C18 column (250 mm \times 4.6 mm), eluting with a gradient of acetonitrile/water [from (5:95) to (30:70)] at a flow rate of 1 mL min^{-1} . All tested compounds have a purity $\geq 95\%$.

1.1 Synthesis of Compounds 9–14

The synthesis of compounds **9–14** was performed as outlined in Scheme S1.

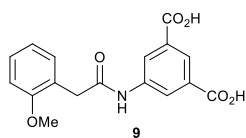


Scheme S1 Synthesis of compounds **9–14**. *Reagents and conditions*. (a) 1. SOCl_2 , CHCl_3 , Δ . 2. dimethyl 5-aminoisophthalate, CH_2Cl_2 , Et_3N , RT. (b) dimethyl 5-aminoisophthalate, HATU, DIPEA, CH_2Cl_2 , RT. (c) 1. LiOH, THF, RT. 2. HCl. (d) *cis*-1,2-cyclohexanedicarboxylic anhydride, DMAP, Et_3N , THF, RT. (e) *trans*-1,2-cyclohexanedicarboxylic anhydride, DMAP, Et_3N , DMF, RT. (f) methyl glycylglycinate, HATU, DIPEA, CH_2Cl_2 , RT. (e) 1. LiOH, THF, RT. 2. Dowex 50W X8–400 (NH_4^+ form).



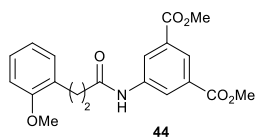
Dimethyl 5-(2-(2-methoxyphenyl)acetamido)isophthalate (43) – A solution of 2-

methoxyphenyl acetic acid (**41**) (114 mg, 0.69 mmol) and thionyl chloride (0.10 mL, 1.38 mmol) in chloroform (2.8 mL) was heated under reflux for 2 h. After cooling to room temperature, solvents were removed under reduced pressure. The resulting residue was dissolved in dry dichloromethane (2 mL) and it was added via canula to a suspension of dimethyl 5-aminoisophthalate (148 mg, 0.69 mmol) and triethylamine (0.14 mL, 1.00 mmol) in dry dichloromethane (3.45 mL). After 3 h, the reaction mixture was diluted with dichloromethane and aqueous HCl (10%). The aqueous layer was separated and the organic extract was washed successively with saturated sodium bicarbonate and brine. The organic extract was dried (anh. Na₂SO₄), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (60:40) diethyl acetate/hexane, to afford the amide **43** (240 mg, 97%) as white solid. Mp: 169–170 °C. ¹H NMR (250 MHz, CDCl₃) δ: 8.37 (s, 1H, H₂), 8.28 (s, 2H, H₄+H₆), 7.90 (s, 1H, NH), 7.39–7.27 (m, 2H, H₃' + H₆'), 6.99 (t, *J* = 8.1 Hz, 2H, H₄' + H₅'), 3.94 (s, 3H, OCH₃), 3.91 (s, 6H, 2×OCH₃) and 3.75 (s, 2H, CH₂) ppm. ¹³C NMR (63 MHz, CDCl₃) δ: 169.8 (C), 165.9 (2×C), 156.9 (C), 138.6 (C), 131.3 (2×C), 131.1 (CH), 129.2 (CH), 125.9 (CH), 124.6 (2×CH), 122.7 (C), 121.4 (CH), 111.0 (CH), 55.6 (OCH₃), 52.4 (2×OCH₃) and 39.8 (CH₂) ppm. IR (KBr) ν: 3300 (NH), 1732 (OCO) and 1678 (NCO) cm⁻¹. MS (ESI) *m/z*: 380 (MNa⁺). HRMS calcd for C₁₉H₁₉NO₆Na (MNa⁺): 380.1105 found, 380.1103.



5-(2-(2-Methoxyphenyl)acetamido)isophthalic acid (9) – A stirred solution of diester **43** (31

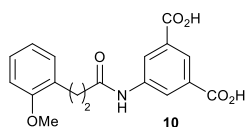
mg, 0.09 mmol) in THF (1 mL) was treated with an aqueous solution of LiOH (0.9 mL, 0.5 M). The reaction mixture was stirred for 2 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with HCl(c). The resulting precipitate was isolated by filtration and washed with MilliQ water. Diacid **9** (29 mg, 99%) was obtained as a white solid. Mp: 323–324 °C. ¹H NMR (250 MHz, CD₃OD) δ: 8.44 (d, *J* = 1.4 Hz, 2H, H₄+H₆), 8.34 (t, *J* = 1.4 Hz, 1H, H₂), 7.29–7.31 (m, 2H, H₄' + H₅'), 6.95 (br d, *J* = 8.1 Hz, 1H, H₆'), 6.89 (dd, *J* = 7.4 and 1.0 Hz, 1H, H₃'), 3.82 (s, 3H, OCH₃) and 3.70 (s, 2H, CH₂) ppm. ¹³C NMR (63 MHz, DMSO-*d*₆) δ: 169.8 (C), 166.6 (2×C), 157.3 (C), 140.0 (C), 131.7 (2×CH), 131.0 (2×C), 128.2 (CH), 124.4 (CH), 123.9 (C), 123.5 (CH), 120.2 (CH), 110.7 (CH), 55.5 (OCH₃) and 37.8 (CH₂) ppm. IR (KBr) ν: 3479 (OH), 3263 (NH), 1693 (OCO) and 1658 (NCO) cm⁻¹. MS (ESI) *m/z*: 328 (M–H). HRMS calcd for C₁₇H₁₄NO₆ (M–H): 328.0816; found, 328.0812.



Dimethyl 5-(3-(2-methoxyphenyl)propanamido)isophthalate (44) – A solution of 3-(2-

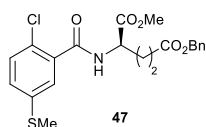
methoxyphenyl)propionic acid (**42**) (212 mg, 1.16 mmol), dimethyl 5-aminoisophthalate (247 mg, 1.16 mmol), *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) (483 mg, 1.28 mmol) and *N,N*-diisopropylethylamine (0.2 mL, 4.64 mmol) in dry dichloromethane (10 mL) was stirred at room temperature for 5 h. The reaction mixture was diluted with dichloromethane and washed with

saturated solution of ammonium chloride, sodium bicarbonate and brine. The organic layer was dried (anh. Na₂SO₄), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (40:60) ethyl acetate/hexane, to give the amide **44** (344 mg, 80%) as white solid. Mp: 138–139 °C. ¹H NMR (250 MHz, CDCl₃) δ: 8.36 (s, 3H, H₂+H₄+H₆), 8.09 (s, 1H, NH), 7.21–7.13 (m, 2H, H₃' + H₆'), 6.84 (m, 2H, H₄' + H₅'), 3.88 (s, 6H, 2×OCH₃), 3.78 (s, 3H, OCH₃), 3.04 (t, *J* = 7.5 Hz, 2H, CH₂) and 2.71 (t, *J* = 7.5 Hz, 2H, CH₂) ppm. ¹³C NMR (63 MHz, CDCl₃) δ: 171.5 (C), 166.0 (2×C), 157.1 (C), 138.7 (C), 131.0 (2×C), 130.0 (CH), 128.5 (C), 127.7 (CH), 125.9 (CH), 124.7 (2×CH), 120.6 (CH), 110.2 (CH), 55.2 (OCH₃), 52.4 (2×OCH₃), 37.5 (CH₂) and 26.2 (CH₂) ppm. IR (KBr) ν: 3361 (NH), and 1699 (CO) cm⁻¹. MS (ESI) *m/z*: 394 (MNa⁺). HRMS calcd for C₂₀H₂₁NO₆Na (MNa⁺): 394.1261; found, 394.1267.



5-(3-(2-methoxyphenyl)propanamido)isophthalic acid (10) – A stirred solution of diester **44**

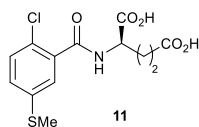
(389 mg, 1.05 mmol) in THF (10 mL) was treated with an aqueous solution of LiOH (11 mL, 0.5M). The reaction mixture was stirred for 2 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with HCl(c). The resulting precipitate was isolated by filtration and washed with MilliQ water. Diacid **10** (356 mg, 99%) was obtained as a white solid. Mp: 305–306 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 13.20 (br s, 2H, 2×OH), 10.26 (s, 1H, H₂), 8.44 (s, 2H, H₄+H₆), 8.15 (s, 1H, NH), 7.17 (m, 2H, H₃' + H₆'), 6.94 (d, *J* = 8.3 Hz, 1H, H₄'), 6.85 (t, *J* = 7.3 Hz, 1H, H₅'), 3.79 (s, 3H, OCH₃), 2.89 (t, *J* = 7.6 Hz, 2H, CH₂) and 2.61 (t, *J* = 7.6 Hz, 2H, CH₂) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆) δ: 171.1 (C), 166.5 (2×C), 157.0 (C), 139.8 (C), 131.6 (2×C), 129.4 (2×CH), 128.6 (C), 127.4 (CH), 124.3 (CH), 123.4 (CH), 120.2 (CH), 110.5 (CH), 55.2 (OCH₃), 36.3 (CH₂) and 25.4 (CH₂) ppm. IR (KBr) ν: 3479 (OH), 3280 (NH), 1718 (CONH), 1697 (CO) and 1660 (CO) cm⁻¹. MS (ESI) *m/z*: 342 (M–H). HRMS calcd for C₁₈H₁₆NO₆ (M–H): 342.0983; found, 342.0980.



1-Methyl-5-benzyl (2R)-2-[2-chloro-5-(methylthio)benzamido]pentanedioate (47) – A solution of

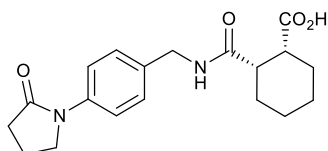
2-chloro-5-(methylthio)benzoic acid (**45**) (146 mg, 0.72 mmol), 5-benzyl 1-methyl D-glutamate (**46**) (182 mg, 0.72 mmol), HATU (330 mg, 0.87 mmol) and *N,N*-diisopropylethylamine (0.5 mL, 2.87 mmol) in dry dichloromethane (7.2 mL) was stirred at room temperature for 3 h. The reaction mixture was diluted with ethyl acetate and washed with saturated solution of ammonium chloride, sodium bicarbonate and brine. The organic layer was dried (anh. Na₂SO₄), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (40:60) ethyl acetate/hexane, to give the amide **47** (292 mg, 94%) as a white solid. [α]_D²⁰ = –12.5 (*c* 1.0, CHCl₃). Mp: 78–79 °C. ¹H NMR (250 MHz, CDCl₃) δ: 7.45 (d, *J* = 2.3 Hz, 1H, ArH), 7.30 (m, 5H, ArH), 7.25 (d, *J* = 8.5 Hz, 1H, ArH) 7.16 (dd, *J* = 8.5 and 2.3 Hz, 1H, ArH), 7.00 (d, *J* = 7.6 Hz, 1H, NH), 5.07 (s, 2H, CH₂), 4.81 (td, *J* = 7.6 and 5.2 Hz, 1H, H₂), 3.72 (s, 3H, OCH₃), 2.42 (s, 3H, SCH₃), 2.55–2.47 (m, 2H, CH₂), 2.40–2.26 (m, 1H, CHH) and 2.17–2.03 (m, 1H, CHH) ppm. ¹³C NMR (62.5 MHz, CDCl₃) δ: 172.5 (C), 171.9 (C), 165.8 (C), 138.5 (C), 135.7 (C), 134.5 (C), 130.5 (CH), 129.3 (CH), 128.6 (C), 128.3 (CH), 127.4 (CH), 127.0 (C), 66.6 (CH₂), 52.7 (OCH₃), 52.3 (CH), 30.3 (CH₂), 27.3 (CH₂) and 15.7 (SCH₃) ppm. IR (KBr) ν: 3315 (NH), 1749 (OCO) 1728 (OCO)

and 1647 (NCO) cm^{-1} . MS (ESI) m/z : 458 (MNa^+). HRMS calcd for $\text{C}_{21}\text{H}_{22}\text{NO}_5\text{SClNa}$ (MNa^+): 458.0799; found, 458.0805.



(2R)-2-[2-chloro-5-(methylthio)benzamido]pentanedioic acid (11) – A stirred solution of diester **47**

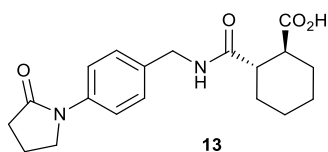
(64 mg, 0.15 mmol) in THF (1.5 mL) was treated with an aqueous solution of LiOH (1.5 mL, 0.5M). The reaction mixture was stirred at room temperature for 1 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with HCl(c). The resulting precipitate was isolated by filtration and washed with MilliQ water. Diacid **11** (48 mg, 99%) was obtained as a white solid. $[\alpha]_{\text{D}}^{20} = +9.4$ (c 1.1, MeOH). Mp: 207–208 °C. ^1H NMR (400 MHz, DMSO- d_6) δ : 8.81 (d, $J = 7.6$ Hz, 1H, NH), 7.74 (d, $J = 8.4$ Hz, 1H, H3'), 7.64 (dd, $J = 8.4$ and 2.1 Hz, 1H, H4'), 7.59 (d, $J = 2.1$ Hz, 1H, H6'), 4.65 (q, $J = 7.8$ Hz, 1H, H-2), 2.82 (s, 3H, SCH₃), 2.67 (t, $J = 7.5$ Hz, 2H, CH₂), 2.36 (m, 1H, CHH) and 2.20 (m, 1H, CHH) ppm. IR (KBr) ν : 3500 (OH), 3309 (NH), 1709 (OCO) and 1643 (NCO) cm^{-1} . MS (ESI) m/z : 330 (M–H). HRMS calcd for $\text{C}_{13}\text{H}_{13}\text{NO}_5\text{SCl}$ (M–H): 330.0208; found, 330.0204.



12

(1R,2S)-2-[(4-(2-oxopyrrolidin-1-yl)benzyl)carbamoyl]cyclohexanecarboxylic acid

(12) – To a solution of *cis*-1,2-cyclohexanedicarboxylic anhydride (39 mg, 0.25 mmol) and dry triethylamine (0.1 mL, 0.76 mmol) in dry DMF (0.8 mL) and under argon was treated with a solution of 1-(4-(aminomethyl)phenyl)pyrrolidin-2-one hydrochloride (**48**) (60 mg, 0.26 mmol) in dry DMF (0.4 mL). The resultant mixture was stirred for 8 h. The solvent was removed under reduced pressure and the resulting residue was partitioned in diluted HCl (0.1M) and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with ethyl acetate ($\times 2$). The combined organic extracts were dried (anh. Na_2SO_4), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (10:90) methanol/ethyl acetate, to give the acid **12** (24 mg, 27%) as a white solid. Mp: 171–172 °C. ^1H NMR (250 MHz, DMSO- d_6) δ : 11.7 (s, 1H, OH), 8.15 (t, $J = 5.7$ Hz, 1H, NH), 7.50 (d, $J = 8.5$ Hz, 2H, H2'), 7.16 (d, $J = 8.5$ Hz, 2H, H3'), 4.16 (d, $J = 5.7$ Hz, 2H, ArCH₂), 3.75 (t, $J = 7.0$ Hz, 2H, NCH₂), 3.56–3.08 (m, 2H, H1+H2), 2.54–2.37 (m, 6H, $3\times\text{CH}_2$), 2.07–1.89 (m, 2H, CH₂) and 1.69–1.40 (m, 4H, $2\times\text{CH}_2$) ppm. ^{13}C NMR (63 MHz, DMSO- d_6) δ : 175.3 (C), 173.7 (C), 173.4 (C), 138.1 (C), 135.5 (C), 127.1 ($2\times\text{CH}$), 119.2 ($2\times\text{CH}$), 48.1 (CH₂), 42.0 (CH), 41.9 (CH), 41.3 (CH₂), 32.3 (CH₂), 27.5 (CH₂), 25.6 (CH₂), 23.9 (CH₂), 22.7 (CH₂) and 17.4 (CH₂) ppm. IR (KBr) ν : 3350 (NH), 1693 (OCO) and 1633 (NCO) cm^{-1} . MS (ESI) m/z : 343 (M–H). HRMS calcd for $\text{C}_{19}\text{H}_{23}\text{N}_2\text{O}_4$ (M–H): 343.1663; found, 343.1659.

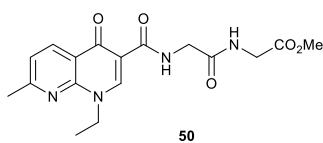


13

(1S,2S)-2-[(4-(2-Oxopyrrolidin-1-yl)benzyl)carbamoyl]cyclohexanecarboxylic acid

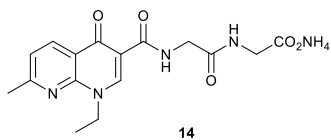
(13) – A solution of *trans*-1,2-cyclohexanedicarboxylic anhydride (109 mg, 0.68 mmol), 1-(4-

(aminomethyl)phenyl)pyrrolidin-2-one hydrochloride (**48**) (161 mg, 0.71 mmol), 4-*N,N*-dimethylaminopyridine (8 mg, 0.07 mmol) and dry triethylamine (0.2 mL, 1.43 mmol) in dry THF (1.9 mL) was stirred for 24 h. The solvent was removed under reduced pressure and the resulting residue was purified by flash chromatography eluting with (10:90) methanol/ethyl acetate to give the acid **13** (118 mg, 51%) as a white solid. $[\alpha]_D^{20} = -4.2$ (*c* 1.0, DMSO). Mp: 208–209 °C. ¹H NMR (250 MHz, DMSO-*d*₆) δ: 11.62 (s, 1H, CO₂H), 8.25 (t, *J* = 5.9 Hz, 1H, NH), 7.50 (d, *J* = 8.6 Hz, 2H, 2×ArH), 7.15 (d, *J* = 8.6 Hz, 2H, 2×ArH), 4.20 (dd, *J* = 15.5 and 5.9 Hz, 1H, CHHN), 4.10 (dd, *J* = 15.5 and 5.9 Hz, 1H, CHHN), 3.74 (t, *J* = 7.0 Hz, 2H, CH₂), 2.45 (m, 2H, 2×CH), 2.04 (m, 2H, CH₂), 1.94–1.82 (m, 2H, CH₂) and 1.71 (m, 2H, CH₂) and 1.23 (m, 6H, 3×CH₂) ppm. ¹³C NMR (63 MHz, DMSO-*d*₆) δ: 176.4 (C), 174.5 (C), 173.7 (C), 138.1 (C), 135.3 (C), 127.0 (2×CH), 119.2 (2×CH), 60.5 (NCH₂), 48.1 (NCH₂), 45.4 (CH), 44.4 (CH), 41.2 (CH₂), 32.3 (CH₂), 29.7 (CH₂), 29.0 (CH₂), 25.2 (CH₂) and 17.4 (CH₂) ppm. IR (KBr) *v*: 3305 (NH+OH), 1730 (CO) and 1647 (CO) cm⁻¹. MS (ESI) *m/z*: 343 (M–H). HRMS calcd for C₁₉H₂₃N₂O₄ (M–H): 343.1652; found, 343.1649.



***N*-[(*N*-(methoxycarbonyl)methyl)carbamoylmethyl] 1-ethyl-7-methyl-4-oxo-**

[1,8]naphthyridine-3-carboxamide (50) – A solution of 1-ethyl-7-methyl-4-oxo-[1,8]naphthyridine-3-carboxylic acid (**49**) (370 mg, 1.56 mmol), diglycine methyl ester (251 mg, 1.56 mmol), HATU (652 mg, 1.71 mmol) and *N,N*-diisopropylethylamine (1.1 mL, 6.31 mmol) in dry dichloromethane (15 mL) was stirred at room temperature for 4 h. The reaction mixture was diluted with ethyl acetate and washed with saturated solution of ammonium chloride, sodium bicarbonate and brine. The organic layer was dried (anh. Na₂SO₄), filtered and concentrated under reduced pressure. The residue obtained was purified by flash chromatography, eluting with (10:90) methanol/dichloromethane, to give the amide **50** (574 mg, 99%) as a white solid. Mp: 276–278 °C. ¹H NMR (400 MHz, DMSO-*d*₆, 70 °C) δ: 10.03 (t, *J* = 5.5 Hz, 1H, NH), 8.93 (s, 1H, H2), 8.57 (d, *J* = 8.1 Hz, 1H, H5), 8.21 (br s, 1H, NH), 7.46 (d, *J* = 8.1 Hz, 1H, H6), 4.58 (q, *J* = 7.1 Hz, 2H, CH₂CH₃), 4.07 (d, *J* = 5.5 Hz, 2H, CH₂), 3.90 (d, *J* = 5.8 Hz, 2H, CH₂), 3.65 (s, 3H, OCH₃) 2.67 (s, 3H, CH₃) and 1.42 (t, *J* = 7.1 Hz, 3H, CH₂CH₃) ppm. ¹³C NMR (100 MHz, DMSO-*d*₆, 70 °C) δ: 175.3 (C), 169.6 (C), 168.8 (C), 163.5 (C), 162.6 (C), 147.9 (C), 147.3 (CH), 135.4 (CH), 120.8 (CH), 119.3 (C), 111.8 (C), 51.1 (CH₂), 45.4 (CH₂), 41.7 (CH₂), 40.3 (CH₂), 24.3 (CH₃) and 14.4 (CH₃) ppm. IR (KBr) *v*: 3292 (NH), 1747 (OCO), 1684 (NCO) and 1647 (NCO) cm⁻¹. MS (ESI) *m/z*: 383 (MNa⁺). HRMS calcd for C₁₇H₂₀N₄O₅Na (MNa⁺): 383.1326 found, 383.1320.



***N*-[(*N*-(carboxyl)methyl)carbamoylmethyl] 1-ethyl-7-methyl-4-oxo-**

[1,8]naphthyridine-3-carboxamide ammonium salt (14) – A stirred solution of the ester **50** (68.6 mg, 0.19 mmol) in THF (0.8 mL) was treated with an aqueous solution of LiOH (1.0 mL, 0.5M). The reaction mixture was stirred for 1 h. The organic solvent was evaporated under reduced pressure and the resultant aqueous solution was washed with ethyl acetate. The aqueous extract was acidified with Dowex 50W X8-400 (NH₄⁺ form). The resulting aqueous phase was evaporated under reduced pressure and freeze-dried to afford the ammonium salt **14** (68 mg, 99%) as a white solid. Mp: 240 °C (dec.). ¹H NMR (250 MHz, DMSO-*d*₆) δ: 10.9 (t, *J* = 5.3 Hz, 1H, NH), 8.97 (s, 1H, H2), 8.55 (d, *J* = 8.1 Hz, 1H, H5), 8.00 (t, *J* = 5.1 Hz, 1H, NH), 7.47 (d, *J* = 8.1 Hz, 1H, H6), 4.59 (q, *J* = 7.0 Hz, 2H, CH₂CH₃), 4.03 (d, *J* = 5.3 Hz,

2H, CH₂), 3.62 (d, $J = 5.1$ Hz, 2H, CH₂), 2.65 (s, 3H, CH₃) and 1.38 (t, $J = 7.0$ Hz, 3H, CH₂CH₃) ppm. ¹³C NMR (63 MHz, DMSO-*d*₆) δ : 175.7 (C), 171.3 (C), 168.3 (C), 163.8 (C), 163.1 (C), 148.0 (CH), 136.0 (CH), 121.4 (CH), 119.7 (C), 111.9 (C), 46.0 (CH₂), 42.2 (2 \times CH₂), 24.9 (CH₃) and 15.6 (CH₃) ppm. IR (KBr) ν : 3566 (N–H), 3269 (NH), 1666 (CO) and 1606 (CO) cm⁻¹. MS (ESI) m/z : 345 (M–NH₄). HRMS calcd for C₁₆H₁₇N₄O₅ (M–NH₄): 345.1204 found, 345.1194.

1.2 Fragment-based screening – It was performed following previously described protocol.²⁷ The enzyme geometries found in PDB code 2WKS^{9m} was used. Taking into account that unfolding and refolding studies of DHQ2 have shown that the trimer⁴⁷ is the biological unit of the enzyme, the trimer was used for these studies. The trimer composed by chains A, B and E in 2WKS was considered.

1.3 Dehydroquinase Assays – The enzyme was purified and assayed as described previously.⁴⁸

1.4 Molecular dynamics simulations – Ligand minimization. Ligand geometries were optimized using a restricted Hartree–Fock (RHF) method and a 6–31G(d,p) basis set, as implemented in the *ab initio* program Gaussian 09.⁴⁹ The resulting wavefunctions were used to calculate electrostatic potential-derived (ESP) charges employing the restrained electrostatic potential (RESP)⁵⁰ methodology, as implemented in the assisted model building with energy refinement (AMBER) suite of programs. The missing bonded and non-bonded parameters were assigned, by analogy or through interpolation from those already present in the AMBER database (GAFF).^{49,51}

Generation and minimization of the DHQ2-ligand complexes. Simulations were carried out using the enzyme geometries found in PDB code 2WKS^{9m} (chains A, B and E).^{9m} The trimer composed by chains A, B and E in 2WKS was considered. Computation of the protonation state of titratable groups at pH 7.0 was carried out using the H++ Web server.⁵² δ and/or ϵ protonation was manually corrected for His102 (dual) of the active site due to mechanistic considerations. Addition of hydrogen and molecular mechanics parameters from the ff14SB⁵³ and GAFF force fields, respectively, were assigned to the protein and the ligands using the LEaP module of AMBER Tools 14.⁵⁴ ⁵⁵ The protein was immersed in a truncated octahedron of ~19000 TIP3P water molecules and neutralized by addition of sodium ions.^{50 56 57} The system was minimized in three stages: (a) minimization of the solvent and ions (5000 steps, first half using steepest descent and the rest using conjugate gradient); (b) minimization of the side chains, waters and ions (5000 steps, first half using steepest descent and the rest using conjugate gradient); (c) final minimization of the whole system (5000 steps, first half using steepest descent and the rest using conjugate gradient). A positional restraint force of 50 kcal mol⁻¹ Å⁻² was applied to the whole protein and α carbons during the first two stages (a–b), respectively.

Simulations. MD simulations were performed using the pmemd.cuda_SPFP^{58 59 60} module from the AMBER 14 suite of programs. Periodic boundary conditions were applied and electrostatic interactions were treated using the smooth particle mesh Ewald method (PME)⁶¹ with a grid spacing of 1 Å. The cutoff distance for the non-bonded interactions was 9 Å. The SHAKE algorithm⁶² was applied to all bonds containing hydrogen, using a tolerance of 10⁻⁵ Å and an integration step of 2.0 fs. Minimization was carried out in three steps, starting with the octahedron water hydrogens, followed by solvent molecules and sodium counterions and finally the entire system. The minimized system was then heated at 300 K at 1 atm by increasing the temperature from 0 K to 300 K over 100 ps and by keeping the system at 300 K another 100 ps. A positional restraint force of 50 kcal mol⁻¹ Å⁻² was applied to all α

carbons during the heating stage. Finally, an equilibration of the system at constant volume (200 ps with positional restraints of 5 kcal mol⁻¹ Å⁻² to α alpha carbons) and constant pressure (another 100 ps with positional restraints of 5 kcal mol⁻¹ Å⁻² to α alpha carbons) were performed. The positional restraints were gradually reduced from 5 to 1 mol⁻¹ Å⁻² (5 steps, 100 ps each), and the resulting systems were allowed to equilibrate further (100 ps). Unrestrained MD simulations were carried out for 100 ns. System coordinates were collected every 10 ps for further analysis. Figures depicting structures were prepared using PYMOL.⁶³ The cpptraj module in AMBER 14 was used to analyze the trajectories and to calculate the root-mean-square deviations (RMSD) of the protein during the simulation.⁶⁴

References

- ⁴⁶ A. B. Maude, A. P. Mehrotra, and D. Gani, *J. Chem. Soc., Perkin Trans. 1* **1997**, *17*, 2513–2526.
- ⁴⁷ N. C. Price, D. J. Boam, S. M. Kelly, D. Duncan, T. Krell, D. G. Gourley, J. R. Coggins, V. Virden, and A. R. Hawkins, *Biochem. J.* **1999**, *338*, 195–202.
- ⁴⁸ C. Sánchez-Sixto, V. F. V. Prazeres, L. Castedo, S. W. Suh, H. Lamb, A. R. Hawkins, F. J. Cañada, J. Jiménez-Barbero, and C. González-Bello, *ChemMedChem* **2008**, *3*, 756–770.
- ⁴⁹ M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian 09, Revision E.01, Gaussian, Inc.: Wallingford CT, 2009.
- ⁵⁰ D. A. Case, T. E. Cheatham, T. Darden, H. Gohlke, R. Luo, K. M. Merz, O. Onufriev, C. Simmerling, B. Wang and R. J. Woods, *J. Comput. Chem.* **2005**, *26*, 1668–1688.
- ⁵¹ J. Wang, W. Wang, P. A. Kollman, and D. A. Case, *J. Mol. Graph. Mod.* **2006**, *25*, 247–260.
- ⁵² (a) J. C. Gordon, J. B. Myers, T. Folta, V. Shoja, L. S. Heath and A. Onufriev, *Nucleic Acids Res.*, **2005**, *33* (Web Server issue):W368. (b) <http://biophysics.cs.vt.edu/H++>.
- ⁵³ J. A. Maier, C. Martinez, K. Kasavajhala, L. Wickstrom, K. E. Hauser and C. Simmerling, *J. Chem. Theory Comput.* **2015**, *11*, 3696–3713.
- ⁵⁴ D.A. Case, V. Babin, J. T. Berryman, R. M. Betz, Q. Cai, D. S. Cerutti, T. E. Cheatham, III, T. A. Darden, R. E. Duke, H. Gohlke, A.W. Goetz, S. Gusarov, N. Homeyer, P. Janowski, J. Kaus, I. Kolossváry, A. Kovalenko, T. S. Lee, S. LeGrand, T. Luchko, R. Luo, B. Madej, K.M. Merz, F. Paesani, D. R. Roe, A. Roitberg, C. Sagui, R. Salomon-Ferrer, G. Seabra, C.L. Simmerling, W. Smith, J. Swails, R. C. Walker, J. Wang, R. M. Wolf, X. Wu and P. A. Kollman (2014), AMBER 14, University of California, San Francisco.
- ⁵⁵ (a) J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman and D. A. Case, *J. Comp. Chem.* **2004**, *25*, 1157–1174. (b) J. Wang, W. Wang, P. A. Kollman and D. A. Case, *J. Mol. Graphics Modell.* **2006**, *25*, 247–260.
- ⁵⁶ J. Aqvist, *J. Phys. Chem.* **1990**, *94*, 8021–8024.
- ⁵⁷ W. L. Jorgensen, J. Chandrasekhar and J. D. Madura, *J. Chem. Phys.* **1983**, *79*, 926–935.
- ⁵⁸ A. W. Goetz, M. J. Williamson, D. Xu, D. Poole, S. Le Grand and R. C. Walker, *J. Chem. Theory Comput.* **2012**, *8*, 1542–1555.

-
- ⁵⁹ R. Salomon-Ferrer, A. W. Goetz, D. Poole, S. Le Grand and R. C. Walker, *J. Chem. Theory Comput.* **2013**, *9*, 3878–3888.
- ⁶⁰ S. Le Grand, A. W. Goetz, and R. C. Walker, *Comp. Phys. Comm.* **2013**, *184*, 374–380.
- ⁶¹ T. A. Darden, D. York and L. G. Pedersen, *J. Chem. Phys.* **1993**, *98*, 10089–10092.
- ⁶² J.-P. Ryckaert, G. Ciccotti and H. J. C. Berendsen, *J. Comput. Phys.* **1977**, *23*, 327–341.
- ⁶³ W. L. DeLano, The PyMOL Molecular Graphics System; DeLano Scientific LLC: Palo Alto, CA, 2008; <http://www.pymol.org/>
- ⁶⁴ D. R. Roe and T. E. Cheatham, *J. Chem. Theory Comput.* **2013**, *9*, 3084–3095.